Antibacterial Activities of *Euphorbia hirta* and *Lantan camara* Extracts on the Growth of some Bacteria Diseases of Banana Plant.

ABSTRACT

Bananas (*Musa acuminata* L.) is confronted with a number of challenges imposed by bacterial diseases which lead to huge yield losses. In order to improve the yields of the banana plantation, one of the recommended management strategies which would be inexpensive and environmentally friendly was developed. The objective of this study was to improve the sanitary state of banana plant. To achieve this objective, bacteria associated with the different banana organs were isolated on Nutrient Agar (culture medium) and their morphological identification was carried out based on the cultural characteristics and the colour of the bacterial walls observed under an optical microscope and using reference documents (bacteriological identification keys). Antibacterial activity of *Lantana camara* and *Euphorbia hirta*extracts were evaluated in vitro on agar medium on the development of *Xanthomonas campestris pv*. *Pseudomonas solanacearum* and *Ralstonia solanacearum*. The results showed that banana plant harbours a diversity of fungal species, the most frequent being *Ralstonia solanacearum* as it was isolated from all infected organs.The extraction yields of the aqueous extracts of *Euphorbia hirta* and *Lantana camara* were particularly high (7% and 13%) respectively, compared to those of the ethanolic extracts which were lower. Aqueous extracts of *Euphorbia hirta*, at 50 mg/ml, had bactericidal activity on the development of *Ralstonia solanacearum* and *Pseudomonas celebensis.* Aqueous extracts of *Lantana camara*, at concentrations of 25 mg/ml and 50 mg/ml, exhibited bactericidal activity on the development of *P. celebensis*. Meanwhile the ethanolic extracts of *L. camara* had bacteriostatic activity on the development of *P. celebensis*. Ethanolic extracts of *E. hirta had* bactericidal activity on the growth of *Xanthomonas campestris* at 25 mg/ml and 50 mg/ml. The same activity was obtained with *P. celebensis* at 50 mg/ml. These results suggest that the aqueous and ethanol extracts of the tested plants at high concentrations could be used as alternatives to chemical products in the fight against banana diseases especially *Xanthomonas*, *Pseudomonas* and *Ralstonia*. Hence further studies need to be undertaken in order to isolate the active compounds from these extracts with bactericidal potential.

Keywords

Banana, Bacteria, *Euphorbia hirta*, *Lantana camara*, Plant Extracts

Introduction

Comment [MT1]: BacterialDiseases

Comment [MT2]: Antibacterial Activities of *Euphorbia hirta* and *Lantan camara* Extracts on the Growth of some Bacteria that Cause Banana Plant Diseases.

Comment [MT3]: are

Comment [MT4]: against

Comment [MT5]: the banana

Comment [MT6]: bacterial

Comment [MT7]: rearrange the sentences, the second sentence should be mentioned first

Comment [MT8]: The yields of the aqueous extracts

Comment [MT9]: against

Comment [MT10]: Meanwhile,

Comment [MT11]: State effective concentration

First domesticated in Southeast Asian regions like the Malasian Peninsula, Indonesia, the Philippines and New Guinea [1], bananas (*Musa acuminata* L.) which are annual and herbaceous perennial plants belonging to the family of Musaceae which are largely cultivated in many tropical and subtropical regions for their fruit rich in carbohydrates, mineral salts (potassium, zinc, magnesium), vitamins such as B6, A, C, K [2], have gone from being the first cultivated fruit to the most consumed and exported fruit in the world [3]. Bananas, along with plantains, are the fourth most important staple crop worldwide and are essential to maintaining food and nutritional security among 400 million people in producing countries [4]. The banana plant which grows in tropical climates with average temperatures of 27°C and more than 200 cm of annual precipitation takes 10 to 18 months to go from planting to producing fruit and are typically harvested green 7 to 10 days before maturing [5].

Since 2016, India, China, and Indonesia have consistently been the largest producing countries to supply their domestic markets. Hence, Latin American countries have primarily remained the biggest exporting countries, with Ecuador, the Philippines, and Costa Rica being the top exporting countries in 2020 (closely followed by Guatemala and Colombia), exporting some 7 Mt, 3.1 Mt, and 2.6 Mt, respectively [6]. Meanwhile the European Union, the United States, and China were the largest importers with about 5.2 Mt, 4.7 Mt, and 1.8 Mt, respectively in 2020[6].

In Africa, Côte d'Ivoire, Cameroon and Ghana are major players in the production of dessert bananas for export on the African continent with 327,852 tons in 2020, 180,879 and 77,286 tons respectively exported in 2020.

Although the global supply of bananas depends greatly on weather and field management practices, producers have generally been able to meet growing global demand [7]. Nevertheless, the surge in demand for bananas that occurred at the outset of the COVID-19 pandemic may have caused supply and demand imbalances in some countries [8,9].The pandemic affected the banana sector in different ways in different parts of the world. On the whole, demand for bananas rose at 1.7% in 2020 from 2019, as it is a nutritious home consumable product that can boost immune systems [10].

