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Development of bacterial resistance to the antimicrobials recovered from an aqueous fruit extract of *Xylopia aethiopica*

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Abstract

Recently, the use of antibiotics for the treatment of numerous infections and diseases increased significantly, and led to noticeable reduction in the rate of mortality and morbidity. The increased development of multidrug resistant bacterial strains that is attributable to the indiscriminate use of antibiotics has led to the search for new antimicrobials of plants origin. This study aimed to assess the potentials of the multidrug resistant bacterial strains to develop resistance to the aqueous fruit extract of *Xylopia aethiopica*. The tested bacterial strains were; *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*. In this study, on using the *in vitro* agar well diffusion assay; the bacterial strains exhibited different diameters of zones of inhibition; ranging from 1.75± 1.06 mm to 12.75± 1.06 mm, on treatment with various concentrations of the aqueous fruit extract. The recorded MIC value for *E. coli* was 250 mg/ ml, while the other bacterial strains recorded 125 mg/ ml. On the other hand, the obtained MBC value for *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* was 2000 mg/ ml, whereas *E. coli* and *P. aeruginosa* recorded 1000 mg/ ml. However, the MBC of *B. cereus* was not detected. The bacterial strains were subjected to a sub-optimal concentration of the extract after exposure for 5, 10, 15 and 20 d. After exposure for 20 d, *P. aeruginosa* expressed sensitivity only at 2000 mg /ml of the extract with a diameter of inhibition of 4.25± 0.35 mm. *E. coli* exhibited sensitivity at 2000 and 1000 mg/ ml, recording diameters of inhibition of 4.5± 0.71 mm and 2.50± 0.71 mm, respectively. The other strains exhibited resistance on treatment with 250 mg/ ml of the extract, except for *B. cereus*, which recorded inhibition diameter of 3.50 ±0.71 mm. This study demonstrated that exposure of the MDR resistant bacterial strains to a sub-optimal concentration of the aqueous fruit extract of *Xylopia aethiopica* could initiate resistance development.

Keywords: Plant antimicrobials, Antimicrobial resistance, *Xylopia aethiopica*, Antibiotics, Multi-drug resistance

1. Introduction

Antimicrobials are antibiotics, which plainly means "against life". Thus, antimicrobials such as antibiotics are chemical substances produced by the microorganisms, which can inhibit the growth and\or kill other microorganisms (Felix and Victoria, 2017). Mortality rate amongst humans due to bacterial infections has greatly reduced; due to the emergence of antibacterial chemotherapy, which is a highly esteemed medical science. In the last half century, the successful discovery; design and synthesis of wide varieties of antibacterial substances have been increasing, which was believed by the medical communities would eradicate the infectious diseases (Ajenifuja and Ariyo, 2019).

Microorganisms are the most successive and adaptive creatures; however, in the past, microbes were exposed to antibiotics produced by other microorganisms such as *P. notatum*, which naturally produces antimicrobial substances (Emad, 2011). Once resistance to some antibiotics develops in bacteria, they pass on this trait to their offspring's through horizontal or vertical transfer. Moreover, the evolution of new antibiotic resistant bacterial strains that are somewhat more lethal compared to the parent strains is increasing; due to the indiscriminate and irrational use of antibiotics (Harish *et al*., 2017).

Hence, it is not shocking that in this modern era microbes have developed resistance against our synthetic and semi-synthetic antibiotics. Cases of prevalent occurrence of resistant bacteria are now very frequent, which leads to many health-related problems. The problem of multi drug resistance is now global; therefore the need for new antimicrobial agents is now greater than ever (Harish *et al*., 2017).

Surprisingly, herbal medicine is currently considered as a fast growing health system worldwide, which is prevalent in developing countries and is increasing drastically in the developed countries; resulting in the shift of focus to the herbal medicine (Ajenifuja and Ariyo, 2019). *Xylopia aethiopica*, is a deciduous tree generally referred to as 'African pepper', which belongs to the plant family called *Annonaceae* (Obajuluwa and Durowaiye, 2020). *Xylopia aethiopica* is used as a spice in many parts of West Africa (Obajuluwa and Durowaiye, 2020). Furthermore, the fruit extract of *Xylopia aethiopica* is effective in the treatment of bronchitis; oedema, dysentery and febrile pains, as reported by Obajuluwa and Durowaiye, (2020).

