



In Vitro Antibacterial Activity of Pawpaw (*Carica Papaya* L.) Leaf Extracts Against the Cassava Blight Pathogen (*Xanthomonas axonopodis* pv *manihotis*)

Olahan, G. S. and Anundi, J. E.

Department of Plant Biology, University of Ilorin, Nigeria

ABSTRACT: The antibacterial activity of 10, 30 and 50% (w/v) concentrations of aqueous and ethanolic extracts of pawpaw (*Carica papaya* L.) leaf against the cassava bacterial blight (CBB) pathogen, *Xanthomonas axonopodis* pv *manihotis* was studied *in vitro* using the broth dilution and agar diffusion methods. The ethanolic extracts were generally more active against the test organism than the aqueous extracts. Activity of both the aqueous and ethanolic extracts increased with the concentration. While the 10% (w/v) aqueous extract did not inhibit the growth of *X. axonopodis* pv *manihotis*, the same concentration of ethanolic extract was bacteriostatic with a minimum inhibitory concentration (MIC) of 0.063% (w/v). Using the agar diffusion method, lower concentrations of the aqueous extract did not create any zone of growth inhibition when incorporated into nutrient agar (NA) and streaked with *X. axonopodis* pv *manihotis*. The average diameters of the zones of growth inhibition by the three concentrations of the ethanolic extract were 5.5mm for the 10% (w/v), 6.5mm for the 30% (w/v) and 9.5mm for the 50% (w/v) concentrations. These results show that both the aqueous and ethanolic extracts of *C. papaya* leaf are active against *X. axonopodis* pv *manihotis*.

KEY WORDS: *Xanthomonas*, Pawpaw, Antibacterial, Activity

*Corresponding author

INTRODUCTION

Cassava, a root tuber is in the family *Euphorbiaceae* (Dutta, 1979). It is an important food item and a source of industrial raw materials in many parts of the lowland tropics. It is a perennial shrub, 1-5 metres in height and produces latex in all its parts (Purseglove, 1977).

Cassava bacterial blight (CBB) disease caused by *X. axonopodis* pv *manihotis* is one of the bacterial diseases of cassava. The disease originates from the use of contaminated planting materials and stem punctures and it is disseminated by splashing rain drops (Lozano and Sequeira, 1974a). Symptoms of CBB on susceptible cultivars include leaf spotting, wilting, dieback and gum exudation in young shoots and vascular discoloration in mature stems.

According to Lozano and Sequeira (1974b), CBB is controlled by the planting of disease-free planting materials usually obtained through plant tissue culture techniques. Lozano (1986) and Fanon (1999) have reported that integrated control measures including sanitary, quarantine as well as precautionary measures are the only control measures for CBB. According to O'Hair and Lambert (2004), there are no chemicals approved for the control of CBB in Florida, USA. However, scientists at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria have developed cassava cultivars with high yields and resistance to CBB. Some of these cultivars include TMS 30040, TMS 30211, TMS 30337, TMS 30395, TMS 30555, TIM 30786 and TIM 30001. Plant tissue culture technique is a highly technical and expensive programme and so is not an economic disease control measure. There is need, therefore, for an affordable alternative control measure for CBB.

The use of plant extracts in plant disease control is very potent and holds a lot of promise. The antibacterial activity of pawpaw leaf extract has been previously reported (Emeruwa, 1982). The aim of this study is to determine the activity of various concentrations of aqueous and ethanolic extracts of pawpaw leaf on *X. axonopodis* pv *manihotis* *in vitro*.

MATERIALS AND METHODS

Materials Used

The culture of *X. axonopodis* pv *manihotis* used in this study was obtained from the Plant Pathology Laboratory at IITA, Ibadan. Fresh pawpaw leaves were detached from a tree growing in front of Chemistry Department, University of Ilorin. The two media used are tryptone soy broth (TSB) solution and nutrient agar (NA). They were prepared routinely, stored in the refrigerator and used as required.