While low yields are partly due to poor soil fertility, weather events such as hurricanes, drought, and heavy rains in the different regions of production,several diseases and pests wreak havoc on banana production, especially in areas where multiple pests and pathogens coexist [11].Of these diseases, bacterial diseases have been considered as one of the most important constraints in the production of banana [12].Among the many bacterial diseases, such as banana Xanthomonas wilt (BXW), *Rastonia solanacearum*, *Xanthomonas*

Comment [MT12]: Malaysian

Comment [MT13]: that

Comment [MT14]: , it takes

Comment [MT15]: is

Comment [MT16]: Meanwhile,

Comment [MT17]: by

Comment [MT18]: bananas

campestry and *Pseudomonas celebensis*, affectingbananain the tropical and subtropical areas,*Rastonia solanacearum*, *Xanthomonas campestry* and *Pseudomonas celebensis* holds an important place in the infection of banana and cause significant yield losses [11]. This consequently, lead to a significant yield gap in banana production, especially in locations where bacterial infections, as well as a variety of other pathogens and pests, are present.

Very widespread in the banana plant world today, it is responsible for damages affecting the entire banana tree, attacking from the roots, the comb, pseudostem and consequently the leaves and fruits. Bacterial diseases can be found on the pseudostems of different *Musa* species including plantains and thus, it represents at this point the second major pre-harvest disease of banana after fungi.These diseases can cause up to 100% yield losses, severely affecting food security and livelihoods for banana farmers [13].

Confronted with these diseases, management is often done through the usage of chemical fungicides. Nonetheless, not only is there a continuous increase in the cost of these chemical pesticides, they induce a certain number of problems like environmental pollution, development of resistance by the bacteria and the presence of chemical residues in the fruits which are potentially detrimental to the health of the consumers and workers [14]. Hence, it is important to develop other alternative methods of control other than the use of chemical pesticides. Among these alternative methods, the control method through the usage of natural products such as plant extracts, has been shown to be effective against many plant pathogens and considered to be safe for consumers and the environment [14].

Materials and Methods

Collection of Samples

Symptomise organs of banana plant (roots, pseudostems, leaves, fruits) were collected from the BOH Plantation Limited in Tiko subdivision of the South-West Region of Cameroon. Samples were put in appropriate bags, labelled and transported to the Research Unit of Phytopathology and Agricultural Zoology of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang for bacterial isolation.

Isolation and Identification of Bacteria Associated withdifferent Banana Plant Organs

The various symptomized collected organs (roots, pseudostems, leaves, fruit) from the field were washed thoroughly in tap water and cut into small fragments 5 mm² using a sterile scalpel. approximately and soaked in a physiological solution (9% sodium hypochloride solution) for 1 hour to extract the bacteria. Using a microbiological hood near the flame of a Bunsen burner, 0.1 ml of bacterial inoculum were introduced into freshculture

Comment [MT19]: bananas

Comment [MT20]: leads

Comment [MT21]: bananas

Comment [MT22]: Upper case

Comment [MT23]:

Comment [MT24]: was

Comment [MT25]: a fresh

medium containing Petri dishes, spread in a spiral shape on the surface of the culture medium using a sterile loop and then incubated at 30°C for 2 days [15].

After 2 days of incubation, the various visible bacterial colonies were sub-cultured separately on fresh culture medium and incubated again at 30°C for 2 days until pure cultures were obtained, sealed with parafilm paper and stored in the refrigerator at 4°C. Morphological identification of the different bacteria isolates was carried out based on the cultural characteristics and and the colour of the bacterial walls observed under an optical microscope (Olympus brand), with the help of bacteriological identification keys [16].

After identification, the bacteria were gram stained to determine if it was a Gram (-) or Gram (+) bacteria. This involved spreading a 2-day-old bacterial culture on a slide, then drying it and observing under an immersion microscope.

Hence, the appearance of a pink colour revealed that the bacterium was Gram (-) and in the appearance of a blue-violet colour, the bacterium was considered to be Gram (+).

Preparation of plant extracts

The plant extracts were prepared from two plants harvested on Campus A of the University of Dschang. The fresh organs were disinfected separately with a sodium hypochlorite solution at 2%, rinsed with sterile distilled water to remove any impurities, chopped into small fragments using a sterilized knife and dried in darkness for one week. When fully dried, the samples were grinded to powder using an electric grinding machine (trade of machine) [17]. Thereafter, using cold solvent extraction method [18], 100 g of each processed samples of *Euphorbia hirta* and *Lantana camara* were macerated in 500 ml of each solvent (sterile distilled water and ethanol) in a bottle for 48 hours at room temperature. After 48 hours, the mixture was filtered using cheese cloth followed by Whatmann filter paper N˚. 1.

The differentethanolic extracts of the two plants were poured into sterilized stainless-steel trays (plates) and taken to a Burcher brand rotary evaporator flask at 67°C for partial evaporation of the solvent (ethanol) before transferring for drying in a Cornelie brand oven together with the aqueous extracts at a temperature of 40°Cfor complete evaporation of the solvents (distilled water and ethanol). The plant extracts were transferred into labeled sterile bottles and stored at 4˚C in a refrigerator pending utilization for the antifungal activity tests.

The extraction yield (EY) was calculated using the following formula:

$$EY(\%) = \frac{M1}{M0} \times 100$$

Where: M0 = the mass of the initial plant material and M1 = the mass of the crude extract.