Bacteria are increasingly developing resistance to the modern antibiotics that is becoming a major public health concern. There is a little or no information on the bacteria developing resistance to the antimicrobials of plant origins, which have been proven to be more effective. Therefore, the objective this study was to evaluate the development of resistance to the antimicrobials recovered from aqueous extract of the *Xylopia aethiopica* fruit; in the multi-drug resistant bacterial strains including; *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *B. cereus*, *P. aeruginosa* and *E. coli*.

2. Materials and methods

2.1. Collection of plant samples

Dried fruit samples of *Xylopia aehiopica* (Dunal) were purchased from Ojoo market, Ibadan, Oyo State, Nigeria. Identification and authentication of these fruit samples were performed at the herbarium unit, Department of Botany, University of Ibadan, Ibadan, Nigeria.

2.2. Bacterial strains

Multiple drug resistant (MDR) bacterial strains including; *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *E. coli*, *B. cereus* and *P. aeruginosa* used in this study were provided by the laboratory culture of the Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria. Gram

staining, morphological and biochemical identification assays were carried out on these strains, to confirm their identity before being used.

2.3. Preparation of the plant extract

Extraction of antimicrobials from the fruits *Xylopia aehiopica* was carried out at the Pharmaceutical Chemistry Laboratory, Department of Pharmacy, University of Ibadan. According to the method adopted by Abalaka *et al*., (2012), thoroughly washed fruits with clean running water were allowed to air dry at room temperature for 2 weeks till properly dried, and then were transported to the laboratory for pulverization. The fruits were pulverized into fine powder using a mortar and a pestle. About 900 g of the fruit powder was transferred into a clean glass flask containing 3500 ml of dist. water, stirred every 2 h using a glass rod, and then left undisturbed for 72 h. The aqueous solvent containing the extract was separated using a muslin bag, and then filtered using a Whatman no. 1 filter paper. The filtrate was concentrated with the aid of a rotary evaporator (Heidolph laborota 400, model 517-01002-002, Germany) set at 40^{o}C, and then the crude extract was further concentrated using a vacuum oven at 40^{o}C with a pressure of 700 mm Hg. Finally, the extract was collected in an airtight dark bottle and then kept at 4°C till further use.

2.4. Phytochemical screening

The aqueous extract of *Xylopia aethiopica* was screened for the presence of the several bioactive compounds including; anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids and cardiac glycosides. These bioactive compounds were determined both qualitatively and quantitatively, in accordance with the standard method of Pallavi *et al*., (2018) with a little modification.

2.5. Preparation of stock solution of the extract

The stock solution of the extract was prepared by dissolving 10 g of the extract in 5 ml of DMSO (Dimethyl sulfoxide), to obtain a concentration of 2000 mg/ ml. Different concentrations of the stock solution were prepared including; 1000 mg/ ml, 500 mg/ ml and 250 mg/ ml (Pai-Wei *et al*., 2015).

2.6. Detection of *in vitro* antimicrobial susceptibility using the Agar well diffusion assay

Overnight growing broth cultures of the bacterial strains showing an absorbance value of 0.129 - 0.134 at a wavelength of 625nm (i.e., equivalent to 0.5 McFarland of culture), were used for testing the antimicrobial sensitivity. Petri plates containing 20 ml Mueller-Hinton agar were seeded individually with the standardized bacterial strains (0.5 McFarland), by aseptically swabbing the surface of the agar with a sterile swab stick dipped individually in the bacterial broth cultures. Four wells were performed in the seeded plates equidistantly using a sterile 6 mm cock-borer, and then 100 µl of the aqueous extract was added into these wells, and allowed to diffuse into the agar for 2 h. A disc of Ofloxacin (30 µg) was used as a positive control, while 10% DMSO was used as a negative control. The plates were then incubated at 37^{o}C for 24 h. The antimicrobial sensitivity of each bacterial strain was determined by measuring the mean value of the diameters (mm) of the inhibition zones, using a calibrated ruler (Prashant and Nagaiyan, 2020).

