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The Synergistic Potentials of *Platostoma africanum* and *Psidium guajava* against Some Multi-drug Resistant Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Author COE designed the study. Authors CCC and PME carried out the laboratory analyses. Authors PME and ANO performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Authors CCA and FAO revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Multiple drug resistant bacteria (MDR) are a global concern. This study investigates the possible broad-spectrum and synergistic antibacterial activities of ethanol leaf extracts of *Psidium guajava* and *Platostoma africanum* combinations on multi-drug resistant isolates.
Methods: The study used three (3) strains of extended spectrum beta lactamase (ESBL)-producing *Escherichia coli*, one non-ESBL-producing *E. coli* (control), 2 strains of methicillin resistant *Staphylococcus aureus* (MRSA) and one non-MRSA (control).The minimum inhibitory concentrations (MICs) of the plant extracts were determined against the test isolates using the agar dilution method.

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Assessment of synergism of the plant combinations against the test isolates was done by the checkerboard method. Their fractional inhibitory concentrations (FICs) indices were calculated and used to indicate synergism against the test organisms.
Results: *P. guajava* showed broad antibacterial activity against both groups of resistant organisms. *P. africanum* showed poor antimicrobial activity against the Gram-negative organisms compared to the Gram-positive strains. Determination of bactericidal activity of the extracts on the test isolates showed that the killing ability of the plants was time dependent. Most combination ratios of the plant extracts showed synergism against ESBL-producing *E. coli* strains and additivity against MRSA. The 8:2 (*P. guajava*: *P. africanum*) combination showed synergism against all the test isolates, with FIC indices ranging from 0.106 to 0.825.
Conclusions: *P. guajava* and *P. africanum* (8:2) combination has synergistic and broad spectrum antibacterial activity against ESBL-producing *Escherichia coli* and MRSA. The possibility of using the combination for disinfection is foreseeable. Characterization of the active principles from these plants is needed.

Keywords: Antimicrobial synergism; Platostoma africanum; Psidium guajava; multi-drug resistance.

1. INTRODUCTION

The emergence of multiple drug resistant bacteria (MDR) has become a major global concern considering the untold consequences arising from treatment failures [1,2]. The global health is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many, and in some cases all, effective antibiotics [3]. Much like the situation in human medicine, the use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains of microbial pathogens, potentially complicating treatment for plants and animals [4]. Furthermore changing patterns of susceptibility and the availability of new antimicrobial agents require continuous updating of knowledge concerning treatment of disease caused by such pathogens. Infections that have acquired resistance against commonly used therapeutic agents have emerged as a global health security concern with serious economic, social and political implications and need to be addressed urgently and at all levels. There is a need to look for new strategies for the management of infectious diseases caused by resistant bacteria. One of the possible strategies towards this objective involves the rational localization of bioactive phytochemicals which have antibacterial activity [5,6].

Up till date, plants are the almost exclusive source of drugs for the majority of the world population [7]. People in developing countries utilize traditional medicine for their primary health care needs [8]. Also plants are currently being manipulated for possible production of vaccines [9,10] that could reduce the burden of infectious diseases in developing nations. In developing economies, synthetic drugs are not only expensive and inadequate for the treatment of infectious diseases but are at times adulterated [11]. Therefore, there is need to search for new infection-fighting strategies to control microbial infections.

The search for antimicrobial compounds from plants has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms [12,13]. Multiple drug resistant pathogens are increasing in number and pose threat to existing therapeutic agents [14]. There is therefore a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Plants present potential hope in that regard. This study will seek to unveil the synergistic effects of combinations of *Psidium guajava* and *Platostoma africanum* against some disease-causing multidrug resistant bacteria.

2. METHODS

2.1 Extraction

The leaves of *P. africanum* and *P. guajava* were air dried at room temperature after washing with distilled water. They were grounded into fine powder with a mechanical grinder and macerated in 95% ethanol for three days. After maceration, the ethanol solutions of the plants were filtered through No. 1 Whatman filter paper and the resulting solutions dried in a rotary evaporator at 40℃. The dried extracts recovered

were placed in sterilized screw-capped bottles and stored at 4℃.

