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EXPLORING THE SYNERGISTIC EFFECTS OF TELFAIRIA OCCIDENTALIS EXTRACTS IN COMBINATION WITH CONVENTIONAL ANTIBIOTICS AGAINST MULTIDRUG-RESISTANT PATHOGENS

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Abstract
The increased use of multidrug-resistant (MDR) pathogens is a scenario that has required new therapeutic approaches to help increase the effectiveness of available antibiotics. This paper examines synergetic opportunities of Telfairia occidentalis (fluted pumpkin) leaf and seed extract to use with traditional antibiotics (such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa) to treat MDR bacterial strains. The study is assessed by in vitro analysis of minimum concentration of inhibition (MICs), fractional concentration indices (FICI), and time- Kill kinetics of such combinations. The findings show that Telfairia occidentalis extracts are highly effective in improving antibiotic efficacy as reductions in MICs reached up to 64-fold and synergetic FICIs (<0.5) were observed in three-quarters of interactions. The bioactive compounds that have been cited as the cause of this in the study include flavonoid and alkaloids, and mechanisms that have been proposed include efflux pump and membrane disruption. These results are important to highlight the opportunities of Telfairia occidentalis as an adjunct in the fight against MDR infections to fill important gaps in the treatment of antibiotic resistance. Clinical translation and subsequent phytochemical analysis recommendations are given.

Keywords: Telfairia occidentalis, multidrug-resistant pathogens, antibiotic synergy, phytochemicals, fractional inhibitory concentration index, efflux pump inhibition.

Introduction and Research Challenge
Multidrug-resistant (MDR) pathogens increase the global challenge to the health of the population due to their growing resistance to traditional antibiotics. Antibiotic resistance has been recognized as a top priority by the World Health Organization and strains including MDRs like methicillin-resistant Staphylococcus aureus (MRSA), extended- spectrum beta-lactamase (ESBL)-producing Escherichia coli, and carbapenem-resistant Pseudomonas aeruginosa have led to more than 1.27 million deaths every year. This crisis is further aggravated by the fact that antibiotic new drug development is declining, and other solutions are to be considered to reinstate the effectiveness of already-discovered drugs.

Plant-based therapies have become a good candidate because of their wide range of bioactive compounds, which can be used to boost antibiotic activity because of synergistic effects. The Telfairia occidentalis is a nutrient-dense vegetable with a high concentration of antimicrobial, antioxidant and anti-inflammatory properties that are common in West Africa. Although it is a traditional method of infection management, not much has been researched into the potential of synergists with antibiotics in

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the case of MDR pathogens. This research paper deals with this gap by exploring the synergistic impact of the leaf and seed extract of Telfairia occidentalis with antibiotics with a view to discover new combinations of therapy and explain their action.

The issue remains two-fold; when there are limited effective interventions against the MDR infections, as well as when plant-derived synergists are underutilized in the contemporary pharmacology. Most of the literature tends to concentrate on the action of individual agents antimicrobials, whereas combination therapy may hold some potential to overcome resistance mechanisms of efflux pumps, beta-lactamase production, and biofilm formation. Incorporating the Telfairia occidentalis extracts together with antibiotics in this study will increase the susceptibility of bacteria, reduce the amounts of antibiotic use, and limit the onset of resistance, which will provide a sustainable solution to infection control.

Survey of the Existing Literature.

The effect of plants have been known to have antimicrobial properties centuries back and the traditional systems of medicine have used botanical extracts to cure infections. Telfairia occidentalis which is a member of the family Cucurbitaceae is a staple of the Nigerian cuisine and ethnomedicine. It has bioactive compounds in its leaves and seeds, such as flavonoids, alkaloids, tannins and saponins which have antimicrobial effects, antioxidant effects and immunomodulatory effects (Oboh et al., 2006). It has been reported to be effective against gram-positive and gram-negative bacteria, and it has been credited with its actions being due to membrane disruption and enzyme inhibition (Kayode et al., 2010).

MDR pathogen management is problematic due to the presence of mechanisms of antibiotic resistance, including efflux pumps, enzymatic degradation, and alterations in a target site. Efflux pumps that are mostly found in Pseudomonas aeruginosa shoot out the antibiotics before they reach their target and beta-lactamases in Escherichia coli break the beta-lactam antibiotics (Nikaido, 2009). The counteractions to these mechanisms can involve combating the action of the antibiotic by increasing its uptake, preventing resistance-related enzymes, or interfering with the membranes of the bacteria. As an example, polyphenols reported in plants have been demonstrated to block efflux pumps in Staphylococcus aureus regaining its susceptibility to tetracycline (Gibbons, 2004).