Preparation of the Extracts

Both the water and ethanolic leaf extracts used in this study were extracted over 24hr duration. The 10, 30 and 50% (w/v) aqueous and 70% (v/v) ethanolic extracts were prepared following standard procedures (Adekunle, 2001). They were filter-sterilized using the techniques of Nester *et al.* (1998) and then tested for sterility against fungal and bacterial contaminants. The extracts were stored in a refrigerator set at a temperature of 25°C prior to use.

Preparation of Turbidity (Opacity) Standard Solution

The procedures of Cheesbrough (1984) were adopted. The resulting turbid solution will be used to match the turbidity of the *X. axonopodis* pv *manihotis* culture suspension in TSB solution.

Preparation of Standardized Culture Suspension

A sterile inoculating loop was used to introduce *X. axonopodis* pv *manihotis* culture into a test tube containing 5ml of sterile TSB solution and shaken until the culture was completely dissolved. The turbidity of this suspension was matched against the standard solution to determine the standardized volume of the test organism in the suspension (Cheesbrough, 1984). The test tube was then plugged with cotton wool and stored in the refrigerator for subsequent use.

Activity of the Extracts

The broth dilution (Pelezar *et al.*, 1993) and agar diffusion (Reiner, 1984) methods were modified and adopted for the determination of the activity of the extracts in this study.

Broth Dilution Method

The following doubling dilution ratios of sterile TSB solution to 10% (w/v) aqueous or ethanolic extracts were prepared: 1:2, 1:4, 1:8, 1:16 and 1:32 in ten separate test tubes (2 test tubes/dilution). A small volume (0.1ml) of the standardized *X. axonopodis* pv *manihotis* culture suspension was pipetted into each of the test tubes. The test tubes were plugged with cotton wool and shaken gently to mix the contents. The control experiment comprised four test tubes:

Test tube A: containing 5ml TSB solution amended with 5ml aqueous extract inoculated with 0.1ml of standardized culture suspension.

Test Tube B: containing 5ml TSB solution amended with 5ml ethanolic extract inoculated with 0.1ml of standardized culture suspension.

Test Tube C; containing 5ml TSB solution inoculated with 0.1ml of the standardized culture suspension.

Test tube D: containing 5ml, TSB solution only.

All the test tubes (both the experimental and control) were incubated at 37°C for 24 hours. The turbidity or clearness of the contents of each test tube was determined by observing each test tube against a white background near a source of light. Turbidity would indicate growth while clearness would indicate inhibition of *X. axonopodis* pv *manihotis* growth.

Minimum Inhibitory Concentration (MIC)

This is the lowest concentration of a substance that will have antimicrobial activity. In this study, the MIC was the lowest doubling dilution ratio of the extract at which the growth of *X. axonopodis* pv *manihotis* was inhibited.

Type of Antibacterial Activity

The procedures of Reiner (1984) were adopted to determine whether the extract was bacteriostatic or bactericidal. The content of each of the experimental test tubes in which there was growth inhibition was streaked on separate sterile nutrient agar plates. The inoculated plates were labelled and incubated at 37°C for 24 hr.

The Agar Diffusion Method

The procedures of Reiner (1984) were modified and used for this study. Nine plates of sterile NA were uniformly streaked with *X. axonopodis* pv *manihotis*. A stainless steel cork borer (10mm diam.) was dipped into 70% (v/v) ethanol and flamed to red hot for sterility. It was allowed to cool and then used to bore a well at the centre of each of the nine plates. The plates were arranged in sets of three. One millilitre (1ml) of 10, 30 or 50% (w/v) aqueous extract was dispensed into each well in the first set of plates using a sterile pipette. Another set of three plates received 1ml of the same concentrations of ethanolic extract while 1 ml of sterile distilled water was dispensed into the wells of the last set of plates to serve as control. All the plates were labeled and incubated at 37°C for 24 hr. To determine antibacterial activity, zone of growth inhibition on each plate was measured by drawing two lines on each lid intercepting at the centre of the well. The diameter of the zone of inhibition along the vertical and horizontal lines was measured and an average obtained for each plate.

