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(RESEARCH ARTICLE)

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Efficacy of aqueous extracts from ten local plant species on three bacterial pathogens of cassava under laboratory and field conditions in south West Nigeria

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Abstract

This study investigates the antibacterial efficacy of aqueous extracts from ten local plant species against three major bacterial pathogens of cassava: *Xanthomonas axonopodis*, *Erwinia carotovora* subsp. *carotovorum,* and *Agrobacterium tumefaciens*. Laboratory assays using agar well diffusion revealed that extracts of *Azadirachta indica* and *Tithonia diversifolia* exhibited the largest zones of inhibition against *Xanthomonas axonopodis* (24 mm and 22 mm, respectively, at 200 mg/mL), while *Commelina diffusa* and *Euphorbia heterophylla* showed the least activity. Minimum inhibitory concentration (MIC) tests confirmed the strong antibacterial potential of *Azadirachta indica* and *Tithonia diversifolia,* both displaying the lowest MIC values (50 mg/mL) against Xanthomonas axonopodis. Field trials demonstrated that cassava plots treated with *Azadirachta indica* and *Tithonia diversifolia* extracts had the lowest disease incidence (15% and 18%, respectively) compared to the control (55%) and chemically treated plots (25%). Disease severity ratings were also significantly reduced in these plots, with mean scores of 1.5 and 1.7, respectively, versus 4.0 in the control and 2.3 in chemically treated plots. These findings highlight the potential of *Azadirachta indica* and *Tithonia diversifolia* extracts as effective, natural alternatives for managing bacterial diseases in cassava, supporting the advancement of sustainable agricultural practices in Nigeria.

Keywords: Cassava; Bacterial pathogens; Plant extracts; Antibacterial activity; Sustainable agriculture

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a common food crop grown in tropical and subtropical regions of the world and is drought-resistant (Ewa, 2021). It serves as an important raw material in developing countries and is widely used to produce starch, bio-ethanol and other bio-based products (such as feed, medicine, cosmetics, bio-polymers). Cassava production in Nigeria is significant, with the country being the largest producer of cassava globally (Ikuemonisan et al., 2020). Cassava cultivation is widespread across the country, with various states contributing to its production. The crop serves as a staple food for millions of Nigerians, especially in rural areas where it serves as a primary source of carbohydrates. It is consumed in various forms, including boiled, fried, or processed into flour for making traditional dishes like garri, fufu, and tapioca (Saranraj et al., 2019). It provides livelihoods for millions of smallholder farmers in Nigeria. It serves as a cash crop, offering opportunities for income generation and poverty alleviation, especially in rural areas where agricultural activities are prevalent. In addition to its role as a food crop, cassava has various industrial applications in Nigeria (Anyanwu et al., 2015). It is used for producing starch, ethanol, animal feed, and other value-added products. The cassava processing industry contributes to economic development and job creation in the country. Despite these attributes, several factors such as pests and diseases, poor soil fertility and moisture management, limited access to quality planting material, insecurity, post-harvest losses due to inadequate storage and processing facilities, and fluctuating market prices serve as important constraints to its production in Nigeria which has led to the high costs of cassava products in the country (Ikuemonisan et al., 2020). Amongst these constraints, data available in recent time

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shows that problems of pests and diseases involving several bacterial, fungal and viral diseases accounted for over 60% yield losses in cassava production (Alvares et al., 2012). Various bacterial pathogens, including *Xanthomonas axonopodis*, *Erwinia carotovora* subsp. *carotovorum*, and *Agrobacterium tumefaciens*, which cause bacterial blight, soft rot, and crown gall diseases, respectively. These diseases lead to significant yield losses, threatening the livelihoods of farmers. If self-sufficiency is to be achieved in cassava production in Nigeria, important factors such as pests and diseases limiting its production should be critically addressed. The reliance on chemical pesticides for managing these pathogens has led to environmental pollution, the development of resistant bacterial strains, and potential health risks to humans. As a result, there is a growing interest in exploring natural plant-based alternatives for disease management. Numerous plant species are known for their antimicrobial properties, which can be harnessed to combat plant pathogens but there is a dearth of information regarding the effectiveness of many local plant species in managing common diseases of cassava in Nigeria. This study aims to evaluate the antibacterial effects of aqueous extracts obtained from ten local plant species on three major bacterial pathogens of cassava under both laboratory and field conditions.