2.2 Confirmation of ESBL-Producing Isolates

All the isolates (obtained as stocks from Pharmaceutical Microbiology laboratory of Nnamdi Azikiwe University) of *E. coli* suspected of producing ESBL, were evaluated for ESBL production by using the phenotypic confirmatory test [Double Disk Synergy Test (DDST) method] as described by CLSI [15]. Briefly, a 0.5 McFarland's suspension of each isolate was spread on a Muller-Hinton (MH) agar plate using a sterile swab stick and with a sterile forceps, a disc of amoxicillin/clavulanic acid (20 µg/10 µg) was placed in the centre of the plate. Giving a center-center distance of 20 mm around the co-amoxiclav disc, ceftriaxone (30 µg) and ceftazidime (30 µg) discs were applied. The plates were incubated 18 – 24 h at 37℃.

An isolate was considered to be an ESBL producer if there was zone of inhibition getting stretched towards amoxiclav disc. All positive ESBL producers were further confirmed by combination disc method as recommended by CLSI [15], utilizing third generation cephalosporin disc alone and in combination with clavulanic acid. The observation of ≥ 5 mm increase in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone diameter when tested alone was taken as a confirmation of ESBL production. The increase in the zone diameter was due to the inhibition of the β-lactamase by clavulanic acid.

2.3 Confirmation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates

All the isolates (obtained as stocks from Pharmaceutical Microbiology laboratory of Nnamdi Azikiwe University) of *S. aureus* suspected to be MRSA strains were evaluated for methicillin resistance by using the disc diffusion method as described by CLSI [15]. The direct colony suspension of organisms adjusted to 0.5 McFarland's standard (1×10^8 CFU/mL) was swabbed onto Mueller-Hinton agar and 1 µg oxacillin disc was placed. The plates were incubated at 35℃ for 24 h. Isolates showing inhibition zone sizes ≤ 10 mm were considered as resistant MRSA; 11-12 mm were considered as intermediate resistant and ≥13 mm were considered as susceptible (not MRSA).

2.4 Determination of Antibiotic Susceptibility Profile of Test Isolates

McFarland 0.5 turbidity standard was used to adjust the inocula of the 16 – 20 h old bacterial cultures grown at 37℃. Disc Diffusion susceptibility test (modified Kirby-Bauer method) was carried out as described by Cheesbrough [16]. A sterilized wire loop was used to transfer 3-5 isolated colonies from a Nutrient agar plate into a sterile bijou bottle containing about 4 mL of physiological saline. The colonies were emulsified in the normal saline to obtain a homogenous suspension of the bacterial cells. The turbidity of the suspension was adjusted visually to that of 0.5 McFarland turbidity standard by adding sterile physiological saline to the suspension. This was used as the inoculum. A sterile swab stick was dipped in the standardized inoculum in the bijou bottle; excess fluid was removed from the swab by pressing it against the side of the bottle. The surface of a Mueller Hinton (MH) agar plate previously dried in an incubator was then swabbed. The plate was left on the bench for about 20-30 min. The antibiotic discs were aseptically placed on the inoculated plates. Each disc was gently pressed on the agar surface using a sterilized forceps to ensure proper contact. Plates were inverted within 30 min of applying the discs and incubated aerobically at 35 - 37℃ for 12 - 18 h. The inhibition zone diameter (IZD) around each disc was measured in millimeter (mm) using a plastic transparent ruler.

2.5 Primary Screening of Extracts for Antibacterial Activity

The agar well diffusion technique described by Perez et al. [17] was used to determine the antibacterial activity of the extracts. Dilutions of 250, 125, 62.5 and 31.25 mg/mL were prepared from 500 mg/mL stock solutions of the four extracts. 20 mL of molten Mueller-Hinton (MH) agar were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of 16–20 h old cultures of test isolates were swabbed aseptically on the agar plates and holes of diameter 8 mm were made in the agar plates using a sterile metal cork-borer. 20 µl of the various dilutions of each extract and control were

put in each hole under aseptic condition, kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. Ciprofloxacin was used as positive control, while sterile water and DMSO were used respectively as the negative controls for the aqueous and ethanol extracts of each plant under study. The plates were then incubated at 37℃ for 24 h and the zones of inhibition were measured.

