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EVALUATION OF INHIBITORY AND TOXICITY EFFECTS OF *XYLOPIA AETHIOPICA* FRUIT EXTRACT AGAINST ESBL-PRODUCING BACTERIAL STRAINS

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ABSTRACT

This study investigated the inhibitory and toxicity effects of *Xylopia aethiopica*, widely utilized in traditional medicine, particularly in Nigeria and West Africa. The clinical isolates obtained from Department of Pathology, Federal Medical Center in Nguru, Yobe State which includes *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Klebsiella oxytoca* were identified and confirmed to produce Extended-Spectrum Beta-Lactamase (ESBL) through standard microbiological methods, followed by genotyping via conventional Polymerase Chain Reaction (PCR). The bioactivity of *Xylopia aethiopica* fruit extract against Extended-Spectrum Beta-Lactamase producing bacterial isolates was evaluated using the agar well diffusion method. The quantitative phytochemical analysis carried out through Soxhlet extraction revealed a range of bioactive compounds in varying amounts such as flavonoids, tannins, phenols, saponins and steroids, while alkaloids were not detected in the chloroform extract. Both ethanol and chloroform extracts displayed significant antibacterial activity, with inhibition zones of 27.50 mm at 100% concentration, surpassing the reference drug, Amikacin (18.03 mm). Additionally, *in vitro* toxicity assessment indicated an LD50 of 3,807.9 mg/kg, indicating a slight toxicity level. These findings suggest that *Xylopia aethiopica* not only has promising antibacterial properties against ESBL-producing bacteria but also warrants caution due to its potential toxicity. The study emphasizes the need for careful consideration when incorporating *Xylopia aethiopica* into medicinal practices.

Keywords: *Xylopia aethiopica*, Fruit extract, Antibacterial activity, Toxicity

INTRODUCTION

There has been a global emergence of bacterial strains resistant to commonly prescribed antibiotics, such as Extended-spectrum beta-lactamases (ESBLs) producers. Extended-spectrum beta-lactamases are a group of enzymes produced by certain bacteria that have resistance to a broad range of beta-lactam antibiotics, including penicillins and cephalosporins. These enzymes can hydrolyze the beta-lactam ring, rendering these antibiotics ineffective against the bacteria that produce them (Gashaw *et al*., 2018). The increasing resistance of bacteria to standard antimicrobial drugs significantly limits their effectiveness and contributes to treatment failures for infectious diseases (Ashish *et al*., 2011). The World Health Organization (2021) reported that available antimicrobial drugs are costly, and the deaths resulting from drug-resistant infections pose a significant concern for public health (Osman *et al*., 2020). In the United States and other developed countries, data on this issue is readily available. In contrast, information on Antimicrobial Resistance (AMR) in the African region is hindered by a lack of adequate data. This gap arises because studies on drug resistance are often limited to only a few countries, resulting in incomplete data on the true extent of the problem (Ndihokubwayo *et al*., 2019).

It has been observed worldwide that there is a high dissemination of extended-spectrum beta-lactamase (ESBL)-producing bacteria, methicillin and carbapenem-resistant bacteria. It was also reported that the problem of ESBL-producing bacteria is more severe in developing countries mostly Africa (Gashaw *et al.,* 2018). This has, led to an increasing number of treatment failures reported among in-patients with infections caused by extended-spectrum beta-lactamase (ESBL) pro-ducing bacteria cases (Osman *et al.,* 2020). A recent surveillance report in 22 countries published by the WHO has shown a high level of AMR, reporting *Escherichia coli* and *Klebsiella pneumoniae* as the two most common resistant bacteria (Mansouri *et al.,* 2019).

The fruits of *Xylopiaa aethiopica* are highly reputed for their numerous medicinal properties. In Africa, it is used as a spice and local remedy for diarrhea, stomach ache, snakebite, cardiovascular diseases, diabetes, and treatment and management of sexually transmitted infections in Southern Nigeria (Gbadamosi *et al.,* 2014). It is used as an antiseptic to arrest bleeding, especially after birth (Anika *et al.,* 2017). It has an inhibitory effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and mild toxicity on *Clarias gariepinus* (Akinsanya, 2016). Essential oils or their constituents are odoriferous substances from plants and are extensively used as medicinal products, in the food industry as flavours, and in the cosmetic industry as a fragrance. Almost every morphological part of the plant is used in traditional medicine for managing various ailments including skin infections, candidiasis, dyspepsia, cough, and fever (Burkhill *et al.,* 2005). The present study aimed to determine the inhibitory and toxicity effect of *Xylopia aethiopica* against some bacterial isolates producing extended-spectrum-lactamase's enzymes.