2.7. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous fruit extract

The minimum inhibitory concentration (MIC) of the crude extract was determined using the macro-broth dilution assay adopted by Nweze *et al*., (2004); with slight modifications. To 9 ml of nutrient broth in each test tube, 100 µl of the varying concentrations; 2000, 1000, 500 and 250 mg/ ml of the fruit extract was dispensed individually into the test tubes, and then 100 µl of each the standardized bacterial strains in saline water; showing an absorbance value of 0.129-0.134 at a wavelength of 625 nm (i.e., equivalent to 0.5 McFarland of culture) was inoculated individually into each tube. Test tubes containing only the crude extract (*Xylopia aethiopica*) and nutrient broth were

used as control. All tubes were incubated at 37ºC for 24 h. After incubation, the tubes were observed for the presence of turbidity (bacterial growth). The MIC was determined as the lowest concentration of the extract that showed no visible growth (no turbidity), compared to the control tubes.

The minimum bactericidal concentration (MBC) of the crude fruit extract was determined using the nutrient broth incubated overnight. About 1 ml of the tubes that had no visible growth was collected, inoculated into a sterile nutrient agar plate; and then the plates were then incubated for 24 h at 37ºC. After incubation, the highest concentration that expressed no visible growth (turbidity) was considered as the MBC (Simiat *et al*., 2017; Ibrahim and Magdi, 2021).

2.8. Analysis of the induced multi drug resistance by the tested bacterial strains

In reference to the method of Pai-Wei *et al*., (2015), 100 µl of the MDR bacterial strains in saline water (i.e., equivalent to 0.5 McFarland of culture) was inoculated into each broth that contain a concentration lower than the MIC, which is the sub-MIC concentration of the extract. The controls were broths containing the sub-MIC concentration of the extract only. All treated tubes were incubated at 37^0C for 20 d, to test for the ability of the bacterial strains to develop resistance. Recovery of the bacterial strains was done on the 5^{th}, 10^{th}, 15^{th} and 20^{th} d of incubation. Antimicrobial testing's of the bacterial strains was done using the agar well diffusion assay, to check for resistance of the bacteria; through possible reduction in diameters of the zones of inhibition. The controls were also plated out to assure purity of the treatments.

2.9. Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of two replicates. Statistical significance was determined using the student's t-test. Where, *p*-value < 0.05 was considered significant.

3. Results

3.1. Phytochemical screening

Results of the qualitative phytochemical screening of the aqueous extract of the fruit of *Xylopia aethiopica* revealed the presence of Saponins; Terpenoids, Flavonoids, Alkaloids and Anthraquinone; however, the Tanins, Cardiac-glycosides and Steroids were not detected, as shown in Table (1).

Quantitative analysis of the Saponins, Flavonoids and Alkaloids revealed that Alkaloids had the highest yield of 36.9 g (Table 1).

3.2. *In vitro* antibacterial potential of the plant extract

The MDR bacteria were susceptible to the aqueous extract of *Xylopia aethiopica* at various concentrations of the extract. The mean values of the diameters of the inhibition zones (mm) ranged from 1.75± 1.06 to 12.75± 1.06, as demonstrated in Table (2). *Staphylococcus epidermidis* had the highest zone of inhibition of 12.75± 1.06 mm, while *E. coli* had the least zone of inhibition of 6.50± 0.71 mm at 2000 mg/ ml of the extract.

Ofloxacin antibiotic that was used as the positive control recorded inhibition zone diameters that ranged from 14.50± 0.71 mm to 34.75± 0.25 mm), while the negative control (10 % DMSO) recorded no inhibition zone.

3.3. MIC and MBC of the crude extract

The MIC of the aqueous extract of *Xylopia aethiopica* on the MDR bacteria is shown in Table (3). The MIC values ranged from 250 mg/ ml to 500 mg/ ml. *E. coli* had the highest MIC value of 250 mg\ ml. Fig. (1) demonstrates the MBC results of the aqueous extract, which ranged from 1000 mg/ ml to 2000 mg/ ml. *P. aeruginosa* and *E. coli* recorded the least MBC value (1000 mg/ml), while the MBC of *B. cereus* was not detected.