2.6 Determination of Minimum Inhibitory Concentration (MIC) of the Crude Extracts on Test Isolates

Agar dilution method as described by Russell and Furr [18] was used to determine the MIC of the extracts. The MICs were determined by the agar dilution method on Mueller Hinton (MH) agar using the plant extracts that inhibited the microorganisms. Stock solution (4000 mg/mL) of each extract was prepared by dissolving the extracts in DMSO. The stock solutions were further diluted in a 2-fold dilution to obtain the following concentrations: 2000, 1000, 500, 250 and 125 mg/mL. The MIC of the control drug (ciprofloxacin) was also determined at dilutions of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. Agar plates were prepared by pouring 19 mL of MH agar into sterile petri plates containing 1mL of the various dilutions of each of the extracts and control drug. The final plate concentrations were 200, 100, 50, 25, 12.5 and 6.25 mg/mL for the extracts and 50, 25, 12.5, 6.25, 3.125, and 1.5625 µg/mL for the control drug. Bacteria were grown for 18 h in Nutrient broth and culture suspensions adjusted to McFarland 0.5 were applied to the surface of the agar plates containing dilutions of the extracts. Plates were incubated at 37℃ for 24 h, after which all plates were observed for growth. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC.

2.7 Determination of Bactericidal Activity of the Extracts on the Test Isolates (Time-Kill assay)

The test isolates (2 strains of MRSA, 3 strains of ESBL-producing *E. coli*, one control strain of *S. aureus* and one control strain of *E. coli*) were used in this experiment. Standardized concentrations (McFarland 0.5, equivalent to $1x10^8$ CFU/mL) of logarithmic phase culture of test isolates were prepared. An appropriate quantity of the extract was added to a sterile test tube containing nutrient broth to give a concentration of 2 x MIC of the extract. A volume of 1 mL of the standardized test culture ($1x10^8$ CFU/mL) was added to 9mL of the extract-broth mixture to give a microbial concentration of $1x10^7$ CFU/mL. The extract-broth-culture mixture (0.2 mL) was placed in a sterile Petri plate and molten MH agar poured into the plate and left to solidify. This is to give control time 0 min count. Samples were taken after 2, 4, 6, and 8 h intervals. The procedure was carried out in triplicates to ensure accuracy. Plates were incubated at 37℃ for 24 hr before counting the colonies. For positive controls, control strains (non-ESBL *E. coli* and non-MRSA respectively) were subjected to the above procedure while for a negative controls, control strains of *S. aureus* and *E. coli* were grown in tubes containing broth with no added plant extract and samples taken at the indicated time intervals. Positive and negative control plates were also incubated. The number of colony forming unit (CFU) were counted after the period of incubation. A graph of percentage viable count against time (h) was plotted.

2.8 Evaluation of Possible Synergistic Antimicrobial Activity of Plants in Combination

The most popular method used to detect antimicrobial interaction is the chessboard or checkerboard titration test in which two drugs are cross-titrated against each other. After incubation, an isobologram is constructed by plotting the inhibition of growth observed at each drug concentration on an arithmetic scale. The line of additivity joins the MICs of the individual drugs acting alone; a deviation of this line towards the axes of the graph suggests synergy; a deviation away from the axes indicates antagonism, although indifference may also produce this result. The summation of the Fractional Inhibitory Concentrations index (ΣFIC) can be calculated [19].

$$\Sigma FIC \text{ index} = FIC_A + FIC_B = \frac{\text{Conc. of A in MIC of A+B}}{\text{MIC of A alone}} + \frac{\text{Conc. of B in MIC of A+B}}{\text{MIC of B alone}}$$

According to Okore [20], the FIC index is interpreted as:

> **Synergism**, if its value is less than 1.0;
> **Additivity**, if it is equal to 1.0;
> **Indifference**, if more than 1.0; and
> **Antagonism**, if more than 2.0