Comment [MT26]: gram-stained

Comment [MT27]: remove a

Comment [MT28]:

Comment [MT29]: the cold

Comment [MT30]: samples

Comment [MT31]: Whatman

Comment [MT32]: Remove that word

Comment [MT33]: antibacterial

***In Vitro* Evaluation of AntiBacterial Activities of Plant Extracts on the Growth of Different Bacteria**

Three bacteria; *Rastonia solanacearum*, *Xanthomonas campestry* and *Pseudomonas celebensis*were selected for this test. The choice of these three bacteria was related to high frequencies of occurrence during the inventory of bacteria associated with banana. The efficacy of plant extracts against different bacteria was assessed using the method of[19], which consisted of introducing 1 ml of bacterial suspension into 20 ml of cold but not solidified culture medium (Nutrient agar), previously sterilised at 121°C for 15 minutes. After the mixture had solidified, sterilised discs of 5 mm in diameter were placed in the centre of fresh culture medium containing Petri dishes. These discs separately received 30 µl of the different plant extracts at concentrations of 12.5 mg/ml, 25 mg/ml and 50 mg/ml and all incubated at 30°C for 48 hours. Petri dishes with discs containing 30 µl of penicillin and sterile distilled water were used as positive and negative controls respectively. This test for In vitro evaluation of plant extracts was done in a complete randomized design with 3 replicates.

After 48 h of incubation, the diameters of the zones of inhibition, which materialised as clear zones around the discs, were measured using a graduated ruler. Plant extract concentrations that induced a zone of inhibition around the disc greater than 3 mm in diameter were considered to have antibacterial properties.Bacteria were classified according to inhibition diameter, in one of the following categories: resistant, limited sensitivity, medium sensitivity, very sensitive [20].

Table 1: Microbial strain sensitivity scale

Diameter of inhibition	>8mm	8-14mm	14-20mm	<20mm
Sensitivity of Bacteria	Resistant	Limited sensitivity	Averagely sensitive	Highly sensitive
Degree of activity	(-)	(+)	(++)	(+++)

Nature of toxicity of plant extracts

The evaluation of the toxicity of plant extracts consisted of seeing if the colony growth, where complete inhibition was observed, was accompanied by a bacteriostatic or bactericidal activity. In this case, the explants of the colony where complete inhibition was observed on the supplementary Nutrient Agar on plant extracts were retaken and placed aseptically on a none bactericidal or plant extract containing Nutrient Agar in a sterilized hood lighted by a Bunsen flame. After 48 hours of re-incubation, at a temperature of 30˚C,

Comment [MT34]: Antibacterial

Comment [MT35]: bananas

Comment [MT36]: sterilized

Comment [MT37]: sterilized

Comment [MT38]: the fresh

Comment [MT39]: materialized

Comment [MT40]: Bacteriostatic and bactericidal activity of the plant extracts

Comment [MT41]: What does it mean?

the activity of plant extracts was considered as bacteriostatic if there was colony regrowth of the bacterial pathogen and bactericidal if there was no colony growth of the pathogen.

Comment [MT42]: This paragraphneeds to be rephrased to make it more understandable

Statistical analysis of the data

Data collected on the prevalence and severity of bacterial disease and inhibition of bacterial growth were subjected to analysis of variance (ANOVA) using the SPSS software version 22.0 and mean values were separated using the Duncan Multiple range Test (DMRT) at 5 % probability level.

Comment [MT43]: Range

RESULTS AND DISCUSSION

Isolation, purification and identification of bacteria associated with different organs of the banana plant

Comment [MT44]: Identification of bacterial Species can t be done by only microscopicobservation. You have to identify them by 16s-rDNA

The various organs of the banana plant were seen to harbour three major bacterial species which includes *Rastonia solanacearum, Xanthomonas campestry* and *Pseudomonas celebensis.* Seen under an ordinary microscope, *Xanthomonas campestry* and *Pseudomonas celebensis* were cocci due to their round shape. *Rasltonia solanacearum* was a bacillus. These species were isolated from different organs of the plant. However, both species were seen to be associated with the pseudosterm as summarised in table 2. All species were Gram

Comment [MT45]: summarized

Table 2: Characterisation of bacteria isolated from different banana organs

Comment [MT46]: Characterization

Banana plant organ	Bacteria	Form of the bacteria	Gram
Leaf	- *Pseudomonas celebensis*	Round	Gram -
	- *Rastonia solanacearum*	Stick	Gram -
Fruit	- *Xanthomonas campestry*	Round	Gram -
	- *Rastonia solanacearum*	Stick	Gram -
Pseudo-trunk	- *Pseudomonas celebensis*	Round	Gram -
	- *Xanthomonas campestry*	Round	Gram -
	- *Rastonia solanacearum*	Stick	Gram -
Roots	- *Rastonia solanacearum*	Stick	Gram -

In vitro evaluation of the antibacterial activity of plant extracts on the development of various bacteria

Comment [MT47]: Against the isolated bacteria

Characteristics and yield of plant extracts

The extraction yields of the different plants varied from one plant species to another, depending on the plant organ used and the extraction solvents used. Aqueous extracts gave higher extraction yields than ethanolic extracts (table 3). The aqueous extract of *Euphorbia hirta* gave an extraction yield of 13 % and that of *Lantana camara*, 7 %.

The aqueous extracts were thick and the ethanolic extracts were creamy.