Table 1: Qualitative and Quantitative phytochemical constituents of the aqueous fruit extract of *Xylopia aethiopica*

Phytochemical compounds	Qualitative	Quantitative (g)
Saponins	+	15.5
Tanins	-	ND
Cardiac glycosides	-	ND
Flavonoids	+	4.9
Terpenoid	+	ND
Steroids	-	ND
Alkaloids	++	36.9
Anthraquinone	+	ND

Where; - = Absent, + = Present, ++ = Present, ND = Not detected

Table 2: Antimicrobial potency of the aqueous extract of *Xylopia aethiopica* against the MDR bacterial strains

Bacterial strains	Diameters of inhibition (mm)				Controls	
	2000 mg/ ml	1000 mg/ ml	500 mg/ ml	250 mg/ ml	Ofloxacin (30 µg)	DMSO (10 %)
Staphylococcus epidermidis	*12.75± 1.06^{a}	6.75± 1.06^{b}	5.50± 0.71^{b}	3.51± 0.71^{b}	21.00± 1.41	(-)
Staphylococcus saprophyticus	8.50± 2.47^{a}	7.50± 0.71^{a}	3.75± 1.06^{b}	1.75± 1.06^{b}	14.50± 0.71	(-)
E. coli	6.50± 0.7 ^{a}	4.50± 0.71^{a}	3.25± 0.35 ^{a}	0.00	34.75± 0.25	(-)
P. aeruginosa	10.50± 0.71^{a}	8.50± 0.71^{a}	6.50± 0.71^{b}	3.50± 0.71^{b}	30.75± 2.75	(-)
B. cereus	8.25± 0.35^{a}	6.50± 0.71^{a}	5.75± 1.06^{a}	5.00± 0.00^{b}	31.00± 1.00	(-)

Means within the same row having different superscript letters are significantly different from each other at $p\leq$ 0.05. Where; - = No activity. *Values are mean ± standard deviation of the duplicate treatments

Table 3: MIC's of the aqueous extract of *Xylopia aethiopica* against the tested bacterial strains

Bacterial strains	Concentration of extract (mg/ ml)				
	2000	1000	500	250	125
Staphylococcus epidermidis	-	-	-	-	+
Staphylococcus saprophyticus	-	-	-	-	+
E. coli	-	-	-	+	+
P. aeruginosa	-	-	-	-	+
B. cereus	-	-	-	-	+