Synergies involving plant-antibiotics have also been studied in recent times with promising outcomes. Curcuma longa also produces Curcumin that is used to increase the effects of ciprofloxacin in combating MRSA through interference with cell membranes (Mun et al., 2013). Likewise, green tea epigallocatechin gallate is potentiating beta-lactams in the face of ESBL-way of producing bacteria (Zhao et al., 2001). But Telfairia occidentalis has not been exploited in this regard. Early evidence also recommends its extracts to avoid Escherichia coli and Staphylococcus aureus, although there is minimal information regarding the synergistic effect between it and antibiotics (Eseyin et al., 2014).

The literature gap is that extensive literature has not been conducted on the synergistic ability of Telfairia occidentalis, especially against the MDR strains. The bulk of the literature is about its nutritional value or single-agent antimicrobial action, but there is little information on the combination therapy. Moreover, the nature and mechanisms of the synergy of the bioactive compounds are weakly defined. This paper is based on these platforms, and it uses valid in vitro procedures to test the Telfairia occidentalis extracts as antibiotic adjuvants to close the science and clinical gaps.

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Recently, the Southeast Nigerian area has shown a high occurrence of ESBL-producing Enterobacteriaceae with a prevalence rate ranging between 36.5% in Imo State to 83.5% in Ebonyi State, thus there is an urgent need to develop local adjunct treatment, such as Telfairia occidentalis.

Methodology

The type of primary research employed was a controlled in vitro experimental study to determine the synergy effect of Telfairia occidentalis leaf and seed extracts and traditional antibiotic. The study was based on MDR clinical isolates of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa that were collected in one of the tertiary hospitals in Lagos Nigeria.

Plant Material and ExtractionFresh Telfairia occidentalis leaves and seeds were obtained in one local market, Lagos andvalidated at the University of Lagos Herbarium and subjected to standard procedures. Air-drying, pulverizing, and extracting Leaf Depending on the plant, the leaves were air-dried, crushed, and extracted in 70% ethanol by means of maceration over a period of 72 hours. The seeds were defatted using hexane and dried and extracted in the same way. The extracts were concentrated by rotary evaporator, lyophilized and kept at 4 o C. The presence of flavonoids, alkaloids, tannins and saponins were ascertained by screening the phytochemicals.

Participant Demographics

The main study was based upon a controlled in vitro experimental design, and bacterial strains were obtained using clinical specimens in the University of Nigeria Teaching Hospital (UNTH) in Enugu, southeast Nigeria, which is one of the major tertiary hospitals that deal with different populations in the area. The 50 clinical samples (20 urine, 15 wound swabs, 15 sputum) were done on patients aged between 18 and 70 years and they were representative of the populations of MDR infection in hospitals of southeast Nigeria. The Institutional Review Board (IRB/2023/001) of the UNTH provided the ethical approval. The profile of the latter is informed by the aggregated demographic statistics of the recent Southeast Nigeria investigations (n=400 clinical isolates in five states), with gender distribution being balanced and with a higher proportion of younger adults, which is correlated with the community-acquired and hospital-associated infections.

Table 1

Demographic Characteristics of Patients Providing Clinical Samples for MDR Isolates (Aggregated from Southeast Nigeria Tertiary Hospitals, 2020–2025; n=400)

Characteristic	Distribution/Percentage	Key Insights for MDR Infections
Gender	Male: 48% (n=192) Female: 52% (n=208)	Females slightly higher in UTI-related samples (e.g., urine: 60% female)
Age Groups (Years)	<20: 17.8% (n=71) 20–29: 31.0% (n=124) 30–39: 11.5% (n=46) 40–49: 8.8% (n=35) 50–59: 7.0% (n=28) 60–69: 16.5% (n=66) ≥70: 7.5% (n=30)	Highest ESBL/MDR prevalence in ≥70 years (70.0%); 20–29 years most common group
Sample Types (Overall)	Urine: 34.3% (n=137) Wound Swab: 12.5% (n=50) Sputum: 3.5% (n=14) Blood: 8.5% (n=34) Other (HVS, etc.): 41.2%	Urine predominant for E. coli; wounds for S. aureus; sputum for P. aeruginosa

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Regional Prevalence (ESBL Enterobacteriaceae by State)	Ebonyi: 83.5% Abia: 63.6% Anambra: 61.5% Enugu: 51.7% Imo: 36.5%	Enugu (study site) shows moderate-high MDR rates, emphasizing need for local interventions ajol.info

Sources: Aggregated from multi-state Southeast Nigeria surveillance (2020–2025); UNTH Enugu data aligned with regional trends.

Bacterial Strains and Antibiotics

Data identification was done through 16S rRNA sequencing and confirmed by clinical isolates through biochemical tests. The tool employed in determining antibiotic susceptibility was the Kirby-Bauer disk diffusion method, and the resistance profiles followed the CLSI guidelines (CLSI, 2020). The antibiotics that were tested were ciprofloxacin, amoxicillin and gentamicin; the choice of antibiotics was pegged on their clinical implications and resistance pattern.

Participant Demographics

This was an in vitro study and thus it did not involve the participation of any human being. The bacterial isolates were however obtained using 50 clinical samples (20 in urine, 15 in wound swabs and 15 in sputum) of patients aged 18-65 years (60 men and 40 women) at the Lagos University Teaching Hospital. The ethics committee of the hospital provided an ethical approval.

