Discover Agriculture

Research

In vitro inhibition of *Xanthomonas vasicola* pv. *musacearum*, the causal agent of banana Xanthomonas Wilt, using medicinal plant extracts from North Kivu, Eastern Democratic Republic of Congo

Franchement Mukeshambala[1,2] · Angele Ibanda[3,4] · Ludivine Lassois[5] · Gakuru Semacumu[1] · Dhed'a Djailo[6] · Leon Nabahungu[7] · Guy Blomme[8] · Godefroid Monde[9]

Received: 11 March 2024 / Accepted: 15 October 2024
Published online: 24 October 2024

Abstract
Banana Wilt caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*), has emerged as a significant threat to food security in eastern Democratic Republic of Congo (Kivu). Currently, the only means of combatting this biotic constraint is through best agricultural practices. The aim of this study was to evaluate the effectiveness of medicinal plants used in the Kivu provinces in inhibiting *Xvm*. Three in vitro experiments were conducted at laboratories of Uganda's National Agriculture Research Organization (NARO) and the International Institute of Tropical Agriculture (IITA) in South Kivu. The bacterial samples were collected from infected field-grown banana plants in South Kivu and isolated on Yeast Extract Peptone Agar (YPGA). Pure *Xvm* colonies were used for identification via i) Polymerase Chain Reaction (PCR) with specific primers and, ii) greenhouse inoculation trials. A completely randomized design was used for the three inhibition tests (1) on Mueller Hinton Agar (MHA) using disc diffusion with 10 plant extracts; (2) in liquid YPG Broth using 10 plant extracts; and (3) on MHA using disc diffusion with 19 plant extracts. The first two trials used plant extracts diluted in petroleum ether, while the third trial used 19 plant extracts diluted in methanol. After maceration, filtration, and solvent evaporation, 10 mg of extract was diluted in 80 µl of distilled water + 10 µl of Dimethylsulfoxide (DMSO). Ten µl of this solution was impregnated on perforated discs of Whatman filter paper. *Zingiber officinale* (ginger) and *Ricinus communis* (castor) were the most effective plant extracts in suppressing *Xvm*. Of the thirteen plant species identified as effective against the pathogen, the Myrtaceae and Euphorbiaceae families were the most represented. Based on these results, evaluating the effectiveness of the most promising plant extracts in disinfecting the metal blades of garden tools is recommended. In addition, various phytochemical groups present in plant extracts could be evaluated for their effectiveness in suppressing *Xvm*, especially phenols and tannins.

Keywords Banana Xanthomonas Wilt · Methanolic extract · Petroleum extract · Phytochemical groups · Biological control

✉ Franchement Mukeshambala, franckmukeir@gmail.com | [1]Department of Crop Science, Université de Goma, P. O. Box 204, Goma, North Kivu, Democratic Republic of Congo. [2]Department of Health, Environment and Sustainable Development, Public Health Faculty, Université Libre Des Pays Des Grands Lacs, P. O. Box 368, Goma, Democratic Republic of Congo. [3]Regional Center for Crop Improvement, Makerere University, P. O. Box 7062, Kampala, Uganda. [4]Faculty of Renewable Natural Resources Management, University of Kisangani, P. O. Box 2012, Kisangani, Tshopo, Democratic Republic of Congo. [5]Gembloux Agro-Bio Tech, TERRA Teaching and Research Center, University of Liège, Passage Des Déportés 2, 5030 Gembloux, Belgium. [6]Biotechnology Department, University of Kisangani, P. O. Box 2012, Kisangani, Tshopo, Democratic Republic of Congo. [7]International Institute of Tropical Agriculture, Campus of Kalambo, P. O. Box 1860, Bukavu, South Kivu, Democratic Republic of Congo. [8]Bioversity International,c/o ILRI, P.O. Box 5689, Addis Ababa, Ethiopia. [9]Department of Crop Science, Agriculture University of Yangambi, P. O. Box 1232, Tshopo, Kisangani, Democratic Republic of Congo.

1 Introduction

The East African Great Lakes region accounts for more than 11% of the world's banana production. A wide range of varieties are cultivated, which are cooked, processed into beer, eaten as dessert, processed into flour and even biscuits [1]. Additionally, novel uses are emerging, e.g. the use of fiber extracted from the leaf sheaths, which is used to produce paper, hair extensions, or other artistic items [2, 3].

In 2018, bananas and plantains occupied 1.2 million hectares in the Democratic Republic of Congo alone, yielding 5.1 million tons [4]. Unfortunately, banana production has decreased by 20 to 60% in the last few decades in the DRCongo [5], this due to abiotic and biotic constraints (pests and diseases) [6]. The biotic constraints primarily include nematodes and weevils as pests, Xanthomonas wilt (BXW), banana bunchy top disease (BBTD), fusarium wilt, and black leaf streak (BLS) [7]. Among these threats, BXW has a significant impact on banana production landscapes in the region due to its rapid spread [8].

The emergence of banana bacterial wilt in the Great Lakes Region has prompted all stakeholders in the banana value chain to take action, recognizing its dire implications for the region's economy and food security [9]. Xanthomonas wilt was first reported on enset (1968) (*Ensete ventricosum*) and banana (1974) in Ethiopia [10, 11]. In 2001, the disease emerged in central Uganda and eastern DRCongo [12–14]. By 2010, Rwanda, Tanzania, Kenya, and Burundi had also reported cases [16].

Petsakos et al. [17] suggest that if the disease is left uncontrolled within 10 years, banana production can decrease by as much as 55%, compared to a BXW-free baseline scenario, resulting in economic losses of around 25 billion USD in Africa south of the Sahara. At the same time, the population at risk of hunger in countries that highly depend on bananas as a staple food is projected to increase by more than 4.6%.