Where; -: No Growth, +: Positive growth

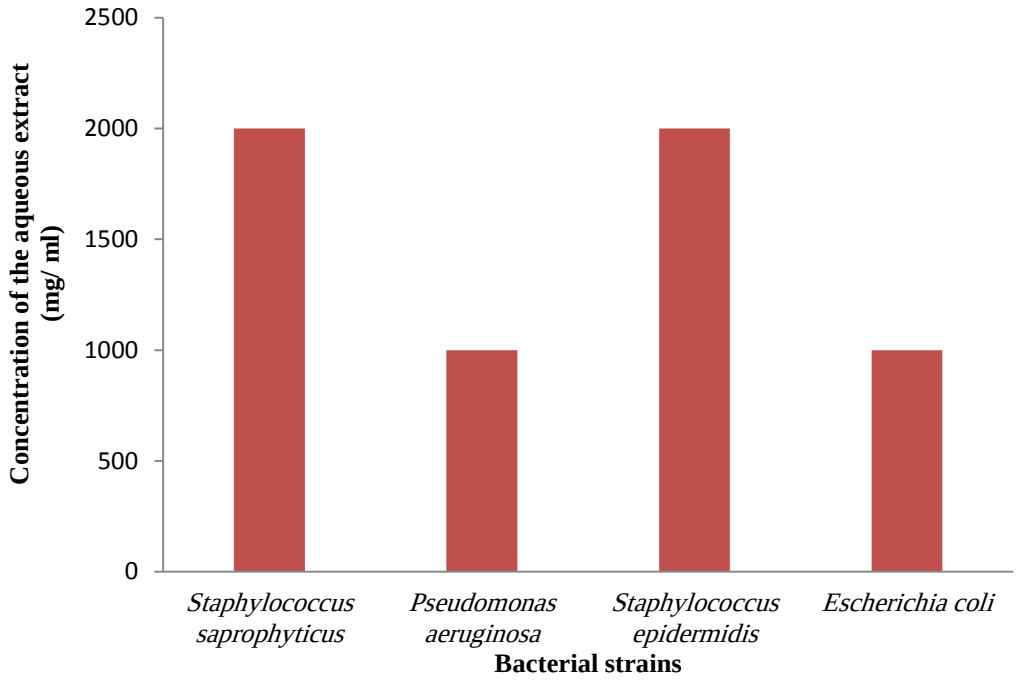

Fig. 1: A chart showing the MBC of the aqueous extract of *Xylopia aethiopica* against the tested bacterial strains

3.4. Detection of multi drug resistance of the tested bacterial strains

The MDR bacteria were subjected to a sub-optimal concentration of the aqueous extract of *Xylopia aethiopica* for 20 d. The resistance pattern of the isolates were tested at the 5th, 10th, 15th and 20th d, which are represented in Tables (4, 5, 6 and 7), respectively.

After exposing the MDR bacteria to the sub-optimal concentration of the extract for 5 d, all the bacteria strains were susceptible to the extract at the various concentrations; except for *E. coli* that showed resistance to the extract (no visible zone of inhibition) at the concentration of 250 mg/ ml. There was considerable reduction in the recorded diameters of the zones of inhibition; however, *B. cereus* had the highest zone with a mean value of 8.00± 0.00 mm, whereas *E. coli* recorded the lowest zone diameter of 5.25± 0.35 mm at the tested concentration of 2000 mg/ ml (Table 4). On 10th day, there was further reduction in the diameters of the zones of inhibition (Table 5). At a concentration of 250 mg/ ml, all the tested bacterial strains were resistant to the extract; except *Staphylococcus epidermidis* and *B. cereus*, which were susceptible expressing diameters of inhibition of 5.25± 0.35 mm and 3.50± 0.71 mm, respectively.

E. coli and *P. aeruginosa* were resistant to the extract at the concentration of 500 mg/ ml; whereas the remaining strains were susceptible to the extract on day 15 (Fig. 6). Further reduction in the diameters of the inhibition zones was observed manifesting resistance of all the tested strains to the extract at the concentration of 250 mg/ ml; except for *B. cereus* expressing a diameter of inhibition of 3.50± 0.71 mm. On the 20th d, *P. aeruginosa* was resistant to the extract at all the tested concentrations; except for the concentration of 2000 mg/ ml. At the concentration of 250 mg/ ml of the extract, all the bacterial strains were resistant to the extract except for *B. cereus*; recording a diameter of 3.50± 0.71 mm (Table 7).

4. Discussion

Plants play a vital role in medicinal applications and have gained wide acceptance, because they are inexpensive; have better compatibility with the human body, and induce minimal side effects on their hosts. Thus, plants serve as alternatives to the conventional therapy for treatment of the microbial infections (Abubakar *et al*., 2018).

About 5 MDR bacterial strains were obtained and used in this study. The phytochemical analysis of the aqueous extract of *Xylopia aethiopica* fruit revealed the presence of several medically bioactive compounds. The presence of saponins; flavonoids, terpenoids, alkaloids and anthraquinones agrees with the previous study conducted by Aguoru *et al*., (2016), who reported the presence of alkaloids, saponins, steroids and flavonoids in the fruit extract of *Xylopia aethiopica*.