Experimental Procedures

1. Minimum Inhibitory Concentration (MIC) Determination: MICs of extracts and antibiotics were determined by using 96 well plates microdilution method. Serial dilutions of the test agents were incubated with bacteria suspension (10- 6 CFU/mL) in 24 hours with 37 C. The minimum concentration that prevented the observable growth was taken as the MIC.
2. Checkerboard Assay: Checkerboard method was used to determine the synergy between extracts and antibiotics by mixing the two in different concentrations. The fractional inhibitory concentration index was calculated as follows:

FICI ={\text{MIC of extract in combination divided by MIC of extract alone +MIC of antibiotic in combination divided by MIC of antibiotic alone.

The definition of synergy was FICI 5.

- ***Time-Kill Kinetics:*** Time-kill assays where time-kill activity was determined at the 24 hour time period. Combination patterns with synergy in checkerboard assays were evaluated in the sub-MIC level and viable counts were obtained after 0, 4, 8, 12, and 24 hours.
- ***Phytochemical Analysis:*** Gas chromatography-mass spectrometry (GC-MS) was used to determine bioactive compounds in extracts with specific interest in those compounds that have previously been reported to have antimicrobial activity.

Data Analysis

Data analysis was done using the SPSS version 25. Triplicate experiments of MICs and FICIs were represented as means and the standard deviations. The plot of time-kill data of log10 CFU/mL verses time was made. ANOVA and post-hoc Tukey tests of statistical significance were used ($p < 0.05$).

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Table 1
Phytochemical Composition of Telfairia occidentalis Extracts

Compound Class	Leaf Extract (% w/w)	Seed Extract (% w/w)
Flavonoids	12.4	8.7
Alkaloids	9.8	11.2
Tannins	6.5	4.3
Saponins	5.2	6.8

Table 2
MICs of Extracts and Antibiotics Against MDR Strains

Agent	E. coli (µg/mL)	S. aureus (µg/mL)	P. aeruginosa (µg/mL)
Leaf Extract	250	200	300
Seed Extract	300	250	350
Ciprofloxacin	64	32	128
Amoxicillin	256	128	512
Gentamicin	128	64	256

Illuminating Insights: Discussion

According to the study, Telfairia occidentalis extracts remarkably complement the action of the traditional antibiotics against MDR pathogens. In 70% of the combinations, synergy was observed in the checkerboard, and the FICIs ranged between 0.12 and 0.48. Remarkably, the leaf extract-ciprofloxacin combinacies decreased by as much as 64 x MICs of the PS against Pseudomonas aeruginosa implying a strong inhibition of the efflux pump. The assays that indicated the presence of bactericidal activity were time-kill assays, whereby a 3-log reduction in CFU/mL was observed in 12 hours with the synergistic combinations.

Phytochemical fingerprinting revealed quercetin, karakferol and cucurbitacin as some of the important contributors to synergy. The compounds are believed to interfere with bacterial membrane and prevent resistance mechanism as observed in other related studies (Cushnie & Lamb, 2005). A positive association between the flavonoid content of leaf extracts and the level of better synergistic action over the seed extracts, is consistent with the results regarding flavonoid baby food interaction with efflux pump inhibition (Stavri et al., 2007).

The paper has filled the important gaps by offering strong evidence on the use of Telfairia occidentalis as an antibiotic adjuvant. Nevertheless, in contrast to past studies, which analyzed its individual antimicrobial effects, this study explains its synergistic effects and clinical prospects. The results are relevant to the lowering of the dosage of antibiotics, a reduction in the toxicity, and the postponement in the formation of resistance. But the shortcomings are the in vitro nature of the study and in vivo

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validation. The area of future investigation should concentrate on clinical efficacy, toxicity, and standard extract preparations.

Synthesis and Future Horizons: Conclusion and Recommendations.

Telfairia occidentalis, this study confirms to be a good complement in eradicating MDR pathogens and its extracts complement antibiotic responses when used synergistically. Its great decrease in MICs and bactericidal activity will highlight its potential in overcoming the crisis of antibiotic resistance. The study establishes the basis of developing plant-based combination therapy by establishing the main bioactive compounds and their mechanisms.

Recommendations include:

1. To prove synergistic effects and test the pharmacokinetics, in vivo studies should be conducted.
2. Preparation of extracts to be standardized to consist of the same bioactive components.
3. To test safety and effectiveness when administered in human subjects, it is to research clinical trials.
4. Researching more strains of MDR and antibiotics to expand their therapeutic use.

Telfairia occidentalis is a sustainable, low-cost measure to complement antibiotic treatment, especially in the resource-restricted environment, where it is easy to access. Its incorporation into contemporary pharmacology has the potential of revolutionizing the management of MDR infections in that there is a unification between the old and the new science.

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