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh fruits of *Xylopia aethiopica* were collected from the Damaturu Central Market in Yobe State, Nigeria. The plant was identified at the Herbarium section of the Department of Plant Biology at Bayero University Kano, where it was assigned the accession number BUKHAN 302. The collected fruits were air-dried and subsequently ground using a mechanical mill. A soxhlet apparatus was employed for the extraction process using two different solvents (ethanol and

chloroform). One liter of 80% ethanol was used to extract 200 g of the plant material at a temperature of 78.4°C. The same extraction method was applied using chloroform at 61.2°C. The resulting filtrates were concentrated using a rotary evaporator set at 45°C. Lastly, the extracts were stored in sterile bottles under refrigerated conditions at 4°C until further use. The percentage yield of all extracts was calculated using the following formula:

$$Yield\ (\%) = \frac{Weights\ of\ solvent - free\ extract(g) \times 100}{Dried\ extract\ weight}$$

Clinical Sample Collection, Isolation and Identification of Bacteria

Clinical samples including urine, blood, sputum, catheter tips, stool, urogenital specimens, and abscesses were collected from the Department of Pathology at the Federal Medical Center in Nguru, Yobe State, Nigeria. These samples were inoculated onto Nutrient Agar (NA) and incubated at 37°C for 24 hours. After incubation, they were sub-cultured onto Petri dishes containing Nutrient Agar using the streak method to obtain pure cultures. The pure cultures obtained after 24 hours of incubation at 37 ºC were subjected to Gram staining to distinguish between Gram-positive and Gram-negative bacteria. The isolates were then tested using various biochemical methods for identification. Species-level identification was performed using the Entero System 18R (Analytical Profile Index, API). Finally, the isolates were screened for Extended Spectrum Beta-Lactamase (ESBL) producing enzymes through both phenotypic and genotypic process, following standard procedures outlined by Clinical and Laboratory Standards Institute, 2020. Bacterial isolates resistant to third-generation cephalosporins were evaluated for ESBL production using disk approximation and combination disc diffusion methods. The ESBL producers were tested for genotyping via conventional PCR.

Quantitative Phytochemical Screening

A quantitative phytochemical screening was carried out to identify the bioactive constituents present in the fruit extract. This assessment was based on the protocols established by Trease and Evans (2002). The process involved systematically extracting the fruit samples and analyzing them for various phytochemicals, including alkaloids, flavonoids, tannins, and saponins, phenols and sterols. Each step of the analysis was conducted with precision to ensure the accuracy and reliability of the results.

Antibacterial Activity

The agar-well diffusion method, as described by Mostafa *et al*. (2018) was employed for the assessment of the antibacterial activities of the plant extract. Six wells were uniformly bored in previously inoculated Mueller Hinton agar plates containing 10^8 cfu/mL (0.5 McFarland's standard) of each bacterial strain tested. Exactly 0.2 mL of each extract at varying concentrations (20%, 40%, 60%, 80%, and 100%) was carefully introduced into the respective wells and allowed to diffuse into the agar for one hour at room temperature. A control was set up in the same manner using Amikacin (1 mg/mL) as the positive control and sterile distilled water as the negative control. The plates were incubated at 37°C for 24 hours. All tests were conducted in triplicates, and the antibacterial activity was quantified as the mean diameter of the clear zone (mm) surrounding the wells, produced by the plant extract. The results were expressed as mean ± standard deviation (SD).

Acute Toxicity, LD_{50}

The acute toxicity of the extract from *Xylopia aethiopica* was assessed in Albino rats (*Rattus norvegicus)* using the method outlined by Lorke (1983). The study was conducted in two phases. In the first phase, three groups of Albino rats were treated orally with the extract at doses of 10, 100, and 1000 mg/kg body weight, corresponding to the first, second, and third groups respectively. The rats were then monitored for 24 hours for any signs of toxicity or death.

In the second phase, three Albino rats were given more specific doses of the extract (1600, 2900, and 5000 mg/kg) orally, based on the findings from the initial phase, and were again observed for 24 hours for symptoms of toxicity and mortality. This procedure was conducted for both chloroform and ethanol extracts. Subsequently, the median lethal dose (LD50) was estimated using the following relationship:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D_0 = Highest dose that gave no mortality,

RESULTS AND DISCUSSION

Physical Features of Fruit Extract of *Xylopia aethiopica*

The chloroform extract of *Xylopia aethiopica* fruits exhibited the highest yield at 34.4%, whereas the ethanol extract yielded the lowest yield at 16.4%, as displayed in Table 1 below.