Xanthomonas vasicola pv. *musacearum* (*Xvm*) [15], is the causal agent of BXW [16–19]. On culture media, the bacterium is characterized by the production of a yellow exudate and circular colonies with a dome-shaped surface. It is gram-negative, positive for catalase and Potassium hydroxide (KOH), and does not reduce nitrate [20]. To date, the molecular characterization of the bacterium primarily uses the primer coding for the general secretor protein D (GspDm), amplifying the 265-bp fragment of *Xvm*gspD [21].

Unfortunately, no chemical formula is currently available to cure the disease, and chemical control of bacterial diseases is not economically viable in the field [22–24]. As a result, cultural practices which include early male bud or flower removal, sterilizing garden tools, controlling animal movement, and using the 'Single Diseased Stem Removal (SDSR)' method have been used as the only means of combating the disease [7, 25]. Additional methods for the elimination of *Xvm* bacteria on e.g. garden tools, or other surfaces could help in disease control. Yemata et al. [26–28] have explored alternative measures using antibiotics and plant extracts in vitro and found that certain antibiotics and plant extracts from native Ethiopian plant species have the potential to inhibit *Xvm*, the same bacterium which is also responsible for bacterial wilt in Enset.

There is great potential for plant extract-based treatments, as shown in ethnobotanical research conducted in DRCongo [29, 30], e.g., in Kinshasa and Kasangulu [30–32], and in North and South Kivu [33–35]. These studies have identified numerous medicinal plant species from families such as *Euphorbiaceae, Rubiaceae, Papilionaceae, Malvaceae, Lamiaceae, Fabaceae, Asteraceae*, *Caricaceae, Poaceae* and *Myrtaceae* that are effective in controlling infectious diseases. Especially in the *Euphorbiaceae, Myrtaceae* and *Asteraceae* families, an important number of species are used for their antibacterial proprieties [36–38]. These effects are attributed to the presence of an estimated 104 chemical components, including glycosidic, phenolic acids, coumarins, monoterpenoids, triterpenoids, diterpenoids, flavonoids, and 4α-methyl steroids [39]. As such, this study aimed to identify the plant species capable of inhibiting the growth of *Xanthomonas vasicola* pv. *musacearum* through in vitro experimentation, specifically targeting antibacterial plant species commonly used by traditional practitioners in Eastern DRCongo.

2 Materials and methods

To identify medicinal plant species in Kivu's traditional medicine that can inhibit *Xanthomonas vasicola* pv. *musacearum (Xvm)*, two complementary experimental sites were utilized. The first was the "National Agriculture Research Organization" (NARO) laboratory in Kawanda/Kampala, Uganda (0°24′51"N 32°32′05"E), while the second was the laboratory at the International Institute of Tropical Agriculture (IITA) Olusegun Obasanjo campus in Kalambo/Bukavu, eastern DRCongo (2°30′50″S; 28°54′43″E).

2.1 Preparation of inoculum

Infected banana pseudostem tissue samples were collected from home gardens in banana growing areas in Mahagi, Ituri province, Eastern DRCongo [40]. The collected tissue sample was cleaned with 70% ethanol and a smaller 4 cm^2 portion was ground in 9 ml of sterile distilled water to release the bacteria. 20 µl of the ground material was then inoculated onto a petri dish containing YPGA medium (Yeast extract, Peptone, Glucose, and Agar) [41]. The inoculated plates were incubated at room temperature for 48 h. For colony purification, only pure yellow colonies were collected using a platinum loop and re-seeded onto YPGA. The inoculum was prepared by diluting pure colonies in sterile distilled water to an absorbance reading of 0.5 Optical Density (OD) at 600 nm wavelength using a nanodrop 2000c spectrophotometer[42], corresponding to 10^8Colony-Forming Units per ml (CFU/ml).

2.2 Extraction of deoxyribose nucleic acid (DNA)

As per Mahuku [43] protocol, the DNA extraction process involved purifying bacterial cells through centrifugation of colonies in a 1 ml tube at 6000 rpm for 10 min in TES buffer: Tris, Ethylene-Diamine-Tetra-acetic acid (EDTA), and Sodium Dodecyl Sulphate (SDS). Furthermore, the proteins were denatured by heating the solution at 65 °C in a water bath. Precipitation of the denatured proteins was achieved by adding 250 µl of 7.5% concentrated ammonium acetate solution to the mixture, followed by keeping it on ice for 10 min and then centrifuging it at 13,000 rpm for 10 min. The resulting solution was collected and mixed with an equal volume of isopropanol to precipitate nucleic acids. Finally, the collected pellet was cleansed with ethanol and diluted in distilled water, ultimately constituting the nucleic acids (DNA).

2.3 Polymerase chain reaction (PCR)

The purified nucleic acid was used to identify the *Xvm* strain using the method described by Adriko et al. [21]. The GspDm-F2 (GCGGTTACAACACCGTTCAAT) and GspDm-R3 (AGGTGGAGTTGATCGGAATG) Primers were specific to amplify a 265 bp DNA fragment from the isolated *Xvm*. The simplex PCR cycles consisted of (i) initial denaturation step at 95 °C for 10 min; (ii) 25 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s, elongation at 72 °C for 90 s; then (iii) a final extension step at 60 °C for 30 min. The amplicons were separated by electrophoresis in a 1% agarose gel in 0.5X TBE buffer at 100 V for 45 min. Gels stained with ethidium bromide were visualized and images captured with the Vilber UV Transilluminator.