Checkerboard assay of antimicrobial combinations was performed as described by Akinpelu and Kolawole [21] with modifications. Briefly, crude extracts 'A' and 'B', each with 4000 mg/mL initial concentrations, were diluted to 1/10 of their MICs along the ordinate and abscissa respectively. The solutions of these extract combinations (2000 mg/mL) were further diluted in a 2-fold dilution process to obtain the following concentrations: 2000, 1000, 500, 250, 125, and 62.5 mg/mL. Agar plates were prepared by pouring 19 mL of MH agar into sterile Petri plates containing 1mL of the various dilutions of each of the extracts. The final plate concentrations were 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL for the extracts. Bacteria were grown for 18 h in Nutrient broth and culture suspensions adjusted to 0.5 McFarland were applied to the surface of the agar plates containing dilutions of the extracts. Plates were incubated at 37℃ for 24 h, after which all plates were observed for growth. The MICs were determined for each crude extract combination and the Fractional Inhibitory Concentrations (FIC) calculated. The test was carried out in duplicate.

3. RESULTS

3.1 Confirmation of Test Isolates

The double disk synergy test (DDST) confirmed the ESBL-producing test isolates (Fig. 1, Plate A and B) while disc diffusion test confirmed the MRSA isolates (Fig. 1, Plate C). In the DDST method, the observation of a ≥ 5 mm increase in the zone diameters produced by the cephalosporins which was tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed the presence of ESBL production by the organism. *S. aureus* isolates showing inhibition zone sizes ≤ 10 mm were considered as MRSA. Here, an IZD of 0 mm was recorded for the test organism which confirms it as an MRSA.

Table 1 shows the antibiotic susceptibility profile of test organisms. The Inhibition Zone Diameter shown is the mean ± SEM of three readings. The multidrug resistant nature of the test isolates (strains of MRSA and ESBL) can be observed as they are resistant to most of the antibiotics tested.

Table 2 shows that *P. guajava* extract displayed more antibacterial activity against the test isolates than *P. africanum.* The *P. guajava* extract was equipotent against the *S. aureus* strains (both MRSA and non-MRSA). The *P. africanum* extract was equipotent against MRSA 2 and control *S. aureus* but less potent against MRSA 1. Comparatively, *P. guajava* extract was more potent than *P. africanum* extract against the *E. coli* strains while the reverse is almost the case against the *S. aureus* strains.

It can be observed in Fig. 2 that the ethanol extracts of the two plants had bactericidal activity against the various test organisms during the time-kill assay and the killing ability of the plants was time dependent.

Most combinations of *P. guajava* and *P. africanum* showed *in vitro* synergistic activity on ESBL-producing *E. coli* 1 and 3, while all combinations showed *in vitro* synergistic activity on ESBL-producing *E. coli* 2 (Table 3). A different picture was seen with non-ESBL-producing *E. coli* (control) were indifference was shown in most cases. Synergism appeared only in three combinations (8:2, 4:6 and 3:7). The implication of this is that, in disease situations where ESBL-producing *E. coli* are implicated, these synergistic combinations of the extract will be preferred.

From Table 4, it can be seen that the combinations showed mostly additivity against the MRSA 1 but antagonism against MRSA 2. The situation was also different from the response shown by the non-MRSA (control). There, indifference dominated followed by synergism and lastly, antagonism. The best combination showing synergism against all the *S. aureus* strains was observed to be 8:2 (*P. guajava* and *P. africanum*) combination.

4. DISCUSSION

The investigation of plant extracts effective against multidrug resistant bacteria [including extended spectrum beta-lactamase - producing organisms and methicillin-resistant *S. aureus*] provides an example of prospecting for new compounds which may be particularly effective against infections that are currently difficult to treat.