The significantly higher yield obtained with chloroform than ethanol suggests that chloroform may be a more efficient solvent for extracting oil from *Xylopia aethiopica*. This finding aligned with previous studies indicating that polar solvents, like ethanol, are often less effective for oil extraction compared to non-polar solvents like chloroform (Gishoma *et al*., 2018). The observed differences in color also offer insights into the chemical makeup of the extracts: the chloroform extract is dark brown, while the ethanol extract is light brown. The darker color of the chloroform extract may indicate a higher concentration of certain compounds, potentially including more pigments or heavier oils. In contrast, the lighter color of the ethanol extract may point to a prevalence of more volatile or lighter components (Stojanović *et al*., 2019).

Moreover, the textures of the extracts reveal differences in consistency: the chloroform extract is oily, while the ethanol extract has a gummy texture. The oily nature of the chloroform extract suggests a successful extraction of lipophilic compounds, such as essential oils and fatty acids. On the other hand, the gummy texture of the ethanol extract may indicate a greater presence of polar compounds like waxes or resins, which are typically less desirable in essential oil extraction (Nweze, *et al*., 2010).

Table 1: Physical features of fruit extract of *Xylopia aethiopica*

Solvents	Weight of spices	Weight of oil extract	% yield of oil extract	Colour	Texture
Chloroform	200g	68.8g	34.4	Dark brown	Oily
Ethanol	200g	32.8g	16.4	Light brown	Gummy

Distributions and percentage occurrence of isolated bacterial isolates from the clinical samples

The results presented in Table 2 highlights the distribution and frequency of various bacterial isolates from the clinical samples, indicating a varied prevalence of specific pathogens in the analyzed samples. *Escherichia coli* emerged as the most prevalent organism, constituting 42.8% of the totals. It is notably highest in urine (16.2%) and stool samples (38.1%), which aligns with its common role as a pathogen in urinary tract infections and gastrointestinal infections (Gupta *et al*., 2021). *Pseudomonas aeruginosa* is also significant, representing 21.1% overall. Its occurrence in clinical samples like sputum and wound swabs reflects its role as a critical pathogen in respiratory and wound infections, particularly in immunocompromised patients (Uhegbou, *et al*., 2020). *Shigella boydii* demonstrates a unique profile, with a high prevalence in stool samples (73.1%) but absent in urine, sputum, or wound swab samples. This might indicate a specific outbreak or localized infections in gastrointestinal cases, as Shigella is predominantly associated with diarrheal diseases (Jaggi, 2012). *Proteus vulgaris* and *Klebsiella oxytoca* show moderate prevalence, with Proteus being significant in wound swabs and blood cultures. *Klebsiella oxytoca* also indicates notable isolation from stool and sputum, underscoring its role in respiratory and gastrointestinal infections (Ilusany *et al*., 2020).

Table 2: Distributions and percentage occurrences of isolated bacterial isolates to the clinical samples

Organisms	Urine (%)	Stool (%)	Sputum (%)	C/tip (%)	Blood (%)	W/Swab (%)	Abscess (%)	Total (%)
Escherichia coli	53 (16.2)	125 (38.1)	23 (7.0)	44 (13.4)	31 (9.5)	43 (13.1)	9 (2.7)	328 (42.8)
Proteus vulgaris	18 (14.6)	35 (28.2)	34 (27.4)	6 (4.8)	20 (16.1)	4 (3.2)	7 (5.6)	124 (16.2)
Pseudomonas aeruginosa	22 (13.6)	42 (25.9)	3 (1.9)	21 (13.0)	23 (14.2)	36 (22.2)	15 (9.3)	162 (21.1)
Shigella boydii	0 (0.0)	57 (73.1)	6 (7.7)	3 (3.9)	12 (15.4)	0 (0.0)	0 (0.0)	78 (10.2)
Klebsiella oxytoca	10 (13.5)	31 (41.9)	8 (10.8)	17 (23.0)	8 (10.8)	0 (0.0)	0 (0.0)	74 (9.7)
Total	103	290	74	91	94	83	31	766

Phenotypic Screening for Extended-Spectrum Beta-Lactamase (ESBL) Bacterial Isolates