RESULTS AND DISCUSSION

Results show that pawpaw (*Carica papaya* L.) leaf extracts have antibacterial activity against *X. axonopodis* pv *manihotis* under the conditions of this study. The level of activity of the extracts varied with the extraction medium and the final concentration of the extract. The aqueous extract was active against *X. axonopodis* pv *manihotis* only at higher concentrations. The test bacterium grew fairly well at 10% (w/v) aqueous extract concentration. The ethanolic extracts were on the whole more active against the bacterial blight pathogen at all the concentrations tested. The dilution ratio of the extracts affected the activity as well as the minimum inhibitory concentration (MIC). While the 10% aqueous extract did not inhibit the growth of *X. axonopodis* pv *manihotis*, the same concentration of ethanolic extract inhibited the growth of the bacterium at the doubling dilution ratios of 1:2, 1:4, 1:8 and 1:16 (Table 1a). At the dilution ratio of 1:32 of the 10% ethanolic extract, there was no growth inhibition. The MIC of this extract was, therefore, 1:16 as shown in Table 1a. Results of the control experiment showed that growth was only inhibited in test tube C corresponding to the 10% ethanolic extract.

Table 1a: Effect of *C. papaya* leaf extract on the growth of *X. axonopodis* pv *manihotis* using the broth dilution method

*Extracts	Doubling dilution ratio				
	1:2	1:4	1:8	1:16	1:32
10% (w/v) aqueous extract	-	-	-	-	-
10% (w/v) ethanolic extract	+	+	+	+	-
30% (w/v) aqueous extract	+	+	-	-	-
30% (w/v) ethanolic extract	+	+	+	+	+
50% (w/v) aqueous extract	+	+	+	-	-
50% (w/v) ethanolic extract	+	+	+	+	+
CONTROLS	Test tube A	Test tube B	Test tube C	Test tube D	
	-	-	+	-	

+ = inhibition; - = no inhibition.; *There was significant difference at 5% between aqueous and ethanolic extracts but not between concentrations of any of the extracts.

Only the ethanolic extract showed antibacterial activity at all the concentrations tested in this study. The active principles in pawpaw leaves responsible for this activity may be more soluble in ethanol than in water. Another factor that may affect the efficiency of extraction apart from solubility and the extraction medium is the duration of extraction. However, duration of extraction was not tested in this

study. Emeruwa (1982) has reported that pawpaw has some medicinal properties. Alcoholic extracts of various parts of medicinal plants have been shown to exhibit antimicrobial activity over and above their aqueous equivalents (Ebana *et al.*, 1993; Sale *et al.*, 2001).

Type of Antibacterial Activity

X. axonopodis pv *manihotis* culture showed some growth activities at all the dilution ratios of both the aqueous and ethanolic extracts tested in this study. Though inhibition was observed, complete arrest of growth did not occur at any of the dilution ratios tested (Table 1b). The extracts were, therefore, only bacteriostatic after 24 hr of incubation at 37°C. According to Agrios (1978), bacteriostatic agents prevent multiplication of bacteria without killing them. Growth of the bacterial culture when streaked on sterile NA plates was an indication that the bacterial cells did not die after 24hr incubation at 37°C. Pelczar *et al.*, (1993) have reported that the broth dilution method is better than the agar diffusion method for determination of antibacterial activity of any agent because it allows for serial dilution as well as facilitates the determination of the minimum inhibitory concentration (MIC). Based on the findings of this study, the ethanolic extracts of pawpaw leaf have great potential in the eventual control of the cassava bacterial blight (CBB) disease.

Table 1b: Type of Antibacterial Activity of 10% (w/v) Ethanolic Extract of Pawpaw Leaf Against *X. axonopodis* pv *manihotis*

Dilution ratio of extract	Reaction of <i>X. axonopodis</i>	Type of activity
1:2	+	Bacteriostatic
1:4	+	Bacteriostatic
1:8	+	Bacteriostatic
1:16	+	Bacteriostatic

+ = Growth; - = no Growth

Growth Inhibition

The aqueous extracts did not show any antibacterial activity at lower concentrations and so produced very narrow inhibition zones only at 30% and 50% concentrations. The ethanolic extracts created various diameters of growth inhibition zones at all the concentrations and dilutions tested (Table 1c). A zone of growth inhibition occurs as a result of repulsion of the test organism by the action/effects of the active principles in the test extract. Generally, the more concentrated the extract is, the wider the zone of inhibition and vice versa. At $P < 0.05$, there was a significant difference between the radial growth of *X. axonopodis* in the different extracts but there was no significant difference between growth in the different concentrations of both aqueous and ethanolic extracts under the conditions of this study.