Table 3:Plant yield and characteristics of extracts

	Yield (%)		Physical aspect	
Plant	Ethanolics	Aqueous	Ethanolics	Aqueous
Euphorbia hirta	8	13	Creamy	Thick
Lantana camara	5	7	Creamy	Thick

Effect of plant extracts on the inhibition of the growth of different bacteria

The results showed that the aqueous and ethanolic extracts of *Lantana camara* and *Euphorbia hirta* had a depressive effect on the growth of the various bacteria isolated from banana. This depressive effect was depended on the solvent, the plant, the concentration and the bacteria tested. It was found that the higher the concentration of the extract applied, the greater the percentage of inhibition.

Effect of aqueous extracts on the growth inhibition diameter of different bacteria

Aqueous extracts of *Euphorbia hirta* and *Lantana camara* gave fairly varied inhibition diameters (table 4**)**. The aqueous extract of *E. hirta at* a concentration of 50 mg/ml showed a significantly greater diameter of inhibition of the growth of *Ralstonia solanacearum* (20.67 mm) and *Pseudomonas celebensis* (17 mm) than the other concentrations and the two controls (negative control and positive control) according to Duncan's test 5 %. This *E. hirta* extract, at concentrations of 12.5 mg/ml and 25 mg/ml, showed growth inhibition diameters for *R. solanacearum* and *P. celebensis* that weresignificantly identical to those of the positive control. These growth inhibitions for both bacteria varied between 9 and 10.33 mm. With *Xanthomonas campestry*, aqueous extracts of *E. hirta* at all concentrations showed growth inhibition diameters (ranging from 10.33 to 14 mm) significantly identical to those of the positive control, according to Duncan's test at the 5% probability threshold.

The aqueous extracts of *Lantana camara*, at concentrations of 25 and 50 mg/ml, showed diameters of inhibition of growth of *R. solanacearum* to beidentical to that of the positive control according to the 5 % Duncan test. Similarly, at concentrations of 12.5 and 25 mg/ml, no significant difference was observed with the positive control. These growth inhibition diameters ranged from 7.66 to 11 mm. At all concentrations, *L. camara* exhibited diameters of growth inhibition on *Pseudomonas celebensis* (ranging from 10 to 17 mm), identical to that of the positive control (10.33 mm) according to the Duncan test at 5 % and greater than those of the negative control (0 mm). At a concentration of 25 mg/ml, *Lantana camara* extract showed an inhibition diameter of 17.67 mm on *Xanthomonas campestry*.

Comment [MT48]: Remove letter s

Comment [MT49]: Deletewas

Comment [MT50]: Delete

Comment [MT51]: Inhibition zone

Comment [MT52]: Rephrase this paragraph

This growth inhibition diameter was greater than that of the other concentrations and the controls.

Table 4: Diameters of growth Inhibition as a function of concentration of aqueous extracts

	Ralstonia solanacearum	*Pseudomonas celebensis*	*Xanthomonas campestry*
		Euphorbia hirta	
T-	0,00 ± 0,00^{c}	0,00 ± 0,00^{c}	0,00 ± 0,00^{b}
T+	10,33 ± 0,57^{b*}	10,33 ± 0,57^{b}	10,33 ± 0,57^{a}
12.5 mg/ml	9,00 ± 1,00^{b}	9,00 ± 5,19^{b}	11,00 ± 3,46^{a}
25 mg/ml	10,00 ± 3,60^{b}	10,33 ± 4,16^{b}	11,00 ± 4,58^{a}
50 mg/ml	20,67 ± 1,15^{a}	17,00 ± 7,21^{a}	14,00 ± 3,00^{a}
		Lantana camara	
T-	0,00 ± 0,00^{c}	0,00 ± 0,00^{b}	0,00 ± 0,00^{c}
T+	10,33 ± 0,57^{ab}	10,33 ± 0,57^{a}	10,33 ± 0,57^{b}
12.5 mg/ml	7,66 ± 1,52^{b}	10,00 ± 2,00^{a}	7,67 ± 4,93^{b}
25 mg/ml	11,00 ± 1,73^{ab}	15,66 ± 8,14^{a}	17,67 ± 4,04^{a}
50 mg/ml	15,66 ± 8,14^{a}	17,00 ± 7,00^{a}	11,67 ± 2,89^{b}

* Means with the same superscript letter in the column are not significantly different according to the duncan test at 5%. T+ = positive control (Penicilline), T- = negative control (Distilled water).

Effect of ethanolic extracts on the growth inhibition diameter of different bacteria

The ethanolic extract (table 5)of *Euphorbia hirta at* concentrations of 25 mg/ml and 50 mg/ml, showed diameters of inhibition growth of 14.33 mm and 12 mm respectively on *Ralstonia solanacearum*. These inhibitions were significantly identical to that of the positive control (10.33 mm) and greater than that of the negative control (0 mm) according to Duncan's test at the 5% probability threshold. With *Pseudomonas celebensis*, the extract, at a concentration of 50 mg/ml, showed an inhibition diameter of 18 mm, which was greater than that of the controls and the other concentrations. At 12.5 mg/ml and 25 mg/ml, this extract showed a growth inhibition diameter of 8.66 mm and 7.33 mm respectively on *P. celebensis*, which were significantly identical to those of the positive control.