2.4 Inoculation of shoots

For confirmation of *Xvm* identity, 1 ml of a solution containing 10^8 CFU/ml was injected into the midrib of a leaf of five, 60-days-old banana plants, of the AAA-EAH cooking banana "Mbwazirume". The inoculation was carried out in the greenhouse of the IITA Kalambo laboratory. The presence of chlorotic spots on the leaves, leaf yellowing/wilting, and yellow ooze/exudates were recorded at 48 h, 10 days, and thereafter every 15 days after inoculation [16].

2.5 Plant sample collection

Two in vitro trials were conducted at NARO-Uganda to test 10 Petroleum Ether extracts of medicinal plants that are traditionally used in Kivu's medicine for the control of human bacterial infections. Plant species were collected between 9 a.m. and 3 p.m. in the vicinity of the Kalambo IITA research center in South Kivu, eastern DRCongo after the disappearance of dew. The species were*: "Allium sativum* L. *(Liliaceae) AS", "Aloe Vera* (L.) Burm.f. *(Xanthorrhoeaceae) AV", "Azadirachta indica* A. Juss. *(Meliaceae) AI", "Bidens pilosa* L. *(Asteraceae) BP", "Capsicum frutescens* L. *(Solanaceae) CF", "Carica papaya* L. *(Caricaceae) CP", "Persea americana* Mill. *(Lauraceae) PA", "Ricinus communis* L. *(Euphorbiaceae) RC", "Solanum lycopersicum* L. *(Solanaceae) SL", "Tetradenia riparia* (Hochst.) *(Lamiaceae) TR"* [45]. These plant species were sent to the Centre de Recherche en Science Naturelles (CRSN),Lwiro, South Kivu, for species identification.

At the IITA Kalambo research facility in eastern DRCongo, an additional trial was conducted to assess *Xvm* colony growth inhibition using extracts from plant species indigenous to eastern DRCongo and known for their antibiotic properties. The test involved 19 methanolic extracts of the following plant species: "*Bidens pilosa* L *(Asteraceae*) BP", "*Citrus limon* (L.) Osbeck (*Rutaceae*) CL", "*Conyza sumatrensis* (Retz.) (*Asteraceae*) CS", "*Eucalyptus eugenioides* Sieber ex Spreng. (*Myrtaceae*) EE", "*Euphorbia heterophylla* L. (*Euphorbiaceae*) EH", "*Euphorbia hirta* L. (*Euphorbiaceae*) EHi"," Flower

of *Ageratum conyzoides* L. (*Asteraceae*) ACf", "Fruit of *Ricinus communis* L. (*Euphorbiaceae*) RCf", "Gymnanthemum amygdalinum (*Vernonia amygdalina* Delile) (*Asteraceae*) VA", "*Lantana camara* L. (*Verbenaceae*) LC", "*Lantana trifolia* L. (*Verbenaceae*) LT", "Leaves of *Ageratum conyzoides* L. (*Asteraceae*) ACl"," Leaves of *Ricinus communis* L. (*Euphorbiaceae*) RCl", "*Phyllanthus niruri* L. or *Diasperus niruri* (L.) Kuntze (*Euphorbiaceae*) PN", "*Piper nigrum* L. (*Piperacea*) PNi", "*Psidium guajava* L. (*Myrtaceae*) PG", "*Solanum aculeastrum* Dunal (*Solanaceae*) SA", "*Tephrosia vogelii* Hook.f. (*Fabaceae*) TV", "rhizome of *Zingiber officinale* Roscoe (*Zingiberacea*) ZO" [45]. These plant species were sent to the Centre de Recherche en Science Naturelles (CRSN),Lwiro, South Kivu, for species identification.

The vicinity of the IITA laboratory at Kalambo as the plant species collection area is characterized by a humid tropical climate tempered by elevation. According to the Köppen-Geiger classification, its climate type is Aw3. The region benefits from two seasons: a rainy season of nine months (from September to May) and a dry season of three months (from June to August) [46]. The plant species collection region has the following soil characteristics: Ph between 4.5 and 5.5; organic matter content: 2.5 to 4.5%; Nitrogen rate content 0.15 to 0.3%; and a clay-loamy texture [47].

The choice of the 26 plant species was the result of a literature review on antibacterial plant species commonly used in the Kivu Provinces of eastern DR Congo and a selection from the 47 plant species tested. Various authors have compiled lists of plant species that are effective in the management of bacterial pathologies of plants and humans by traditional practitioners, for example, Shalukoma et al. [29] reported 77 plant species, Balagizi [44] reported 129 species, while Korangi et al. [45] mentioned 12 species. Insights provided in the above-mentioned publications guided the selection of the 26 plant species. In addition to the review work, unpublished work financed by the Agence Universitaire de la Francophonie (AUF) made it possible to constitute in the microbiology laboratory of Université Libre des Pays des Grands Lacs a herbarium of 52 species of antibiotic plants based on an ethno-botanical survey in the city of Goma.

2.6 Extract preparation

The various plant parts of the collected plant species were dried at room temperature in the shade. Preparation of plant extracts involved diluting and macerating for 24 h, 62.5 g of dry powder of each plant material in 250 ml of the extracting solvents (petroleum ether or methanol) using glass containers. Each dilution was duly labelled. The samples were shaken constantly and filtered using Whatmann No 1 filter paper after 24 h [48]. The container of filtrate was put in a Water bath until a thick pomace remained. The thickened extract was stored in sterile plastic bottles of 50 ml volume in the refrigerator at 4 °C.

2.7 Inhibition test with plant extract

The inhibition test of *Xvm* was performed using perforated discs of sterile Whatman filter paper. These discs were soaked, for an hour, with 10 µl of a solution containing 10 mg of plant extract, 10 µl of Dimethyl Sulfoxide (DMSO), and 80 µl of sterile distilled water.