Table 1. Mean IZD (mm) produced by commercial antibiotics against the test organisms

Antibiotics	ESBL 1	ESBL 2	ESBL 3	*E. coli* (control)	MRSA 1	MRSA 2	*S. aureus* (control)
Ciprofloxacin (5 µg)	0±0	0±0	0±0	24±0	14±0	20±0	42±0
Ofloxacin (5 µg)	0±0	0±0	0±0	22±0.33	8±0	22±0.88	35±0
Chloramphenicol (30 µg)	18±0	0±0	0±0	10±0	35±0	18±0	37±0
Erythromycin (30 µg)	9±0	0±0	0±0	11±0.67	35±0	10±0	15±0
Nalidixic acid (30 µg)	0±0	0±0	0±0	22±0.67	11±0	0±0	16±0.67
Ceftazidime (30 µg)	0±0	0±0	0±0	10±0.33	0±0	0±0	8±0
Meropenem (10 µg)	34±0	15±0.33	13±0.67	16±0.67	0±0	0±0	11±0.33
Imipenem (10 µg)	31±0	30±0	34±0	37±0.33	50±0	18±0	52±0
Ampicillin (10 µg)	0±0	0±0	0±0	0±0	13±0	0±0	30±0
Oxacillin (1 µg)	0±0	0±0	0±0	12±0	0±0	0±0	15±0
Gentamicin (10 µg)	12±0	10±0.33	12±0	24±0.58	24±0.67	20±0	30±0
Co-trimoxazole (25 µg)	0±0.33	0±0	0±0	32±0.33	32±0	35±0	32±0
Tetracycline (30 µg)	0±0	0±0	16±0.33	20±0	12±0.33	20±0	27±0
Co-amoxiclav (30 µg)	14±0	0±0	0±0	0±0	7±0.33	0±0	18±0
Cefotaxime (30 µg)	11±0	0±0	0±0	26±0.67	0±0	0±0	0±0

SEM: standard error of mean.

Table 2. MIC (mg/mL) of crude ethanolic extract of the plants and control drug on test Isolates

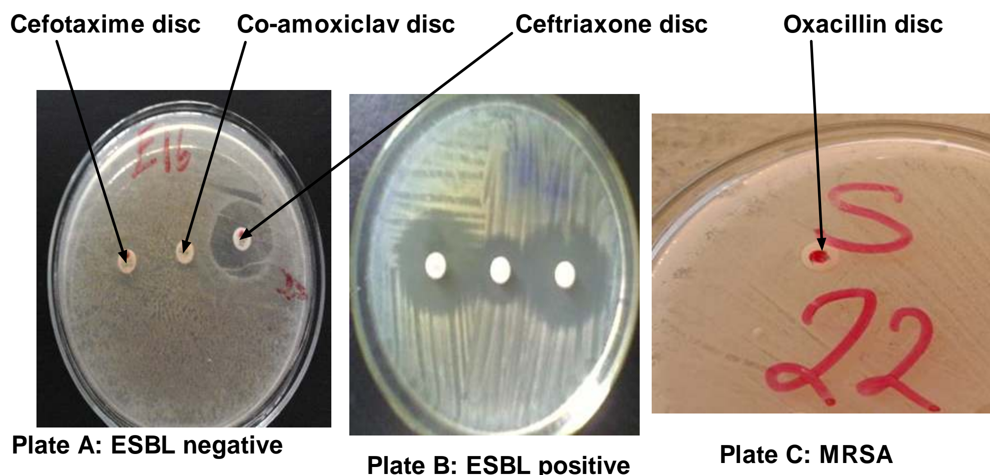
Test Isolates	*P. guajava*	*P. africanum*
ESBL 1	25	200
ESBL 2	50	200
ESBL 3	50	200
E. coli (control)	12.5	100
MRSA 1	25	25
MRSA 2	25	6.25
S. aureus (control)	25	6.25

Table 3. Activity of combinations of *Psidium guajava* and *Platostoma africanum* against test ESBL producing and non-ESBL producing (Control) *E. coli*