Table 1c. Effect of *Carica papaya* leaf extracts on the radial growth of *X. axonopodis* pv *manihotis**

Type of Extract	Conc. of Extract (%) (w/v)	Diameter of Inhibition (mm)
Aqueous	10	-
	30	0.3 ^a
	50	1.5 ^a
Ethanolic	10	5.5 ^b
	30	6.5 ^b
	50	9.5 ^b
Control	Control	-

*Values followed by the same letter not significantly different at 5%

ACKNOWLEDGEMENT

The authors sincerely acknowledge the assistance of the scientists in the Plant Pathology Unit of the

REFERENCES

- ADEKUNLE, A. A. (2001). Ethnobotanical studies of some medicinal plants from Lagos State of Nigeria. *Nig. Journal Bot.* 14:71-79.
- AGRIOS, N. G. (1988). *Plant Pathology*. Academic Press, London. 803pp.
- CHEESBROUGH, M. (1984). *Medical Laboratory Manual for Tropical Countries Vol. 2*, 158 PH. 479 pp.
- EBANA, R. U. B., Madunagu, B. E. and Etok, C. A. (1993). Antimicrobial effect of *Strophantus hispidus* and *Secamone afzeli* on some pathogenic bacteria and their drug resistant strains. *Nig. J. Bot.* 6:27-31.
- DUTTA, A. C. (1979). *Botany for Degree Students 4th Edn.* Oxford University Press, Delhi. 909pp.
- EMERUWA, A. C. (1982). Antimicrobial substance from *Carica papaya* fruit extracts. *Llydia* 5(2):123-127.
- FANON, A. (1999). Epidemiological and Ecological Investigations on Cassava Bacterial Blight (CBB) and development of integrated methods for its control in Africa. Ph. D. Thesis, University of Gottingen, Germany.
- LOZANO, J. C. (1986). Cassava Bacterial Blight (CBB): Manageable Disease. *Plant Disease* 70:1089-1093.
- LOZANO, J. C. and Sequeira, L. (1974a). Bacterial blight of Cassava in Columbia: Etiology. *Phytopathology* 64:74-82.
- LOZANO, J. C. and Sequeira, L. (1974b). Bacterial blight of Cassava in Columbia: Epidemiology and Control. *Phytopathology* 64:83-88.
- NESTER, E. W., Evans Roberts, C., Nancy, N. P., Denise, G. A. and Martha, T. N. (1998). *Microbiology: A Human perspective*. 2nd edn., WCB McGraw-Hill, New York. 848 pp.
- O'HAIR, S. and Lambert, M. (2004). University of Florida Extension Institute of Food and Agricultural Sciences. Cassava Production in Miami-Dade County, Florida, USA. (Internet).
- PELCZAR, M. J., Chan, E. C. S. and Krieg, N. R. (1993). In: Sule, I. O. (1999). Antibacterial Activity of Some Commercial Detergents. B.Sc. Thesis, University of Ilorin, Nigeria. 31 pp.
- PURSEGLOVE, J.W. (1977). *Tropical Crops. Dicotyledons. Volumes 1 and 2 Combined*. Longman group Ltd., London 719 pp.
- REINER, R. (1984). *Antibiotics: An Introduction* New Horn Publishing Co., Ibadan, Nigeria. 172 pp.
- SALE, M., Bassey M. and De, M. (2001). Antibacterial Spectrum of Extracts of *Zingiber officinale* (Ginger) and *Allium sativum* (Garlic). Book of Abstracts. 25th Annual Conference of Nigerian Society of Microbiology, Ado-Ekiti, Nigeria. Dec. 2001.