In the first NARO-based trial, MHA was used to assess the level of bacterial inhibition via diffusion on filter paper discs soaked in plant extracts. For the positive control two antibiotics (tetracycline and amoxicillin) were used, while distilled water with DiMethylSulfOxide (DMSO) was used as the negative control. This trial was conducted using a completely randomized experimental design featuring 13 treatments (comprising 10 plant extracts, 1 amoxicillin control, 1 tetracycline control, and 1distilled water + DMSO control) in 3 replications.

The second in vitro experiment carried out at the NARO laboratory in Kawanda/Kampala, featured the same treatments as the previous trial. It was also conducted using a completely randomized design and utilized a YPG liquid culture medium (or Broth). This time, the level of inhibition was measured by examining the turbidity (OD) of the medium at 600 nm using the NANODROP 2000c spectrophotometer, as detailed by [42].

The IITA Kalambo-based in vitro experiment was conducted using a completely randomized design with 21 treatments, which included 19 plant extracts, 1 tetracycline positive control, and 1 distilled water + DMSO negative control. The study was replicated 3 times. The Sisvar 5.6 software was used for analysis of variance, and the means were separated using the Tukey test at a significance level of 0.05. The diameter of inhibition was initially measured on Mueller Hinton Agar plates after inoculating the medium with 100 µl of 10^8 CFU/ml of *Xvm* for one hour. The extract-soaked discs were then placed on the agar. For the liquid medium Yeast Peptone Glucose (YPG), inhibition was measured using a nanodrop 2000c spectrophotometer at a wavelength of 600 nm. 10 µl of inoculum was added to 9 ml of YPG Broth medium, mixed an hour later with 1 ml of the plant extract solution, DMSO, and sterile distilled water [49].

2.8 Phytochemical screening

For the Alkaloids test, 20 mg of plant powder was dissolved in 2% Hydrochloric acid (HCl) and filtered using a glass funnel. 1 ml of Wagner's reagent (1.27 g of iodine + 2 g of potassium iodide in 100 ml of distilled water) was added to 2 ml of the filtrate. A brown solution was an indication for the presence of alkaloids [51]. For the flavonoids screening, 0.5 g of plant powder was placed in test tubes. Ten ml of distilled water was added to each test tube and mixed by shaking, with subsequent filtration. Then 3 ml of the aqueous filtrate was mixed with 5 ml of ammonia (10%) added with 1 ml of concentrated sulphuric acid. The formation of a yellow color confirmed the presence of flavonoids [50, 52]. For detection of phenolic compounds, 2 ml of the extract were mixed with 3–4 drops of 5% Ferric Chloride (FeCl3) solution. The formation of a black color indicated the presence of phenols [28, 50, 52].

For the terpenoids screening, 0.5 g of plant powder was put in a test tube, 10 ml of methanol was subsequently added, and the mixture was centrifuged. Then 5 ml of the supernatant was mixed with 2 ml of chloroform, with addition of 3 ml of sulfuric acid. The formation of a brown layer between the two solutions confirmed the presence of terpenoids [50, 53]. The test for Saponins was carried out using 1 g of extract boiled in 5 ml of distilled water, and shaken vigorously for five minutes. The persistence of the frothing confirmed the presence of saponins [50, 54]. For Tannins screening, 0.2 g of plant extract was dissolved in 5 ml of distilled water, heated in a water bath and filtered. Subsequently, 2 ml of a 5% ferric chloride solution was added to 1 ml of the filtrate. The apparition of a blue color meant a positive tannins test [50, 54].

3 Results and discussion

3.1 PCR results

The PCR results as indicated in Fig. 1, confirmed the presence of *Xvm* in diseased banana leaves from Mahagi under code 32a (Fig. 1), detected upon amplification of GspDm. This aligns with previous findings by [55], indicating the positive presence of *Xvm* in these samples. Thus, the bacterium used in the experiments is *Xvm*. For several years, researchers in the Great Lakes region have relied on the GspDm primer as a popular molecular biology tool to diagnose Xanthomonas wilt [56] The GspDm primer has proven to be effective in diagnosing bacterial wilt in the field, even without PCR amplification, as confirmed by the loop-mediated isothermal amplification (LAMP) method [57].

3.2 Plant inoculation

No disease symptoms were observed till 7 days after inoculation (Fig. 2A), with yellowing of the inoculated leaf (Fig. 2B). A second leaf showed symptoms after about 18 days (Fig. 2C), while all leaves wilted by the 36th day (Fig. 2D). Complete wilting of the plant was observed on the 47th day (Fig. 2E). Interestingly, leaf yellowing and wilting symptoms appeared earlier on the plantlets compared to similar/previous studies [55]. Uwamahoroet al. [55], reported first leaf symptom appearance at 12 days after inoculation and the latest at 35 days. In the same study, complete plant wilting occurred between day 46 and day 80 after inoculation. The rapid development of bacterial wilt symptoms, which occurred from day 8 to day 47 in this experiment, could possibly be explained by the use of the highly susceptible highland cooking banana genotype 'Mbwazirume' (*Musa* AAA-EAH).