Table 3 presents the prevalence of extended-spectrum beta-lactamase (ESBL)-producing bacterial isolates among various tested organisms. *Escherichia coli* demonstrates a significant prevalence of 50.0%, with 286 positive isolates identified out of 295 tested. This finding is consistent with existing literature that frequently highlights *Escherichia coli* as a major contributor to antibiotic resistance (Patel *et al*., 2020). *Proteus vulgaris* exhibits a prevalence rate of 15.73%, with all 90 tested isolates confirmed as positive. This underscores its importance as a pathogen, particularly in urinary tract infections, as reported in previous studies (Murray *et al*., 2017). *Pseudomonas aeruginosa*, another significant pathogen, has a similar prevalence rate of 15.39%, based on 88 tested isolates. This organism is often recognized for its resilience and resistance patterns, which emphasize its relevance in nosocomial infections (Khan *et al*., 2019). *Shigella boydii*, with a prevalence of 9.09%, and *Klebsiella oxytoca*, slightly higher at 9.79%, notably contribute to the isolates tested. Shigella's role in gastrointestinal infections is well-documented, while Klebsiella's increasing resistance to beta-lactams raises growing concerns (Kader *et al*., 2018; Gupta *et al*., 2021).

The genotypic identification of Extended-Spectrum Beta-Lactamase (ESBL) genes among the bacterial isolates was conducted using Polymerase Chain Reaction (PCR) techniques. This analysis successfully detected the presence of two specific ESBL genes: the TEM gene and the SHV gene. The TEM gene was identified at a fragment size of 450 base pairs (bp), while the SHV gene was found at a larger size of 950 bp. These findings highlight the genetic markers associated with antibiotic resistance in the tested isolates (Figure 1).

Table 3: Distribution of confirmed ESBL producing bacterial isolates

Organisms	No tested	No positive	% prevalence
Escherichia coli	295	286	50.0
Proteus vulgaris	90	90	15.73
Pseudomonas aeruginosa	88	88	15.39
Shigella boydii	54	52	9.09
Klebsiella oxytoca	58	56	9.79