Results of the *in vitro* antibacterial assay of the aqueous extract of *Xylopia aethiopica* against the tested MDR bacterial strains demonstrated that these strains were susceptible to the extract at various concentrations (i.e. 2000, 1000, 500 and 250 mg\ l); with recorded different diameters of zones of inhibition. The highest zone of inhibition was observed in *Staphylococcus epidermis* (12.75± 1.06 mm) at 2000 mg/ ml, whereas the least diameter was recorded in *Staphylococcus saprophyticus* (1.75± 1.06 mm) at an extract concentration of 250 mg/ ml. These results are in accordance with the previous work of Edet *et al*., (2016), who worked on the antibacterial activity of the aqueous extract of *Xylopia aethiopica* fruit against the bacterial pathogens of *E. coli* and *P. aeruginosa*. They observed that the bacterial pathogens were susceptible to this aqueous extract with recorded diameters of zones of inhibition of; 12.00± 0.41 and 12.00± 2.83 mm, respectively. Currently, the MIC values of the fruit extract ranged from 250 mg/ ml to 500 mg/ ml, while the MBC values ranged from 1000 mg/ ml to 200 mg/ ml; illustrating that the current fruit

Table 4: Antimicrobial efficacy of the aqueous extract of *Xylopia aethiopica* against MDR bacterial strains exposed to a sub-optimal concentration of the extract for 5 d

Bacterial strains	Zones of inhibition (mm)				Control
	2000 mg/ ml	1000 mg/ ml	500 mg/ ml	250 mg/ ml	(10 % DMSO)
Staphylococcus epidermidis	*6.75± 0.35^{a}	6.25± 0.35^{a}	3.00± 0.00^{b}	2.50± 0.00^{b}	-
Staphylococcus saprophyticus	6.25± 1.77^{a}	5.00± 1.41^{a}	4.00± 0.71^{a}	2.25± 0.35^{b}	-
E. coli	5.25± 0.35^{a}	4.00± 0.00^{a}	2.25± 0.35^{b}	0.00^{c}	-
P. aeruginosa	6.50± 1.41^{a}	5.25± 1.06^{a}	2.75± 1.06^{b}	1.50± 0.71^{b}	-
B. cereus	8.00± 0.00^{a}	6.25± 0.35^{a}	5.00± 0.00^{b}	4.25± 0.35^{b}	-

Means within the same row having different superscript letters are significantly different from each other at $p \leq 0.05$. Where; - = No activity. *Values are Mean± standard deviation of duplicate treatments

Table 5: Antimicrobial potency of the aqueous extract of *Xylopia aethiopica* against MDR bacterial strains exposed to a sub-optimal concentration of the extract for 10 d

Bacterial strains	**Zones of inhibition (mm)**				**Control**
	2000 mg/ ml	1000 mg/ ml	500 mg/ ml	250 mg/ ml	(10 % DMSO)
Staphylococcus epidermidis	*11.50± 0.71^{a}	9.25± 0.35^{b}	6.25± 0.35^{c}	5.25± 0.35^{c}	-
Staphylococcus saprophyticus	9.50± 0.71^{a}	6.75± 0.25^{b}	3.75± 1.06^{c}	0.00^{d}	-
E. coli	4.00± 0.00^{a}	2.50± 0.71^{a}	1.50± 0.71^{a}	0.00^{b}	-
P.aeruginosa	9.50± 0.71^{a}	7.50± 0.71^{b}	4.50± 0.71^{b}	0.00^{c}	-
B. cereus	7.75± 0.35^{a}	6.00± 0.00^{a}	4.50± 0.71^{b}	3.50± 0.71^{b}	-

Means within the same row having different superscript letters are significantly different from each other at $p \leq 0.05$. Where; - = No activity. *Values are Mean± standard deviation of duplicate treatments

Table 6: Antimicrobial activity of the aqueous extract of *Xylopia aethiopica* against MDR bacterial strains exposed to a sub-optimal concentration of the extract for 15 d