3.3 Plant extract yield by selected plant species

Expressed in percentage, the plant extract yield (% extract yield) was the ratio by weight of the extract harvested from the 62.5 g of plant powder macerated in the solvent. In the first and second experiments, utilizing petroleum ether as a solvent, as presented in Fig. 3, *Aloe vera* (9.44%), *Capsicum frutescens* (8.64%),, and leaves of *Ricinus communis* (6.88%) exhibited high plant extract yields, while in the third experiment, using methanol as a solvent (Fig. 4), *Psidium guajava* (12.09%), leaves of *Ricinus communis* (10.72%), and *Phyllanthus niruri* (9.12%) had the highest plant extract yields. The yield of plant extracts is influenced by various factors, including the type of solvent used and the duration of extraction [57]. Methanol is a well-known solvent for extracting numerous phytochemical groups and is recognized for its positive polarity, leading to good yields in extractions. It was observed that the extract yield from plant material was less than 13% (Figs. 3 and 4) for all plant species studied when using a single maceration process. This was mainly due to the lack

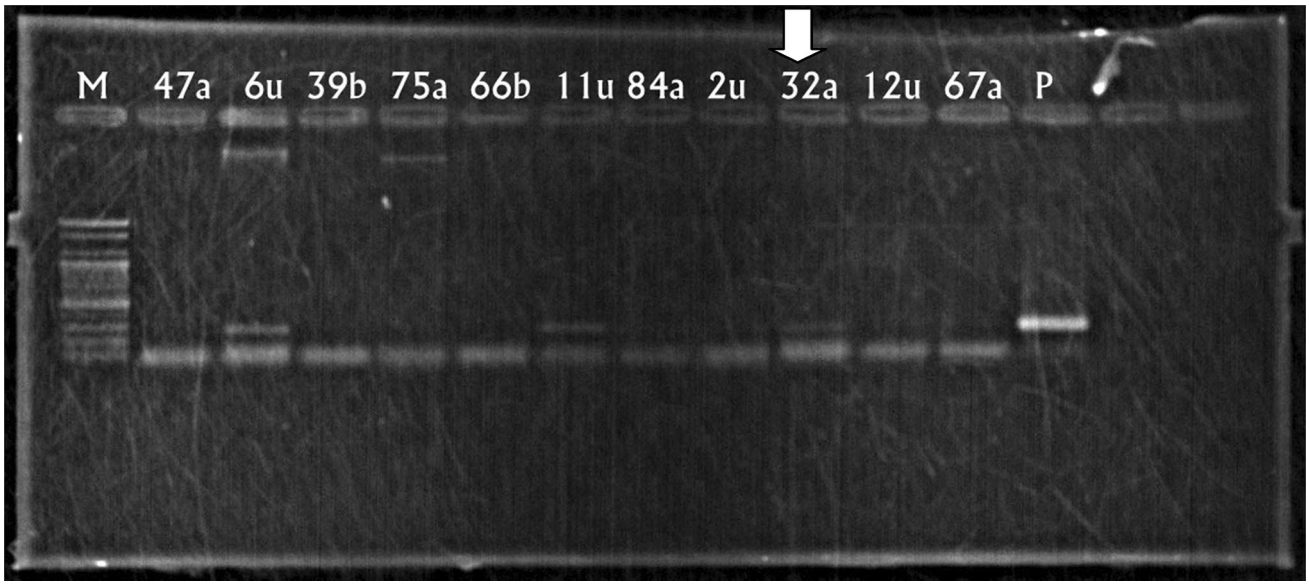

Fig. 1 PCR result on 1% Agar Gel Electrophoresis after GspDm amplification

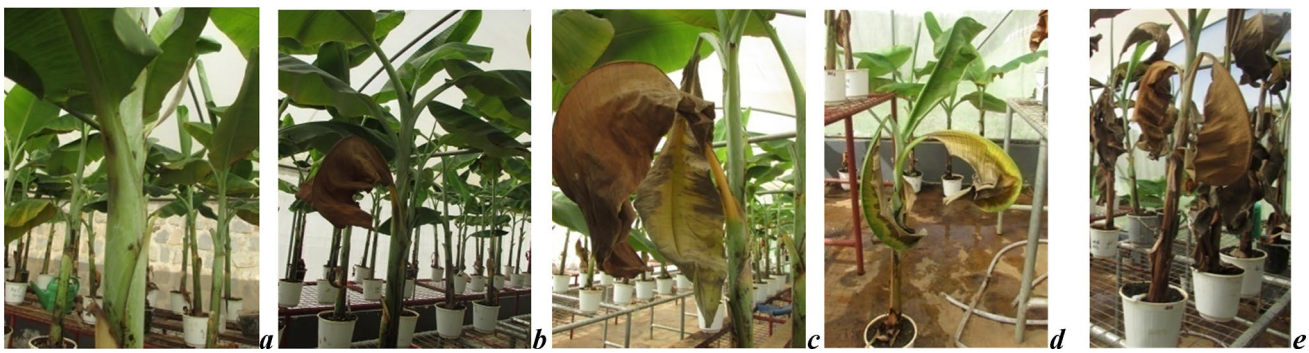

Fig. 2 Inoculation results at IITA-Kalambo: **A** at 48h after inoculation; **B** at 10 DAI (days after inoculation); **C** at 25 DAI, **D** at 40 DAI, and **E** at 55 DAI

of specialized equipment for solvent recycling after evaporation. On the other hand, when a solvent recycling apparatus like Soxhlet is used, the extraction process is optimized, but nevertheless takes several days, with extract yield increasing to over 40% [58]. It is important to note that the yield of plants varies due to the age of leaves and plants, as well as environmental factors [59].