However, the extract of *Lantana camara*, at a concentration of 50 mg/ml, showed diameters of inhibition of growth of 12.33 mm and 10.33 mm on *Ralstonia solanacearum* and *P. celebensis* respectively *that were* significantly identical to those of the positive control. With *Xanthomonas campestry*, ethanolic extract of *L. camaraa* at all concentrations showed growth inhibition diameters (ranging from 9.33 mm to 13.33 mm) which were significantly identical to those of the positive control, according to Duncan's test at 5% probability threshold.

Comment [MT53]: Supplement the results with figures of the inhibition zones

Comment [MT54]: Inhibition zone

Comment [MT55]: Inhibition zone

Table 5: Diameters of growth Inhibition as a function of concentration of ethanolic extracts

	Ralstonia solanacearum	*Pseudomonas celebensis*	*Xanthomonas campestry*
		Euphorbia hirta	
T-	0,00 ± 0,00c	0,00 ± 0,00c	0,00 ± 0,00c
T+	10.33 ± 0.57ab	10,33 ± 0,57b	10,33 ± 0,57b
12.5 mg/ml	7,00 ± 1,00b	8,66 ± 2,08b	11.33 ± 7.57ab
25 mg/ml	14,33 ± 6,02a	7,33 ± 1,15b	20,66 ± 4,04a
50 mg/ml	12.00 ± 2.64ab	18,00 ± 3,60a	16.66 ± 7.63ab
		Lantana camara	
T-	0,00 ± 0,00c	0,00 ± 0,00c	0,00 ± 0,00b
T+	10.33 ± 0.57ab	10,33 ± 0,57a	10,33 ± 0,57a
12.5 mg/ml	8,00 ± 2,00b	7,00 ± 1,00b	10,33 ± 1,52a
25 mg/ml	9,00 ± 1,00b	8,33 ± 1,52b	9,33 ± 3,05a
50 mg/ml	12,33 ± 2,51a	10,33 ± 1,52a	13,33 ± 3,51a

* Means with the same superscript letter in the column are not significantly different according to the duncan test at 5%. T+ = positive control (Penicilline), T- = negative control (Distilled water).

Sensitivity of bacteria to different plant extracts

The sensitivity of the plant extracts varied according to the bacteria, the extraction solvent, the plant and the concentration applied.

Sensitivity of bacteria to aqueous extracts of different plants

The various bacteria were moderately sensitive to aqueous extracts of *Euphorbia hirta* at concentrations of 12.5 and 25 mg/ml and in the presence of the positive control. At a concentration of 50 mg/ml, *Ralstonia solanacearum* was highly sensitive and the other two bacteria (*Pseudomonas celebensis* and *Xanthomonas campestry*) were moderately sensitive.

With aqueous extracts of *Lantana camara*, at a concentration of 12.5 mg/ml, *R. solanacearum* and *X. campestry* were resistant. *P. celebensis*, at this concentration, showed limited susceptibility. Average sensitivity was obtained with *R. solanacearum* and *P. celebensis* at 50 mg/ml.

Table6: Sensitivity test as a function of concentration of aqueous extracts

	Ralstonia solanacearum	*Pseudomonas celebensis*	*Xanthomonas campestry*
		Euphorbia hirta	
T+	+	+	+
12.5 mg/ml	+	+	+
25 mg/ml	+	+	+
50 mg/ml	+++	++	++

	Lantana camara		
T+	+	+	+
12.5 mg/ml	-	+	-
25 mg/ml	+	++	++
50 mg/ml	++	++	+

*- = resistant; + = limited sensitivity; ++ = moderately sensitivity and +++ = very sensitive

Sensitivity of bacteria to ethanolic extracts from different plants

Ethanolic extracts of *Euphorbia hirta*, at concentrations of 25 and 50 mg/ml, showed that *Ralstonia solanacearum was* moderately susceptible. The same sensitivity was obtained with *Pseudomonsa celebensis* and *Xanthomonas campestry* at 50 mg/ml. At a concentration of 25 mg/ml, *X. campestry* was highly susceptible and *P. celebensis* was resistant.

All bacteria in the presence of ethanolic extracts of *Lantana camara showed* limited sensitivity, with the exception of *R. solanacearun* and *P. celebensis* which were resistant at 12.5 mg/ml.

Table 7: Sensitivity test as a function of concentration of ethanolic extract

	Ralstonia solanacearum	*Pseudomonas celebensis*	*Xanthomonas campestry*
	Euphorbia hirta		
T+	+	+	+
12.5 mg/ml	-	+	+
25 mg/ml	++	-	+++
50 mg/ml	++	++	++
	Lantana camara		
T+	+	+	+
12.5 mg/ml	-	-	+
25 mg/ml	+	+	+
50 mg/ml	+	+	+

*- = resistant; + = limited sensitivity; ++ = moderately sensitivity and +++ = very sensitive

Bacteriostatic and bactericidal activity of plant extracts

Aqueous extracts of *Euphorbia hirta*, at 50 mg/ml, had bactericidal activity on the development of *Ralstonia solanacearum* and *Pseudomonas celebensis.* Aqueous extracts of *Lantana camara*, at concentrations of 25 mg/ml and 50 mg/ml, exhibited bactericidal activity on the development of *P. celebensis.* The other concentrations of aqueous extracts, positive controls and ethanolic extracts of *L. camara* had bacteriostatic activity. Ethanolic

Comment [MT56]: against

extracts of *E. hirta had* bactericidal activity on the growth of *Xanthomonas campestry* at 25 mg/ml and 50 mg/ml. The same activity was obtained with *P. celebensis* at 50 mg/ml.