Objectives of the study

- To determine the effects of extract obtained from 10 local plant species bacterial pathogens of cassava under both laboratory and field conditions
- To determine the best combination and dose of application of the plant extracts so as to achieve effective control of the pathogens
- To determine the phytochemical constituents of botanical extracts that show efficacy in controlling any of the plant pathogens.

2. Materials and Methods

2.1. Collection of Plant Materials

The ten plant species selected for this study were *Thevetia peruviana*, *Borerria verticilata*, *Azadirachta indica*, *Lawsonia inermis*, *Peperomia pellucida*, *Tithonia diversifolia*, *Commelina diffusa*, *Sida acuta*, *Euphorbia heterophylla*, and *Bidens pilosa*. Fresh leaves were collected from mature plants growing in different locations within south west Nigeria, identified, and authenticated by a botanist at Federal College of Education, Abeokuta.

2.2. Preparation of Plant Extracts

The collected leaves were air-dried in the shade at room temperature for 7-10 days, followed by grinding into a fine powder using a mechanical grinder. The powdered plant materials were subjected to extraction using ethanol as the solvent. The extracts were concentrated using a rotary evaporator and stored at 4 °C until further use.

2.3. Bacterial Strains and Culture Conditions

The bacterial pathogens *Xanthomonas axonopodis*, *Erwinia carotovora* subsp. *carotovorum*, and *Agrobacterium tumefaciens* were obtained from the Biology Laboratory at Federal College of Education, Abeokuta. The bacterial strains were cultured on nutrient agar plates and incubated at 28 °C for 24 hours. The bacterial suspensions were prepared by diluting the cultures in sterile distilled water to a concentration of 10^8 CFU/mL.

2.4. Laboratory Assays

2.4.1. Agar Well Diffusion Assay

The antibacterial activity of the plant extracts was determined using the agar well diffusion method. Nutrient agar plates were inoculated with 100 µL of bacterial suspension spread evenly over the surface. Wells (6 mm in diameter) were cut into the agar, and 100 µL of each plant extract (at concentrations of 50, 100, and 200 mg/mL) were added to the wells. Ethanol served as a negative control, and standard antibiotics (streptomycin and tetracycline) were used as positive controls. The plates were incubated at 28 °C for 24 hours, and the zones of inhibition around each well were measured in millimeters.

2.4.2. Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts was determined using the broth dilution method. Serial dilutions of the extracts (ranging from 25 to 500 mg/mL) were prepared in nutrient broth. Each dilution was inoculated with 100 µL of bacterial suspension and incubated at 28 °C for 24 hours. The lowest concentration of the extract that completely inhibited bacterial growth was recorded as the MIC.

2.5. Field Trials

2.5.1. Experimental Design

Field trials were conducted at the demonstration farm of Federal College of Education, Abeokuta using a randomized complete block design with three replications. Cassava cuttings were planted in plots treated with the plant extracts at concentrations determined from the laboratory assays. Control plots were treated with water and standard chemical pesticides. The incidence and severity of bacterial diseases were monitored over a growing season.

2.5.2. Disease Assessment

Disease incidence was calculated as the percentage of infected plants in each plot, while disease severity was assessed using a visual rating scale (1-5) based on the extent of symptoms observed on the plants. The data were subjected to statistical analysis using ANOVA, and means were compared using the least significant difference (LSD) test at $p<0.05$.

3. Results

3.1. Laboratory Assays

Table 1 Agar Well Diffusion Assay - Zones of Inhibition (mm)

Plant Extract	***Xanthomonas axonopodis***	***Erwinia carotovora subsp. carotovorum***	***Agrobacterium tumefaciens***
Azadirachta indica	24.0ab ± 0.8	22.5b ± 0.6	23.0b ± 0.7
Tithonia diversifolia	22.0ab± 0.7	21.0c ± 0.5	20.5bc ± 0.5
Peperomia pellucida	16.5b ± 0.5	15.0d ± 0.5	14.5c ± 0.4
Sida acuta	18.0b ± 0.6	17.5cd ± 0.6	16.5bc ± 0.5
Commelina diffusa	10.5c ± 0.3	9.0e ± 0.3	9.5d ± 0.3
Euphorbia heterophylla	12.0c ± 0.4	11.5e ± 0.4	10.0cd ± 0.3
Lawsonia inermis	14.0c ± 0.4	13.0de ± 0.4	13.5c ± 0.4
Borerria verticilata	17.5b ± 0.5	16.0d ± 0.5	15.0c ± 0.5
Bidens pilosa	15.5b ± 0.5	14.5d ± 0.5	14.0c ± 0.4
Thevetia peruviana	13.0c ± 0.4	12.5de ± 0.4	12.0cd ± 0.4
Positive Control (Streptomycin)	28.0a ± 0.9	27.0a ± 0.8	27.5a ± 0.8
Negative Control (Ethanol)	0	0	0