Combinations by continuous variations		Activity against test ESBL producing *E. coli* 1		Activity against test ESBL producing *E. coli* 2		Activity against test ESBL producing *E. coli* 3		Activity against test *E. coli* (Control)	
P. guajava	***P. africanum***	**FIC index (ΣFIC)**	**Results interpretation**	**FIC index (ΣFIC)**	**Results interpretation**	**FIC index (ΣFIC)**	**Results interpretation**	**FIC index (ΣFIC)**	**Results interpretation**
10	0	0.913	-	-	-	-	-	-	-
9	1	0.413	Synergism	0.231	Synergism	0.463	Synergism	1.825	Indifference
8	2	0.738	Synergism	0.106	Synergism	0.213	Synergism	0.825	Synergism
7	3	0.650	Synergism	0.097	Synergism	0.388	Synergism	1.475	Indifference
6	4	0.281	Synergism	0.088	Synergism	0.350	Synergism	1.300	Indifference
5	5	0.475	Synergism	0.079	Synergism	0.313	Synergism	1.125	Indifference
4	6	0.775	Synergism	0.138	Synergism	0.275	Synergism	0.950	Synergism
3	7	1.200	Indifference	0.238	Synergism	0.475	Synergism	0.775	Synergism
2	8	1.700	Indifference	0.400	Synergism	0.800	Synergism	1.000	Additivity
1	9	0.913	Synergism	0.650	Synergism	1.300	Indifference	1.700	Indifference
0	10	-	-	-	-	-	-	-	-

Table 4. Activity of combinations of *Psidium guajava* and *Platostoma africanum* against MRSA and non-MRSA (Control)

Combinations by continuous variations		Activity against test isolate MRSA 1		Activity against test isolate MRSA 2		Activity against test isolate non-MRSA (control)	
P. guajava	***P. africanum***	**FIC index (ΣFIC)**	**Results interpretation**	**FIC index (ΣFIC)**	**Results interpretation**	**FIC index (ΣFIC)**	**Results interpretation**
10	0	-	-	-	-	-	-
9	1	1.000	Additivity	1.300	Indifference	1.300	Indifference
8	2	0.500	Synergism	0.800	Synergism	0.800	Synergism
7	3	1.000	Additivity	1.900	Indifference	1.900	Indifference
6	4	1.000	Additivity	2.200	Antagonism	2.200	Antagonism
5	5	1.000	Additivity	2.500	Antagonism	2.500	Antagonism
4	6	1.000	Additivity	2.800	Antagonism	1.400	Indifference
3	7	1.000	Additivity	3.100	Antagonism	1.550	Indifference
2	8	1.000	Additivity	3.400	Antagonism	0.850	Synergism
1	9	1.000	Additivity	3.700	Antagonism	0.925	Synergism
0	10	-	-	-	-	-	-



Plates A and B showing ESBL negative and ESBL positive *E. coli* strains repectively.
Plate C showing antimicrobial pattern of a *S. aureus* isolate confirmed to be MRSA.

Fig. 1. Confirmation of test isolates/organisms

As the rapid emergence of drug-resistant organisms necessitates the continuous search for new antimicrobial substances, natural products may act as alternative to antibiotics and chemotherapeutic agents in certain circumstances. Antibacterial activity may be due to active components which are present in plant extracts. Some reasons to support the use of antimicrobial combinations include: decreased emergence of resistant strains, decreased dose-related toxicity as a result of reduced dosage and increased spectrum of activity against polymicrobial infections [22]. Some researchers have reported antibacterial activities of combinations of plant materials with antibiotics against both Gram-positive and Gram-negative bacteria [23,24].

Our study (Table 1) showed that ESBL-producing *E. coli* are usually resistant to several antibiotics including the fluoroquinolones and aminoglycosides. Resistance against the carbapenems (meropenem) is worthy of note in this study. The MRSA was also observed in the study. All these multidrug resistant organisms pose great challenge to clinicians, patients and the healthcare system in general. Several authors have reported the emergence of resistant *E. coli* and *Staphylococcus aureus* [25,-27].