Table 8: Sensitivity test as a function of concentration

	Ralstonia solanacearum		*Pseudomonas celebensis*		*Xanthomonas campestry*	
	Euphorbia hirta					
	Aqueous	Ethanolics	Aqueous	Ethanolics	Aqueous	Ethanolics
T+	b*	b	b	b	b	B
12.5 mg/ml	b	b	b	b	b	B
25 mg/ml	b	b	b	b	b	B
50 mg/ml	B	b	B	B	b	B
	Lantana camara					
	Aqueous	Ethanolics	Aqueous	Ethanolics	Aqueous	Ethanolics
T+	b	b	b	b	b	B
12.5 mg/ml	b	b	b	b	b	B
25 mg/ml	b	b	b	b	B	B
50 mg/ml	B	b	b	b	b	B

*B = bactericidal activity and b = bacteriostatic activity

Discussion

Isolation of the different bacteria associated with different banana organs

This study identified 3 bacteria species (gram-) associated with the fruit, root, leaf and psuedostem of *Musa acuminata*. They include; *Xanthomonas campestris pv*, responsible for bacterial wilt, *Pseudomonas celebensis* responsible for *banana blood disease*, and finally *Ralstonia solanacearum* responsible for moko disease.The presence of this diversity of bacteria could be due to the fact that *Musa acuminata*plant constitutes an important source of nutrients like carbohydrates for these bacteria. Species like *Ralstonia solanacearum* had the highest frequency of occurrance compared to the other bacteria species as it was isolated from all the different organs.

These three species are generally reported to cause significant damage to the leaves, pseudostems, fruits and of course to the entire banana plant. These results are in accordance with those of [21] and those of[22], who reported that the *R. solanacearum* species complex (RSSC) and xanthomonas wilt are a highlydiverse group of bacterial strains found worldwide and are classified among the most destructive plant pathogenic bacteria. However, the bacterium *R. solanacearum* species complex (RSSC) stands out as a highly diverse group of bacterial strains and classified among the most destructive plant pathogenic

Comment [MT57]: Bacteriostatic and bactericidal activity of plant extracts

Comment [MT58]: How the higher concentration (50mg/ml) show bacteriostatic activity

Comment [MT59]:

Comment [MT60]: occurrence

bacteria compared to *Xanthomonas campestris* and *Pseudomonas celebensis* [22]. *R. solanacearum* is a very formidable species for a wide range of hosts, attacking the foliage, stems and fruits of its hosts causing damages in cool climates and is strongly associated with latent infection.

Xanthomonas campestris was less important compared to the *solanacearum* species with respect to its low isolation frequencies (in the fruits and pseudo-trunk only), while *Pseudomonas celebensis* was relatively less important as it had the least isolation frequency (in the Leaves only). Several reports showed the implication of the genus *Ralstonia* and *Xanthomonas* as being responsible for the major economic losses in the production of banana [12]. The results are similar to those of [13], who reported that Banana Xanthosoma Wilt disease is among the most serious biotic diseases affecting banana production in East Africa, which is the largest producer and consumer of banana in the region.

Effect of *in vitro* plant extracts on the growth of various bacteria

Extraction yields of different plants

Plant extraction yields varied depending on the plant species and the type of extraction solvent used; indeed, this variability in extract yield may be due to intrinsic factors such as the botanical species or family, the vegetative cycle of the plant, the stage of development of the plant, and extrinsic factors such as climatic conditions, soil type, and the place and time of harvesting [23].

The variation in yield observed within the same plant between aqueous and ethanolic extracts can be explained by the fact that distilled water, being more polar than ethanol, is less selective with regard to the chemical compounds in the plant, hence the better extraction yields was observed with water as opposed to ethanol.

Effect of Plant Extracts on the Inhibition Diameter of Growth of Bacterial Pathogens

Comment [MT61]: Inhibition Zone Diameter

In this study, we investigated the antibacterial activities of *Euphorbia hirta* and *Lantana camara* extracts against *Ralstonia solanacearum, xanthomonas campestry* and *pseudomonas celebensis*. Our results demonstrated that *Euphorbia hirta* and *Lantana camara* extract at all tested concentrations had greater overall depressive effect on the inhibition diameter growth of these three bacteria strains than the negative control

Comment [MT62]: A greater

This depressive effect is thought to be due to the fact that the plants used for this test contain compounds or substances with antibacterial properties that influence the growth of these three bacteria. These results corroborate those of [24]who showed that certain plants contain compounds with antibacterial properties such as alkaloids, sterols, terpenoids,

flavonoids, anthraquinone phenols, saponins or tannins. Hence their use in traditional medicine.