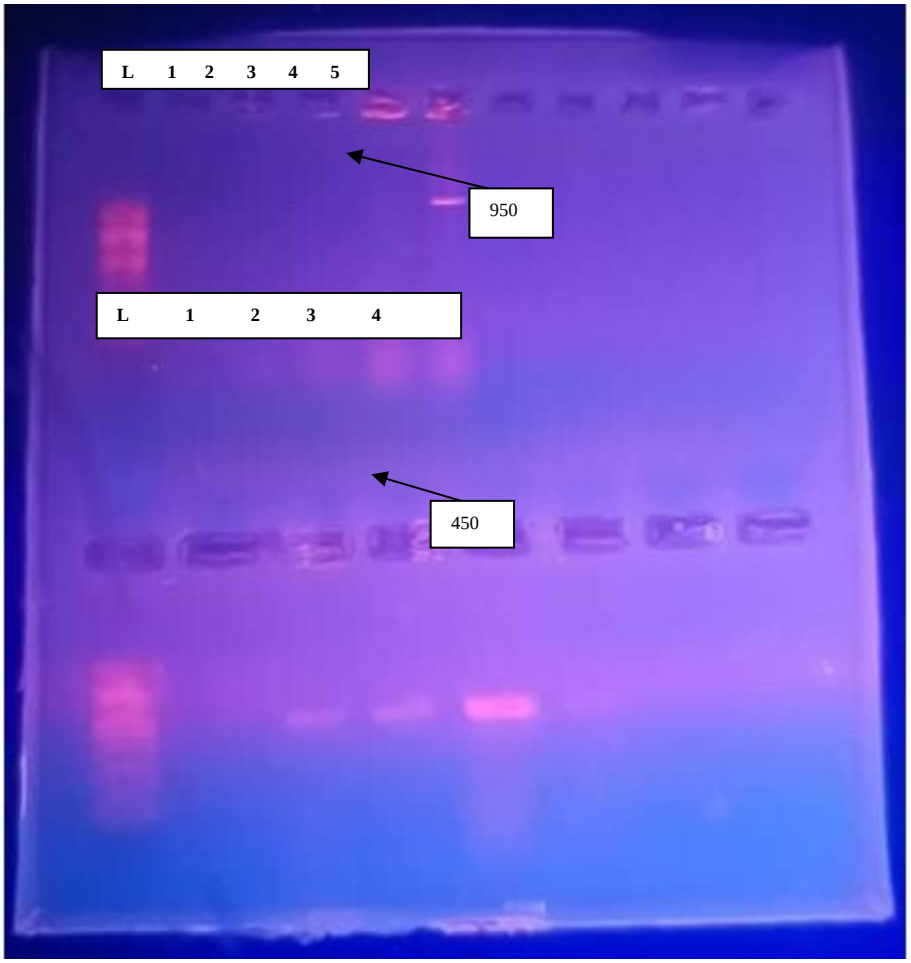

Plate 1: Gel Electrophoretic image showed the results of amplification of fragments of TEM gen and SHV gen from ESBL producing organism by PCR from clinical Bacteria isolates. Lane 1 is *Escherichia coli*, Lane 2 is *Shigella boydii*, Lane3 is *Klebsiella oxytoca*, Lane 4 is *Proteus vulgaris and* Lane5 is *Pseudomonas aeruginosa*, **L** is hyper ladder. [TEM 450bp and SHV 950bp].

Quantitative Phytochemical screening

The quantitative phytochemical analysis of *Xylopia aethiopica* in Table 4 revealed significant variations in the phytochemical constituents. Alkaloids were only detected in the ethanol extract, showing a concentration of 10.37 ± 0.04 mg/g. Its absence in the chloroform extract may suggest that alkaloids in *Xylopia aethiopica* are more soluble in polar solvents like ethanol, which aligns with the findings of other studies indicating that the solvent polarity plays a crucial role in the extraction efficiency of alkaloids (Verma and Joshi, 2020).

The chloroform extract contained a low concentration of saponins (11.80 ± 0.10 mg/g) compared to ethanol (20.17 ± 0.30 mg/g). Saponins are known for their bioactive properties, and their higher extraction yield with ethanol can be attributed to the solvent's ability to solubilize glycosides, reinforcing the observation that saponins are more efficiently extracted using polar solvents (Okwu, 2004). Both extracts showed contrasting levels of tannins, with ethanol revealing 26.15 ± 0.00 mg/g and chloroform a significantly lower concentration (0.96 ± 0.63 mg/g). This difference suggests that tannins, which are polyphenolic compounds, are better solubilized in ethanol, further supported by the literature that indicates tannins' affinity for polar solvents (Makkar *et al*., 1997). The phenolic content was again higher in the chloroform extract (1.49 ± 0.21 mg/g) in contrast to the ethanol extract (0.98 ± 0.01 mg/g). This finding is intriguing as it highlights that certain phenolic compounds may have varying solubility profiles. This observation aligns with previous studies that have explored the solubility of phenolic compounds in different solvents (Singleton *et al*., 1999). It was also observed that there is high concentration of flavonoids in the chloroform extract (26.01 ± 0.01 mg/g) compared to the ethanol extract (19.02 ± 0.71 mg/g). This high yield in chloroform suggests that specific flavonoids within *Xylopia aethiopica* may have a higher affinity for non-polar solvents, which is consistent with findings that indicate the extraction efficiency of flavonoids can vary widely depending on the chemical structure and the solvent used (Stalinski *et al*., 2015). The chloroform extract exhibited a sterol content of 7.10 ± 0.01 mg/g, while the ethanol extract showed 9.10 ± 0.00 mg/g. This suggests that sterols are present in both extracts, though more abundant in ethanol. This can be rooted in the known behavior of sterols to dissolve better in non-polar solvents, but they can also be extracted with polar solvents to a certain extent (Shah *et al*., 2012). In conclusion, the results exhibit the importance of solvent selection when extracting phytochemicals from plants, as it significantly influences the yield and types of compounds obtained.

Table 4: Quantitative Phytochemical features of fruit extracts of *Xylopia aethiopica*

Solvents	Alkaloids	Saponins	Tannins	Phenols	Flavonoids	Sterols
Chloroform	Not detected	11.80±0.10	0.96±0.63	1.49±0.21	26.01±0.01	7.10±0.01
Ethanol	10.37±0.04	20.17±0.30	26.15±0.00	0.98±0.01	19.02±0.71	9.10±0.00