The *P. guajava* and *P. africanum* individually showed better activities against the non-ESBL-producing and non-MRSA (control) strains. However, combinations of the two plants showed better synergistic activity against test ESBL-producing organisms compared to the non-ESBL-producing (control) strain. At high concentrations, bacteriostatic agents may show bactericidal actions. Studies [28,29] show that antimicrobial agents usually have higher MICs (close to the susceptibility breakpoint) against resistant organisms than susceptible ones. The MICs of the *P. guajava* extract on the *S. aureus* (MRSA and non-MRSA) were comparable but that shown by *P. africanum* slightly differs. The high MICs observed in this study was because the pure active principle was not extracted and investigated. It is expected that the MIC of the pure active principle will be in µg quantity.

The antibacterial activities of all the extracts of the plants materials either when used separately or combined were time dependent (Fig. 2) as killing rate increased with time. It had been shown that the efficacy of most plant extracts is time and concentration dependent [30,31].

FIC indices were used as indicators of synergistic activities of the antimicrobial combinations. *P. guajava* + *P. africanum* combinations showed more synergistic than indifferent or antagonistic effects against the test isolates (Tables 3 and 4). The 8:2 combination of the two plants recorded synergistic activities against all the test isolates (both resistant and susceptible Gram-negative and Gram-positive isolates), with FIC indices ranging from 0.106 to 0.825. The synergistic effects of *P. guajava* + *P. africanum* combinations were more pronounced on the Gram-negative organisms.

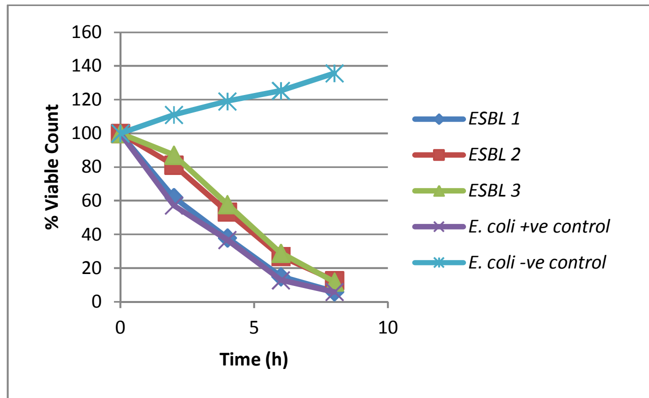


Fig. 2a. Bactericidal activities of ethanol extracts of *P. africanum* on Gram-negative test organisms

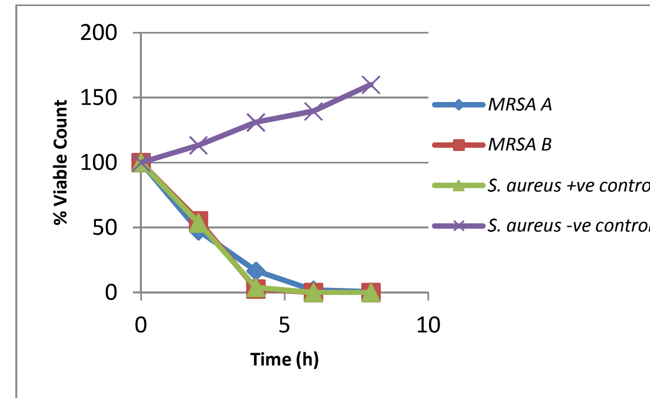


Fig. 2b. Bactericidal activities of ethanol extracts of *P. africanum* on Gram-positive test organisms.

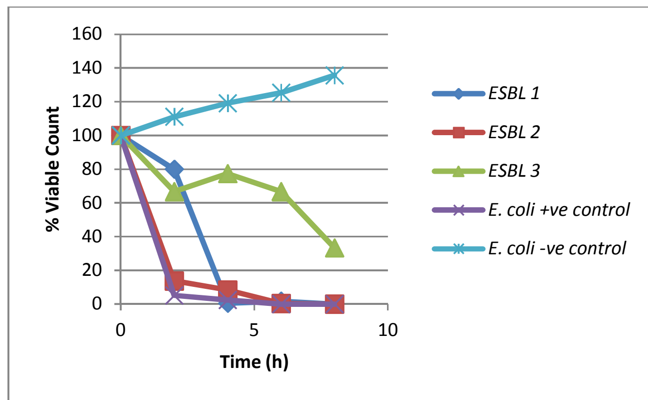


Fig. 2c. Bactericidal activities of ethanol extracts of *P. guajava* on Gram-negative test organisms