The growth inhibition diameter of the different bacteria was influenced by the concentration of extract applied. The growth inhibition diameter was greater with increasing extract concentration which suggest that higher concentrations of extracts have greater antibacterial activity than lower concentrations. Similar results were reported by [17], who showed that higher concentrations of plant extracts had greater antibacterial activity on the development of *Ralstonia solanacearum, the* causal agent of bacterial wilt in potatoes, than lower concentrations. At the same concentrations, different diameters of growth inhibition were observed. This difference in the antibacterial activity of plant extracts against these bacteria could be due to the fact that the plant extracts contain different active ingredients. According to [24], the antibacterial activity of plant extracts is often closely linked to the simultaneous actions of their constituents.

The results obtained with *Euphrobia hirta* extracts on the growth of *Pseudomonas celebensis* corroborate with those obtained by [25], who showed that aqueous and hexanolic extracts of this plant had antibacterial activity on the development of *P. celebensis* responsible for blood disease in banana. The results obtained with *Lantana camara* on the development of *R. solanacearum* are similar to those of [26], who showed that the flavones extracted from the methanol extract of dried leaves of *L. camara* also showed the antibacterial and antifungal properties which inhibited the development of *Colletotrichum gloeosporioides* Penzthe causal agent of Anthracnose disease of mango fruits. Similar results were reported by [27], who showed that ethanolic extracts of *Lantana camara* inhibited the development of *Pseudomonas celebensis* and *Xanthomonas campestris, the* causal agents of blood disease and bacterial wilt in banana, respectively.

The areas of the discs that showed inhibition of bacterial growth were subcultured on *Nutriment* Agar culture medium without extract to demonstrate the bacteriostatic or bactericidal activity of these plant extracts. Bacteriostatic activity was observed with ethanolic extracts of *Lantana camara* and *Euphorbia hirta*. The bacteriostatic activity observed with these extracts was linked to their temporary effect on bacteria or to their concentrations. The aqueous extracts of *Euphorbia hirta* at a concentration of 50 mg/ml had bactericidal activity on *Ralstonia solanacearum* and *Pseudomonas celebensis*. And ethanolic extracts of these extracts at concentrations of 25 mg/ml and 50 mg/ml respectively had bactericidal activity on the growth of *Xanthomonas campestris.* The aqueous extract of *Lantana camara*, at concentrations of 25 and 50 mg/ml, showed bactericidal activity on the growth of *P. celebensis*. These results corroborate those of[28], who showed that the

Comment [MT63]: Inhibition zone diameter

Comment [MT64]: suggests

Comment [MT65]:

Comment [MT66]: bananas

Comment [MT67]: The areas around the discs

Comment [MT68]: sub-cultured

Comment [MT69]: plant extract

aqueous extract of *Lantana camara* was bactericidal against *Staphylococcus aureus* an also of the fact that they are of different concentration and chemical composition.

Conclusions

This study revealed that *Musa acuminata*plant is affected by a diverse range of bacteria of which the most common are those belonging to the genus *Ralstonia* and *Xanthomonas*. The extracts of *A. ampeloprasum* and *C. citratus*can be used for the control of these bacteria. Hence, the results obtained in this study afirms that both ethanol and aqueous extracts *of Euphorbia hirta* and *Lantana camara* plants could be developed as natural pesticides in the control of bacteria that affects banana plant.

Comment [MT70]: ??????? Omit this

Comment [MT71]: affirms

REFERENCES

1. Jones, D.R., 2018. Handbook of Diseases of Banana, Abaca and Enset. CABI, Wallingford. https://doi.org/10.1079/9781780647197.0000
2. Reay, D., 2019. Climate-smart bananas. In D. Reay (Ed.), Climate-smart food(pp. 81–91). *Springer International Publishing*. https://doi.org/10.1007/978-3-030-18206-9_7
3. WorldAtlas, 2020. Most popular fruits in the world. https://www.worldatlas.com/articles/the-mostpopular-fruit-in-the-world.html
4. Banana Link, 2022. Blog: ALDI sends a strong signal with a bold banana purchasing reform.
5. Moyer, K., 2022. 20 interesting banana facts. https://blog.aghires.com/20-interesting-banana-facts/ Organic Produce Network. (2018). *Organics Unlimited carries on family tradition*. https://www. organicproducenetwork.com/article/591/organics-unlimited-carries-on-family-tradition
6. UN Comtrade, 2022. International trade statistics. UN Comtrade Database. https://comtrade. un.org/data/
7. Hommel, A., 2019. Global market update: Banana. Tridge.
8. Cheon, J. and Lee, J., 2021. 2021 banana market report.Tridge.
9. Fresh Plaza, 2021. The logistics and transportation costs of banana exports soar by up to 300% in Colombia.https://www.freshplaza.com/article/9352429/the-logistics-and-transportation-costsof-banana-exports-soar-by-up-to-300-in-colombia/
10. Research and Markets. 2019. Global banana market 2019-2024: Increasing health consciousness driving the banana market. Cision. https://www.prnewswire.com/news-releases/global-bananamarket-2019-2024-increasing-health-consciousness-driving-the-banana-market-300880835. html
11. Tripathi, L., Ntui, V,O. and Tripathi, J.,N., 2020. CRISPR/Cas9 genome editing of banana for disease resistance. *Current Opinion in Plant Biology, 56*, 118–126.