Antibacterial activity

The result in Table 5 clearly demonstrates the remarkable antibacterial activities of ethanol extracts from *Xylopia aethiopica* fruits against various bacterial isolates, showing the effects of different concentrations (20%, 40%, 60%, 80%, and 100%). For *Escherichia coli*, the extract exhibited an impressive inhibition zone of 23.33 mm at 100% concentration, significantly surpassing the 11.33 mm observed at 20%. This substantial increase indicates robust antibacterial properties that escalate with concentration. Similarly, *Proteus vulgaris* displays a pronounced response, reaching a peak inhibition of 26.00 mm at the highest concentration, underscoring the exceptional effectiveness of *Xylopia aethiopica* against this strain. In contrast, *Pseudomonas aeruginosa* shows a comparatively lower response, with a maximum inhibition of 15.67 mm at 100%. This suggests a relative resistance of this strain compared to the other tested bacteria. Both *Shigella boydii* and *Klebsiella oxytoca* also demonstrated significant increases in inhibition zones at higher concentrations, reaching 26.00 mm and 18.67 mm, respectively, at 100%. Notably, the reference antibiotic, Amikacin, revealed varying inhibition across the bacterial strains, with a peak of 18.03 mm against *Proteus vulgaris*. This comparison highlights that while *Xylopia aethiopica* demonstrates considerable antibacterial activity, its effectiveness varies depending on the bacterial strain. Importantly, all tests yield P-values of 0.00, signifying highly significant differences in antibacterial activity between the extract at different concentrations and control conditions. These results unequivocally establish that *Xylopia aethiopica* fruits possess significant antibacterial properties, particularly against *Escherichia coli* and *Proteus vulgaris*. This is consistent with previous studies that have recognized the potent antibacterial effects of plant extracts (Kader *et al*., 2018).

However, the result in Table 6 demonstrates the antibacterial activities of chloroform extracts from *Xylopia aethiopica*. For *Escherichia coli*, the inhibition zones ranged from 11.56 mm at a 20% concentration to 22.60 mm at a 100% concentration, indicating a positive dose-response relationship. This result demonstrates that the extract exhibits increased antibacterial activity as the concentration increases. Similarly, *Proteus vulgaris* showed significant antibacterial effects, with inhibition zones varying from 13.53 mm to 27.50 mm. This strain displayed the most substantial increase in inhibition zone size, suggesting a heightened sensitivity to the extract. *Pseudomonas aeruginosa* yielded inhibition zones ranging from 9.34 mm to 21.00 mm, reflecting moderate sensitivity. While growth inhibition was observed, it was not as pronounced as in the other strains. For *Shigella boydii*, the inhibition zones ranged from 11.54 mm to 26.33 mm, with a relatively high level of activity noted at the 100% concentration, indicating susceptibility to the chloroform extract. *Klebsiella oxytoca* demonstrated the least sensitivity among the tested isolates, achieving a maximum inhibition zone of 21.57 mm at the 100% concentration. The lower inhibition values at reduced concentrations suggest that higher doses are necessary for effective inhibition. Amikacin, a standard antibiotic, exhibited inhibition zones between 14.05 mm and 23.03 mm across the various bacteria. Overall, the chloroform extracts of *Xylopia aethiopica* show comparable antibacterial activity against several isolates at higher concentrations. The overlapping susceptibility profiles highlight the potential of *Xylopia aethiopica* as an alternative antibacterial agent, particularly in light of the growing resistance to conventional antibiotics (Khan *et al*., 2019 and Kawo, *et al*., 2011,). The P-values indicate that the differences in inhibition zones are statistically significant ($P < 0.05$) for *Shigella boydii* and *Klebsiella oxytoca*, suggesting that the tested concentrations significantly influence antibacterial activity and warrant further investigation into the mechanism of action.

Table 5: Antibacterial activities of ethanol extract of *Xylopia aethiopica* fruits

Bacteria isolates	Concentration of extracts (%)						P-Value
	20% (mm)	40% (mm)	60% (mm)	80% (mm)	100% (mm)	Amikacin (mm)	
Escherichia coli	11.33	13.67	16.33	19.67	23.33	15.00	0.00
Proteus vulgaris	8.33	12.00	17.33	22.67	26.00	18.03	0.00
Pseudomonas aeruginosa	4.33	6.33	10.00	12.67	15.67	10.55	0.00
Shigella boydii	11.33	13.00	18.00	22.67	26.00	15.24	0.05
Klebsiella oxytoca	5.67	9.33	11.00	14.33	18.67	15.03	0.00
P-Value	0.00	0.00	0.00	0.00	0.00	0.00	

Table 6: Antibacterial activities of chloroform extracts of *Xylopia aethiopica* fruits