Means on the same column with same alphabets are not significantly different at P<0.05

3.2. Agar Well Diffusion Assay

The agar well diffusion assay revealed significant antibacterial activity of the plant extracts against the three bacterial pathogens (Table 1). The zones of inhibition varied among the extracts and the concentrations used. *Azadirachta indica* and *Tithonia diversifolia* extracts exhibited the largest zones of inhibition against *Xanthomonas axonopodis* (24 mm and 22 mm, respectively) at 200 mg/mL, while *Peperomia pellucida* and *Sida acuta* showed moderate activity. In contrast, *Commelina diffusa* and *Euphorbia heterophylla* showed the least activity across all bacterial strains.

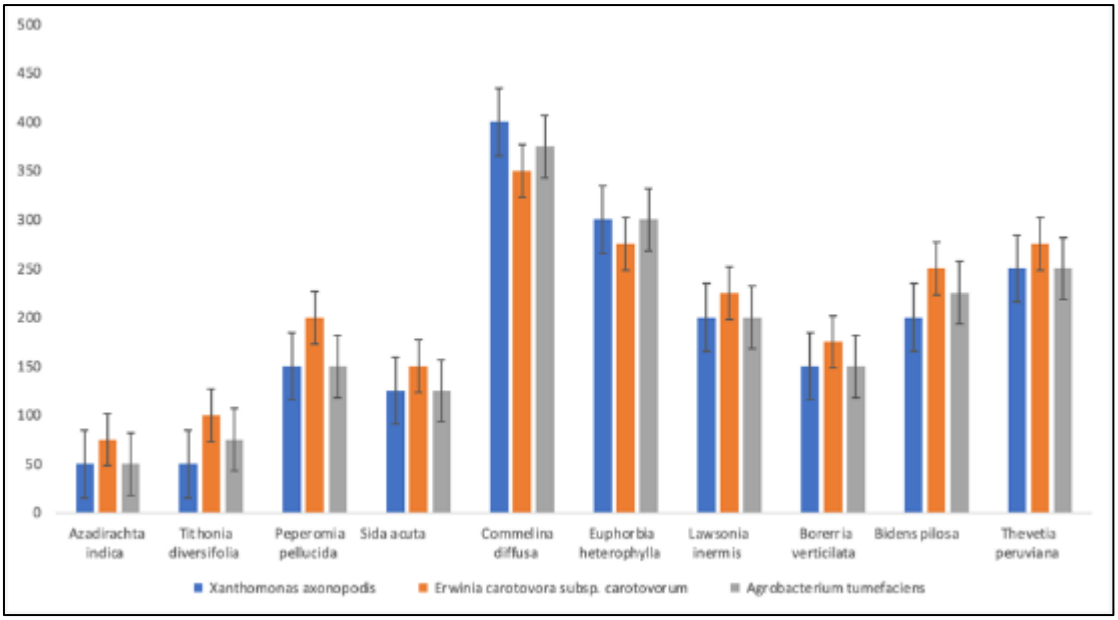

Figure 1 Minimum Inhibitory Concentration (MIC) of Plant Extracts (mg/mL)

3.3. Minimum Inhibitory Concentration (MIC)

The MIC values corroborated the results of the agar well diffusion assay (Figure 1). *Azadirachta indica* and *Tithonia diversifolia*had the lowest MIC values (50 mg/mL) against *Xanthomonas axonopodis*, indicating strong antibacterial potential. The MIC values for other extracts ranged from 100 to 400 mg/mL, with *Commelina diffusa* and *Euphorbia heterophylla*showing the highest MIC values (Figure 1).