3.4 Inhibition tests

The in vitro trials carried out at NARO-Kawanda in Uganda, showed that only the leaf extract of *Ricinus communis* had antibacterial activity (Table 1). The optical density obtained after inhibition with *R. communis* extract was 2.12, while the control displayed 3.35 (Table 1). The other tested plant species did not show any antibacterial activity. According to [28], the phytochemical groups present in *Ricinus communis* allow it to effectively act on gram-negative bacteria species like *Xvm*. It can affect the cell wall, proteins, and DNA synthesis in different ways. The plant's crude extract has a significant antibacterial power due to the presence of alkaloids, terpenoids, or polyphenols that inhibit the growth of some bacterial species. The observed inhibition can also be attributed to the hydrophobicity of the phytochemical compounds allowing them to disrupt the lipids of the bacterial cell membrane and mitochondrial membrane, thereby making the cell more porous to solutes and ions, leading to its imminent death. Yemata et al. [28] observed an inhibition zone ranging from approximately 9 to 21 mm when using extracts from *R. communis* leaves on the same *Xvm* bacterium in Ethiopia, and

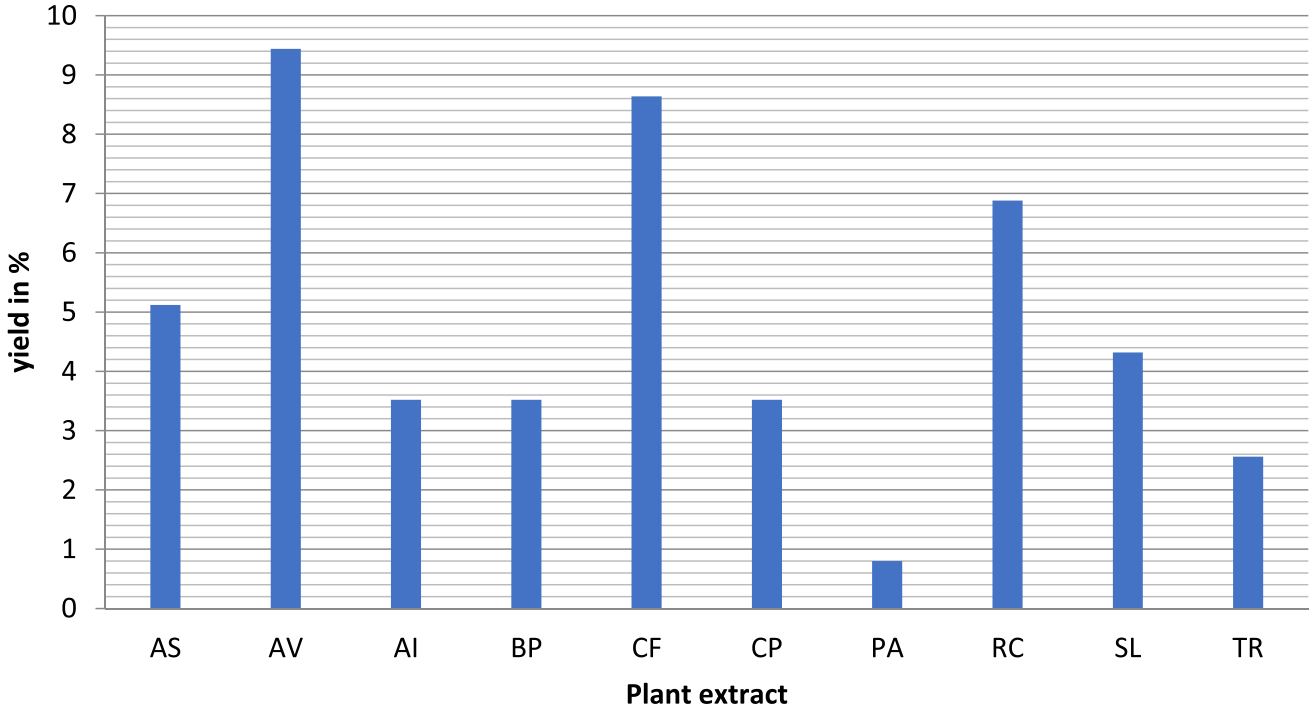

Fig. 3 Percentage yield by plant species in Petroleum Ether Extract at NARO, Kawanda in Uganda. AS, *Allium sativum* L., AV, *Aloe Vera* (L.) Burm.f.; AI, *Azadirachta indica* A. Juss; BP, *Bidens pilosa* L.; CF, *Capsicum frutescens* L; CP, *Carica papaya* L.; PA, *Persea americana* Mill.; RC, *Ricinus communis* L.; SL, *Solanum lycopersicum* L.; *TR*, *Tetradenia riparia* (Hochst.)