Bacteria isolates	Concentration of extracts						P-Value
	20% (mm)	40% (mm)	60% (mm)	80% (mm)	100% (mm)	Amikacin (mm)	
Escherichia coli	11.56	13.95	16.00	20.65	22.60	15.45	0.00
Proteus vulgaris	13.53	15.07	17.05	24.03	27.50	18.21	0.00
Pseudomonas aeruginosa	9.34	13.43	14.00	18.03	21.00	14.05	0.00
Shigella boydii	11.54	15.06	18.50	20.05	26.33	18.24	0.05
Klebsiella oxytoca	4.23	8.35	13.63	18.80	21.57	23.03	0.04
P-Value	0.00	0.00	0.00	0.00	0.00	0.00	

Acute toxicity

Man's intake of some plant extracts has solely increased, and this may be in the form of food, medicines and beverages, and other industrial and household products. These substances are capable of eliciting chronic and acute toxicity, which may be mild or severe, depending upon their nature. The administration of *Xylopia aethiopica* fruit extracts (10 to 1000 mg/kg) did not produce any sign of toxicity or death in the study's first phase. However, mortality was observed with a single mouse that was given 5000 mg/kg of the *Xylopia aethiopica* fruit extracts in the second phase. The oral LD_{50} in mice was thus estimated to be 3807.9 mg/kg body weight. In addition, results from acute toxicity tests could serve as a guide in dosage selection for long term toxicity studies as well as other studies involving animal use. Based on the estimated oral LD_{50}, the *Xylopia aethiopica* fruit extracts are considered slightly toxic in mice. These agreed with Abdullahi *et al.* (2020) that the lower the LD_{50} value, the more toxic the substance is. The higher the LD_{50} value, the less toxicity of the substance.

Table 7: Median lethal dose determination of *Xylopia aethiopica* fruit extracts in Albino rats

Dose(mg/kg)	Number of Albino rats used	Mortality
Phase one		
10	3	0/3
100	3	0/3
1000	3	0/3
Phase two		
1600	1	0/1
2900	1	0/1
5000	1	1/1

LD_{50} = 3807.9 mg/kg

CONCLUSION

The consumption of certain plant extracts has significantly increased among humans, incorporating them into food, medicines, beverages, and various industrial and household products. This study concluded that *Xylopia aethiopica* fruit extracts possess notable inhibitory properties against some bacterial strains that produce extended-spectrum beta-lactamases (ESBL). Caution should be exercised when ingesting *Xylopia aethiopica* fruits, as these substances can induce chronic or acute toxicity, with severity varying based on their concentration. However, the chloroform extracts of *Xylopia aethiopica* fruits exhibit promising antibacterial activity against various bacterial pathogens.

REFERENCES

Abdullahi, H. Y, Abdullahi, B. N. and Ibrahim, A. D. (2020). Preliminary studies on the anti-inflammatory and analgesic effects of methanol leaf extract of *Ficus asperifolic Miq.*, *Trop J.Nat. Prod. Res.*, 4(3):85-90. ISSN 2616-0684 (Print).

Akinsanya, B. (2016). Antimicrobial properties and toxicological effects of *Xylopia aethiopica* in *Clarias gariepinus*. *Journal of Medicinal Plants Research*, 10(3): 25-31.

Anika, S., Iwuno, M. A. and Okafor, J. (2017). The role of *Xylopia aethiopica* in traditional medicine: A medicinal plant utilized for postpartum care. *African Journal of Traditional, Complementary and Alternative Medicines*, 14(2):122-128.

Ashish, S., Mohit, S. M. and Sharma, K. (2011). Antibacterial activity of commercial and wild *Cinnamon* species. *Journal of phytology*, 3(2): 102-106.

Burkhill, H. M., Kew, N. D. and Bradley, J. (2005). The Useful Plants of West Tropical Africa. Royal Botanic Gardens.

Gashaw, T., Challa, D. and Adugna, A. (2018). Global and local insights into the problem of extended-spectrum beta-lactamases (ESBL) production in bacteria. *Water Science and Technology*, 78(10): 2232-2240.

Gbadamosi, I. T., Osho, A. A. and Adetunji, C. O. (2014). Medicinal applications of *Xylopia aethiopica*: A review. *Journal of Ethnopharmacology*, 155(1): 1-10.

Gishoma, C. and Ngabonziza, J. (2019). Addressing the knowledge gaps in antimicrobial resistance in the African region. *BMC Infectious Diseases*, 19(102), 1-7.