Bacterial strains	Zones of inhibition (mm)				Control
	2000 mg/ ml	1000 mg/ ml	500 mg/ ml	250 mg/ ml	(10 % DMSO)
Staphylococcus epidermidis	*9.75± 0.35^{a}	6.00± 0.71^{b}	4.25± 0.35^{b}	0.00^{c}	-
Escherichia coli	4.50± 1.41^{a}	4.25± 1.06^{a}	1.50± 0.71^{b}	0.00^{c}	-
Staphylococcus saprophyticus	9.50± 0.71^{a}	6.50± 2.12^{b}	3.75± 1.06^{c}	0.00^{d}	-
E. coli	4.50± 0.71^{a}	4.00± 0.71^{a}	0.00^{b}	0.00^{b}	-
P.aeruginosa	5.00± 0.71^{a}	3.25± 0.35^{a}	0.00^{b}	0.00^{b}	-
B. cereus	7.25± 0.35^{a}	6.25± 0.35^{a}	4.50± 0.35^{b}	3.50± 0.71^{b}	-

Means within the same row having different superscript letters are significantly different from each other at $p \leq 0.05$. Where; - = No activity. *Values are Mean± standard deviation of duplicate treatments

Table 7: Antimicrobial potential of the aqueous extract of *Xylopia aethiopica* against MDR bacterial strains exposed to a sub-optimal concentration of the extract for 20 d

Bacterial strains	Zones of inhibition (mm)				Control
	2000 mg/ ml	1000 mg/ ml	500 mg/ ml	250 mg/ml	(10 % DMSO)
Staphylococcus epidermidis	*8.25± 0.35^{a}	5.50± 0.35^{b}	4.25± 1.06^{b}	0.00^{c}	-
Staphylococcus saprophyticus	6.25± 1.77^{a}	4.00± 1.41^{b}	2.50± 0.71^{b}	0.00^{c}	-
E. coli	4.50± 0.71^{a}	2.50± 0.71^{a}	0.00^{b}	0.00^{b}	-
P. aeruginosa	4.25± 0.35^{a}	0.00^{b}	0.00^{b}	0.00^{b}	-
B. cereus	6.00± 0.00^{a}	4.75± 0.35^{a}	4.25± 0.35^{a}	3.50± 0.71^{b}	-

Means within the same row having different superscript letters are significantly different from each other at $p \leq 0.05$. Where; - = No activity. *Values are Mean± standard deviation of duplicate treatments

extract had both bacteriostatic and bactericidal potentials against the tested MDR bacterial strains. However, the extract did not express any bactericidal efficacy against *B. cereus*. These results are in agreement with those of the previous work reported by Unimke *et al*., (2017), who determined the MIC and MBC values of the cold and hot aqueous extracts of the *Xylopia aethiopica* fruit against several tested bacterial strains, and observed that these extracts exhibited both bacteriostatic and bactericidal activities against these tested bacterial strains.

The *in vitro* antibacterial assay of the tested strains on exposure to a sub-MIC concentration of the aqueous extract of *Xylopia aethiopica* for 20 d showed the reduction in the diameters of the zones of inhibition; in addition, total resistance was recorded observed through the absence of zones of inhibition. After the 5th d of exposure to the extract; there was a noticeable reduction in the diameters of the zones of inhibition. On the 10th d, *E. coli*, *Staphylococcus saprophyticus* and *P. aeruginosa* became resistant to the extract at 250 mg/ ml. After 15 d of exposure; *E. coli* and *P. aeruginosa* became resistant to the extract at the tested concentration of 500 mg/ ml. On the 20th d, *P. aeruginosa* expressed resistance to the extract at 1000 mg/ ml. These results are contradictory with results of the previous study conducted by Pai-wei *et al*., (2015), who reported that exposure of the tested bacterial strains to a sub MIC concentration of the antimicrobial's recovered from plants; did not induce resistance in these strains. Meanwhile, current results

are in agreement with the study of Vadhana *et al*., (2015), who concluded that there was a possibility for resistance development in microbes towards some common herbal antimicrobial compounds; although the mechanism of this resistance was not precise.

Conclusion

This study demonstrated the exposure of MDR bacterial strains to a plant extract at a sub-optimal concentration, which could influence the development of resistance in these bacteria to the herbal antimicrobials. This was observed through the reduction in diameters of the zones of inhibition (mm) of the treated strains. Therefore, proper use is paramount in order to maintain the long term use of this phyto-therapeutics.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

Non-applicable.

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