12. Blomme G, Dita M, Jacobsen KS, Pérez Vicente L, Molina A,Ocimati W, Poussier S and Prior P 2017: Bacterial Diseases of Bananas and Enset: Current State of Knowledge and Integrated Approaches Toward Sustainable Management. *Frontiere Plant Science*, 8(1290). *doi: 10.3389/fpls.2017.01290*

13. Tripathi, L., Ntui, V.O., Tripathi, J.N. 2022. Control of Bacterial Diseases of Banana Using CRISPR/Cas-Based Gene Editing. *International Journal of Molecular Science,23*, (3619), 1-16. https:// doi.org/10.3390/ijms23073619
14. Hung, T.N., Hung, N.Q., Mostert, D., Viljoen, A., Chao, C.P. and Molina, A.B. 2017. First Report of Fusarium Wilt of Cavendish Bananas, Caused by *Fusarium oxysporum* f. sp. Cubense Tropical Race 4 (VCG 01213/16), in Vietnam. *Plant Disease*, 21, 102-448. https://doi.org/10.1094/PDIS-08-17-1140-PD
15. Kara, M., Soylu, S. 2022. Isolation of endophytic bacterial isolates from healthy banana trees and determination of their *in vitro* antagonistic activities against crown rot disease agent *Fusarium verticillioides*. *MKU. J. Agric. Sci.* 27(1): 36-46. DOI: 10.37908/mkutbd.1021349
16. Duhan, P., Bansa, P., Rani, S. 2020. Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. *South African Journa of Botany*, 134(2020): 43-49. www.elsevier.com/locate/sajb
17. Agyingi, A., Keuete, K.E., Maah, R.N. 2023. Inventory and Management of Fungi Associated with Banana Plant through the Use of *Allium ampeloprasum* and *Cymbopogon citratus* Extracts. *Journal of Agricultural Chemistry and Environment*, 12, 51-64. https://doi.org/10.4236/jacen.2023.122005
18. Yemo, N.Y., Tsopmbeng, N.G.R., Keuete, K.E. and Nchongboh, C.G. 2017. Antifungal Activities of Plant Extracts against Coffee Berry Disease Caused by Colletotrichum kahawae L. *International Journal of Current Research in Biosciences and Plant Biology*, 4, 60-66. https://doi.org/10.20546/ijcrbp.2017.407.007
19. Arias, P., Dankers C., Liu, P., Pilkauskas, P.2003. The world banana economy, 1985-2002. *FAO*: Rome. http://www.fao.org/3/y5102e/y5102e00.htm. Accessed 15th April 2019
20. Schulz B., Sucher J., Aust H.J., Krohn K., Ludwig K., Jones P.J. & Döring D. 1995.Biological active secondary metabolites of endophetic *Pezcula* species.*Mucological Research***,** 99, 1007-1015.
21. Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V. & Machado, M.A. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular plant pathology,* 13(6), 614-629.

22. Uwamahoro F., Berlin A., Bylund H., Bucagu C., Yuen J. 2019. Management strategies for banana Xanthomonas wilt in Rwanda include mixing indigenous and improved cultivars. *Agronomy for SustainableDevelopment*, 39, 22
23. Keuete K.E. 2014. Inventaire des champignons post-recoltes des fruits de l'avocatier et essai delutte antifongique par l'utilisation des extraits de quelques plantes. Thèse, Université de Dschang, 89p
24. Muntean, D., Vulpie, S. 2023. Antioxidant and Antibacterial Activity of Plant Extracts. *Antibiotics*, *12*, 1176. https://doi.org/ 10.3390/antibiotics12071176
25. Abbas, A., Khan, S.U., Khan, W.U., Saleh, T.A., Khan, M.H.U., Ullah, S., Ali A., Ikram, M. 2019. Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: Alternatives to chemical pesticides. C. R. Biologies 342: 124-135.
26. Bashir, S., Jabeen, K., Iqbal, S., Javed, S., Naeem, A. 2018. *Lantana camara*:Phytochemical Analysis And Antifungal Prospective**.** *Planta Daninha***,** 1-7.Doi: 10.1590/S0100-83582019370100137
27. Navarrete, B., Nahir, A., Fadime, E., Pita-Ospina., Melida, R., Mora, S., Emilia, S., Quintero, G., Consuelo, M., Lizarazu, B. 2020. In vitro activity of the ethanolic extracts from Lantana camara L., Petiveria alliacea L. and Lippia dulcis T. against pathogenic bacteria. Nova [online]. 18(33): 53-71, ISSN 1794-2470
28. Navarrete, B., Nahir, A., Fadime, E., Pita-Ospina., Melida, R., Mora, S., Emilia, S., Quintero, G., Consuelo, M., Lizarazu, B. 2020. In vitro activity of the ethanolic extracts from *Lantana camara* L., *Petiveria alliacea* L. and *Lippia dulcis* T. against pathogenic bacteria. Nova [online]. 18(33): 53-71, ISSN 1794-2470
29. Dehou, R.J., Abissi, Y.G., Kpossou, G., Tchogou, P., Lokonon, E., Agbogba, F., Houngbeme, A., Attakpa, E., Anago, E., Baba-Moussa, L And Senou, M. 2021. Bactericidal effect of the aqueous extract of the leaves of *Lantana camara* L. (Verbenaceae), a plant used in Benin in the treatment of skin infections. *Journal of Applied Biosciences* 167: 17406 – 17412, ISSN 1997-5902