Gupta, A., *et al.* (2021). "The role of ESBL-producing Enterobacteriaceae in clinical infections." *Journal of Antimicrobial Chemotherapy*, 76(3): 572-579.

Ilusanya, O. A. F, Odunbaku, O. A., Adesetan, T. O. and Amosun, O. T. (2020). Antimicrobial Activity of Fruit Extracts of *Xylopia Aethiopica* and its Combination with Antibiotics against Clinical Bacterial Pathogens. *Journal of Biology, Agriculture and Healthcare*. 2: 212.

Jaggi, U, (2012). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Res. Int.*, 44:530–536.

Kader, A. A. *et al.* (2018). The prevalence of ESBL-producing Klebsiella pneumoniae in a Saudi Arabian hospital. *Journal of Infection and Public Health*, 11(3): 425-432.

Kawo, A. H., Suleiman, Z. A. and Yusha'u, M. (2011). Studies on the antibacterial activities and chemical constituents of *Khaya senegalensis* and *Ximenia mericana* leaf extracts. *Af r i c a n Journal of Microbiology Research*, 5(26):4562- 4568.

Khan, A. U., *et al*. (2019). Emerging resistance: An acuteness in *Pseudomonas aeruginosa*. *Infection and Drug Resistance*, 12: 3467-3481.

Lorke, D. A. (1983). New approach to practical acute toxicity testing. *Arch Toxicol.*, 54: 275-287.

Makkar, H. P. S., Francis, G., and Becker, K. (1997). Nutritional values and anti-nutritional components of whole and extracted seeds of several Glycine max varieties. *Animal Feed Science and Technology*, 66(3-4):195-207.

Mansouri, S., Alhamdan, N., and Ghabban, A. (2019). Antimicrobial resistance surveillance report: WHO recommendations. *Clinical Microbiology and Infection*, 25(6): 738-747.

Ndihokubwayo, J. B., Murray, C. J. L. *et al*. (2017). Global burden of bacterial antimicrobial resistance in 2015: a systematic analysis. *The Lancet*, 389(10079): 133-148.

Ndihokubwayo, J. B. and Cart-well. A. Z. (2019). Antimicrobial resistance in the African Region: issues, challenges and actions proposed. *Afr Heal Monit*, (16): 27–30.

Nweze, E. I. and Onyishi, M. C. (2010). *In vitro* antimicrobial activity of ethanolic and methanolic fruit extracts of *Xylopia aethiopica* and its combination with disc antibiotics against clinical isolates of bacteria and fungi. J. Rural Trop Public Health, 9: 1-6.

Okwu, D. E. (2004). Phytochemicals, vitamins and mineral contents of some varieties of yam (*Dioscorea* spp). *International Journal of Molecular Medicine and Advance Sciences*, 1(2): 13-
21.

Osman, A. Y., Hassan, A. S. and Mohammed, R. Y. (2020). Antimicrobial resistance patterns in the WHO African region. *Infection and Drug Resistance*, 13: 429-438.

Patel, J. B. *et al*. (2020). "Antimicrobial susceptibility testing methods: A review." *Clinical Microbiology Reviews*, 33(3): e00017-19.

Shah, W. H., Khan, M. I. and Khan, M. I. (2012). Phytochemical evaluation of some medicinal plants from Pakistan. *African Journal of Biochemistry Research*, 6 (1): 1-5.

Stojanović, R. and Lamuela-Raventos, R. M. (2019). Analysis of total phenols and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Methods in Enzymology*, 2(99): 152-178.

Stalinski, R. *et al*. (2015). Flavonoid extraction and characterization from plant material by liquid chromatography. *Journal of Chromatography*, 1381: 259-271.

Trease, G. E., and Evans, W. C. (2002). Pharmacognosy. 15th ed. B Saunders, London. pp. 137- 440.

Uhegbou, F., Donaghy, P. and Badri, M. *et al*. (2020). Assesment of antimicrobial activity of aqueous and ethanolic extracts of Monodoral myristical seed. *Mintage Journal of pharmaceutical and medical sciences*, 4: 1-3.

Verma, S. K. and Joshi, B. (2020). Phytochemical analysis and antimicrobial activity of various extracts of *Hibiscus rosa-sinensis*. *International Journal of Chemical Studies*, 8(4): 769-774.

World Health Organization (WHO). (2021). Global antimicrobial resistance and use surveillance system. Antimicrobial resistance, WHO. This analysis should provide a comprehensive overview of the antibacterial activities highlighted in the provided table.