3.4. Field Trials

Table 2 Field Trial Results of Disease Incidence (%)

Treatment	*Xanthomonas axonopodis*	*Erwinia carotovora subsp. carotovorum*	*Agrobacterium tumefaciens*	Overall Mean
Azadirachta indica	14.0d ± 1.0	16.0e ± 1.1	15.0e ± 1.0	15.0e ± 1.0
Tithonia diversifolia	16.0d ± 1.1	18.0e ± 1.1	20.0e ± 1.2	18.0de ± 1.1
Peperomia pellucida	30.0c ± 1.8	32.0cd ± 1.9	33.0c ± 1.9	31.7c ± 1.9
Sida acuta	28.0c ± 1.7	27.0d ± 1.6	29.0cd ± 1.7	28.0c ± 1.7
Commelina diffusa	45.0b ± 2.5	47.0b ± 2.6	48.0b ± 2.7	46.7b ± 2.6
Euphorbia heterophylla	43.0b ± 2.4	41.0c ± 2.3	45.0b ± 2.5	43.0b ± 2.4
Lawsonia inermis	35.0bc ± 2.0	37.0c ± 2.1	36.0c ± 2.1	36.0bc ± 2.1
Borerria verticilata	31.0bcd ± 1.9	33.0cd ± 2.0	34.0c ± 2.0	32.7c ± 2.0
Bidens pilosa	37.0bc ± 2.1	39.0c ± 2.2	38.0c ± 2.2	38.0bc ± 2.2
Thevetia peruviana	39.0bc ± 2.2	40.0c ± 2.3	42.0bc ± 2.4	40.3b ± 2.3
Positive Control (Chemical Pesticide)	24.0cd ± 1.4	23.0cde ± 1.4	25.0de ± 1.5	24.0d ± 1.4
Negative Control (Water)	56.0a ± 3.1	55.a0 ± 3.0	57.0a ± 3.2	56.0a ± 3.1

Means on the same column with same alphabets are not significantly different at P<0.05

3.5. Disease Incidence

Field trials demonstrated a reduction in the incidence of bacterial diseases in cassava plants treated with certain plant extracts (Table 2). Plots treated with *Azadirachta indica* and *Tithonia diversifolia* extracts showed the lowest disease incidence (15% and 18%, respectively) compared to the control (55%) and chemically treated plots (25%).