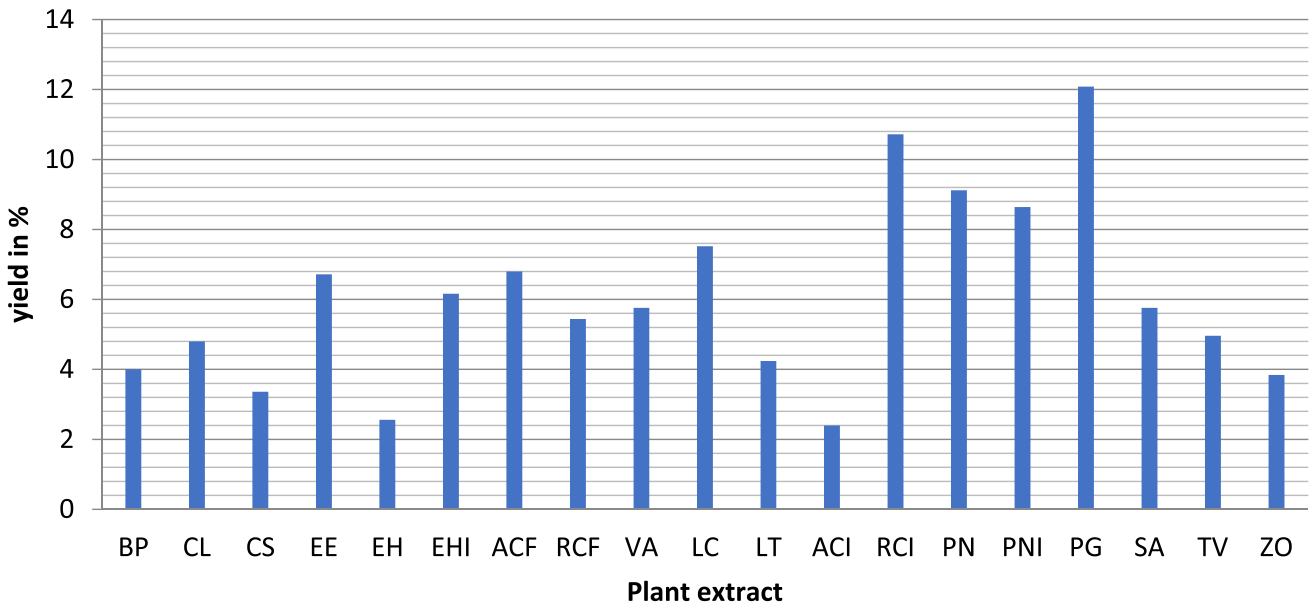

Fig. 4 Percentage yield by plant species of Methanol Extract at ITTA-Kalambo in the South Kivu province, eastern DR Congo. BP, *Bidens pilosa* L; CL, *Citrus limon* (L.) Osbeck; CS, *Conyza sumatrensis* (Retz.); EE, *Eucalyptus eugenioides* Sieber ex Spreng.; EH, *Euphorbia heterophylla* L.; EHI, *Euphorbia hirta* L.; ACF, Flower of *Ageratum conyzoides* L.; RCF, Fruit of *Ricinus communis* L.; VA *Vernonia amygdalina* Delile; LC, *Lantana camara* L.; LT, *Lantana trifolia* L.; ACI, Leaves of *Ageratum conyzoides* L.; RCl, Leaves of *Ricinus communis* L.; PN, *Phyllanthus niruri* L.; PNI, *Piper nigrum* L.; PG, *Psidium guajava* L; SA, *Solanum aculeastrum* Dunal; TV, *Tephrosia vogelii* Hook.f; ZO, rhizome of *Zingiber officinale* Roscoe

varying according to concentrations used. However, as reported by [27], antibiotics are more effective than *R. Communis* leaf extracts in inhibiting *Xvm* bacteria.

The in vitro experiment conducted at IITA Kalambo using plant species collected in the Kivu region further expanded the list of medicinal plants from Kivu that are effective in controlling *Xvm* (Table 2). An additional 5 extract types were found to be effective in suppressing *Xvm* (Table 2). The most effective was the extract of Rhizome of *Z. officinale* ZO, which is locally known as Ginger. According to the Tukey test at 0.05, it was observed that extracts from the leaves of *R. communis*, *T.vogelii* and *E. heterophylla* exhibited a similar inhibition zone comparable to that of ZO (Table 2). In addition, two other plant extracts, namely *Tephrosia vogelii* and fruits of *Ricinus communis*, displayed an inhibition zone similar to RCl, but distinct from that of ZO (Table 2).

The rhizomes of *Z. officinale* are commonly used in the region as part of therapeutic beverages [60]. According to Sebiomo et al. [61], *Z. officinale* is as effective as the antibiotic streptomycin in inhibiting *Xanthomonas oryzae* pv.*oryzae*. Ranjan et al. [62] also found that ginger is more effective in inhibiting certain pathogenic bacteria (30 mm diameter of inhibition) compared to antibiotics and could be used for developing broad-spectrum antibiotics. Sebiomo et al. [61] reported that the ginger root ethanol extract showed the greatest effect on both *Staphylococcus aureus* and *Staphylococcus pyogene* compared to the leaf and root water extract and the leaf ethanol extract.

Yousfiet al. [63] assessed effects of a ginger extract on Gram-positive human pathogenic bacterial strains, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Micrococcus luteus*, and four Gram-negative human pathogenic bacterial strains, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Salmonella typhimurium* and *Escherichia coli*, and found that this extract is highly effective in suppressing bacterial growth. Ginger rhizomes turned out to contain compounds (such as gingerol and shogaol) which showed significant antibacterial potential. The promising antibacterial activity of ginger extracts is a result of bacterial membrane disruption.

Although the mechanism of bacterial inhibition by ginger is not yet fully understood, the phytochemical screening of its extract revealed the presence of alkaloids, phenols, flavonoids, tannins, and terpenoids [64]. Flavonoids are known to inhibit various cellular processes in bacteria, while alkaloids inhibit ATP transport and substitute for some precursors of molecules necessary for bacterial development [65].

3.5 Phytochemical screening

The effective plant species reported in the current study share a rich amount of molecules from the phenol group, while molecules from the tannin and terpenoid groups are present in varying quantities (Table 3). Phenol is a polar compound with well-know antibacterial and antifungal properties, already demonstrated on bananas. Its mechanism of action involves denaturing bacterial cell proteins, which halts all protein-catalyzed metabolic activities [66–68]. Given that the antibiotic tetracycline effectively inhibited *Xvm* by targeting proteins, it seems likely that phenolic compounds found in the plant extracts are key in the observed bacterial growth inhibition. Similar results to this research were obtained for *Z. officinale* [65, 69], *R. communis* [28], for *E. heterophylla* [51], *T. vogelii* [70], *P. nigrum* [71] and for the flowers of *A.*

Table 1 Inhibition zone and absorbance (mm) for plant extracts obtained using petroleum ether, of various plant species, assessed at NARO Kawanda in Uganda