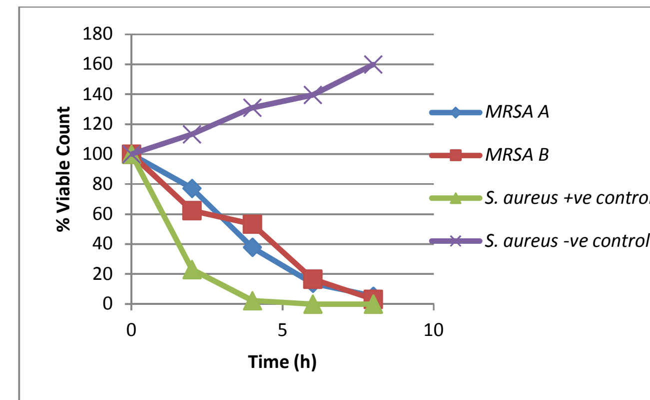


Fig. 2d. Bactericidal activities of ethanol extracts of *P. guajava* on Gram-positive test organisms

Fig. 2. Determination of bactericidal activity of the extract on the test isolates (Time-kill Assay)

Synergistic or additive effects support the use of the plants in combination instead of use in isolation against pathogenic bacteria especially multidrug resistant strains. This synergism might be used advantageously in treating some resistant bacterial infections or disinfecting surfaces suspected to be contaminated by these resistant organisms or better still be investigated as a possible preservative for pharmaceutical product due to its broad spectrum of activity and high microbial killing rate.

According to Cain et al. [32] synergistic activity suggests different mode of actions of the combining compounds. The improvement in the activity of the two plant extracts may be due to the accumulation of inhibitory concentrations at the target sites or due to other mechanisms. Combined antimicrobials are preferred as microbial resistance is less likely to develop against substances having more than one type of modes of action [33].

It could be said that the ethnomedicinal significance of the selected plants for this study corresponds to the pharmacological actions of the secondary metabolites they contain. It is therefore, pertinent that these specific metabolites be screened and separated to undergo pharmacological processes and if active, be developed to become a potent drug.

There is the need to focus research on plants with antimicrobial properties, with the aim of characterizing the active phytocompounds of these plants and screening the secondary metabolites in them against microbial growth. This is necessary because of the microbial resistance manifested by some of the pathogenic microorganisms against the common antibiotics.

Furthermore, toxicity issues also have to be considered and the efficacy of non-toxic extracts have to be evaluated in vivo. Elaborate laboratory and clinical studies of plants are needed in order to better understand their antibacterial properties so as to allow the scientific community to recommend their uses as an alternative to conventional antibiotics [8].

5. CONCLUSION

The present study has shown the combinations of ethanol leaf extracts of *P. africanum* and *P. guajava* to possess synergistic antibacterial activity against ESBL-producing *Escherichia coli* and MRSA, and thus provide the initial steps for further isolation and characterization of the active antibacterial principles from these plants. The study of the synergistic interaction of active phytocompounds is required to exploit these potential plant extracts in the combination therapy against infectious diseases caused by multidrug-resistant organisms. Also, screening of the isolated and fully characterized active antibacterial principles, singly and in combination for possible improved therapeutic results is needed. The extracts, in combination, may be used in wound dressing and in disinfecting surfaces.

HIGHLIGHTS

- *Psidium guajava* and *Platostoma africanum* combinations show broad-spectrum antibacterial property.
- *Psidium guajava* and *Platostoma africanum* leaves extract in the 8:2 combination ratio offers a fresh hope in the fight against multi-drug resistant bacteria.
- Reporting carbapenem resistant ESBL-producing *E. coli* strain.
- *Psidium guajava* and *Platostoma africanum* are potential sources of lead molecules against multi-drug resistant bacteria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

Some parts of this manuscript were previously presented and published as an abstract in the following conference.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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