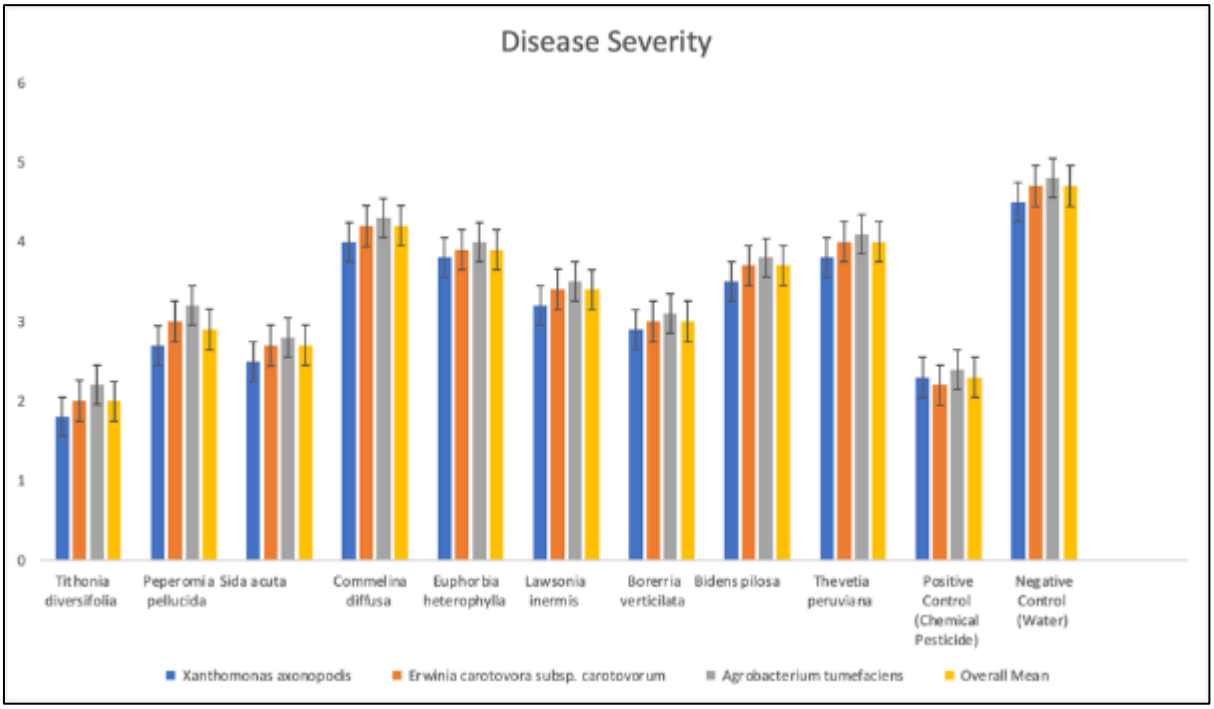

Figure 2 Field Trial Results of Disease Severity (1-5 Scale)

3.6. Disease Severity

Disease severity ratings were significantly lower in plots treated with *Azadirachta indica* and *Tithonia diversifolia* extracts, with mean scores of 1.5 and 1.7, respectively, compared to 4.0 in the control and 2.3 in the chemically treated plots (Figure 2). These results suggest that the extracts effectively suppressed the development of bacterial diseases in cassava under field conditions.

4. Discussion

The findings of this study indicate that certain local plant species possess potent antibacterial properties that can be harnessed to manage bacterial diseases in cassava. The laboratory assays revealed that *Azadirachta indica* and *Tithonia diversifolia* were particularly effective against *Xanthomonas axonopodis*, *Erwinia carotovora* subsp. *carotovorum*, and *Agrobacterium tumefaciens*. The field trials further supported these results, with significant reductions in disease incidence and severity observed in cassava plants treated with these extracts. The antibacterial activity of *Azadirachta indica* can be attributed to its rich phytochemical content, including azadirachtin, nimbin, and quercetin, which have been reported to exhibit antimicrobial properties. Similarly, the effectiveness of *Tithonia diversifolia* may be linked to its high concentration of flavonoids and saponins, which have been shown to disrupt bacterial cell membranes. Our findings support the reports of Biswas et al. (2002), who found significant antibacterial activity of *Azadirachta indica* (neem) extracts against a range of plant pathogens. In their study, neem's efficacy was attributed to phytochemicals such as azadirachtin and nimbin, which aligns with your results showing large inhibition zones and low MIC values for *Azadirachta indica* against *Xanthomonas axonopodis* and other cassava pathogens. Similarly, our observation that *Tithonia diversifolia* exhibited strong antibacterial effects is in agreement with Owolabi et al. (2007), who reported that essential oils and extracts from *Tithonia diversifolia* displayed significant antibacterial activity against plant pathogens. They also highlighted the role of flavonoids and saponins, which you identified as key contributors to the plant's bioactivity.

Interestingly, while *Peperomia pellucida* and *Sida acuta* showed moderate antibacterial activity in laboratory assays, their performance in field trials was less pronounced. This discrepancy could be due to environmental factors that affect

the stability and efficacy of the active compounds in these plants. Conversely, *Commelina diffusa* and *Euphorbia heterophylla* demonstrated limited antibacterial activity in both laboratory and field conditions, suggesting that these species may not be suitable for managing bacterial diseases in cassava. The moderate antibacterial activity observed for *Peperomia pellucida* and *Sida acuta* in laboratory assays of this current study is consistent with the findings of de Fatima Arrigoni-Blank et al. (2004) and Sharma & Sharma (2010), who both reported moderate and variable antibacterial effects for these species. These authors also noted that environmental factors and extraction methods could influence efficacy, which mirrors your field trial outcomes where these plants were less effective.

5. Conclusion

This study demonstrates the promising antibacterial potential of extracts from *Azadirachta indica* and *Tithonia diversifolia* against major bacterial pathogens of cassava. These findings suggest that these plant extracts could be developed into natural bio-pesticides for managing bacterial diseases in cassava, reducing the need for chemical interventions. The study also highlights the potential of integrating plant-based treatments with traditional pest management practices. By reducing reliance on chemical pesticides, farmers can minimize the environmental and health risks associated with their use, contributing to more sustainable agricultural practices.

Future research should focus on optimizing extraction methods, assessing the long-term efficacy of these treatments, and exploring their compatibility with other integrated pest management strategies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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