Treatment	Inhibition zone (mm)	Absorbance OD_{600}
Distilled water with DiMethyl Sulf Oxide (negative control)	7.00 a	3.35 d
Allium sativum (AS)	7.00 a	3.30 d
Aloe Vera (AV)	7.00 a	3.30 d
Azadiracta indica (AI)	7.00 a	3.01 cd
Bidens pilosa (BP)	7.00 a	3.11 cd
Capsicum frutescens (CF)	7.00 a	3.05 cd
Carica papaya (CP)	7.00 a	3.27 d
Persea americana (PA)	7.00 a	3.15 cd
Solanum lycopersicum (SL)	7.00 a	3.26 d
Tetradenia riparia (TR)	7.00 a	3.22 d
Leaves of*Ricinus communis* (RC)	16.33 b	2.12 b
Amoxicillin (positive control)	19.33 c	1.65 b
Tetracycline (positive control)	33.67 d	0.14 a

Means followed by the same letter are not significantly different according to the Tukey's test at 0.05

Table 2 Inhibition zone (mm) for the 19 methanolic plant extracts, assessed at IITA Kalambo in eastern DR Congo

Treatment	Inhibition zone (mm)
Distilled water with DiMethylSulfOxide(negative control)	7.00 a
Conyza sumatrensis (CS)	7.00 a
Solanum aculeastrum (SA),	7.00 a
Bidens pilosa (BP),	7.33 a
Leaves of Ageratum conyzoides (ACl)	7.00 a
Lantana camara (LC)	9.33 ab
Vernonia amygdalina (VA)	9.67 abc
Euphorbia hirta (EHi)	10.7 abc
Lantana trifolia (LT)	12.0 bc
Citrus limon (CL)	13.00 bcd
Eucalyptus eugenoides (EE)	13.00 bcd
Psidium guajava (PG)	13.33 cd
Phyllanthus niruri (PN)	13.33 cd
Flower of Ageratum conyzoides (ACf)	16.00 de
Piper nigrum (PNi)	19.00 ef
Fruit of Ricinus communis (RCf)	19.33 ef
Tephrosia vogelii (TV)	21.67 fg
Euphorbia heterophylla (EH)	21.67 fg
Leaves of Ricinus communis (RCl)	22.00 fg
Rhizome of *Zingiber officinale* (ZO)	23.33 g
Tetracycline (positive control)	31.67 h

Means followed by the same letter are not significantly different according to the Tukey's test at 0.05

conyzoide [72]. The quantity of polyphenols in ethanol extracts of *Z. officinale* is evaluated between 297.63 mg expressed in micrograms Gallic Acid equivalent per milligram of extract (mg EAG/mg extract) and 322.11 mg EAG [63].

4 Conclusion

The study underscores the promising antibacterial potential of 5 medicinal plants commonly used in Kivu against Xvm. Notably, Z. officinale and R. communis warrant special attention, while multiple species from the Euphorbiaceae and Myrtaceae families have also shown effectiveness in inhibiting *Xvm*. All of the plant species with inhibiting properties were rich in phenol, indicating that protein denaturation could be a viable option for inhibiting the bacterium. Moving forward, further research should focus on the practical applications of these findings, e.g.in assessing the potential use of the most promising plant extracts for garden tool sterilization. In addition, various phytochemical groups present in plant extracts could be evaluated for their effectiveness in suppressing *Xvm*, especially phenols and tannins. The use of effective plant extracts, for eliminating bacteria on e.g.garden tools, can reduce or even prevent the application of chemicals, thus save-guarding environmental and human health. It can also prevent the elimination of non-target organisms.

Table 3 Result of phytochemical screening for the five most effective plant species

Plant extract	Alkaloids	Flavonoids	Phenols	Terpenoids	Saponins	Tannins
Piper nigrum (Pni)	+	+	+ + +	+	+ + +	+ + +
Fruit of *Ricinus communis* (RCf)	+ + +	+	+ + +	+	+	+ + +
Euphorbia heterophylla (EH)	–	–	+ + +	+	+ + +	+
Tephrosia vogelii (TV)	–	–	+ + +	+ + +	–	+ + +
Leaves of *Ricinus communis* (RCl)	+	+ + +	+ + +	+	+	+ + +
Rhizome of *Zingiber officinale* (ZO)	+	+	+ + +	+	–	+ + +

+ + +:abundant; + moderate;—absent

Acknowledgements This research was funded by the International Institute of Tropical Agriculture (IITA). Therefore, we extend our appreciation to the IITA team that funded the field and lab work through Canadian funds under an agreement with the Centre de Recherches pour le Développement International (CRDI).

In this study we complied with the International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and the IUCN red list index of threatened species Convention on the Trade in Endangered Species of Wild Fauna and Flora. In this study, we sourced plants from their natural habitats, while ensuring sustainable harvesting practices. No specific permissions were required, and we were committed to ethical standards and environmental responsibility in our research.

Author contribution FM: Trial Management, data collection, analysis, writing and review AI: Writing and Review, LL: PCR analysis and interpretation, review GS: Microbiology (Isolation, culture and review) DD: Preparation of extracts, phytochemical screening and review LN: Supervision of Kalambo/IITA trials and review GB: Uganda's trials supervision and review GM: Analysis, Interpretation of results and review.

Data availability The datasets generated and analyzed during the current study on "Exploring In Vitro Xvm Inhibition of the Causal agent of Banana Xanthomonas Wilt by Medicinal Plant Extracts sourced from North Kivu province, Eastern Democratic Republic of Congo" are available from the corresponding author upon reasonable request. All results relevant to the study are included in the article, and data will be uploaded as supplementary information if required. This ensures transparency and supports the reproducibility of our findings. For further inquiries regarding data access, please contact the Corresponding Author. Sincerely, MukeshambalaFranchement, Department of Crop Sciences, Université de Goma, P.O. Box 204 Goma, North Kivu Province, Democratic Republic of Congo Uganda. Email: franckmukeir@gmail.com (corresponding author).

Declarations

Ethics approval and consent to participate We confirm that this manuscript has not been published previously in any peer-reviewed journal, and it is not being considered for publication elsewhere. All co-authors have read and approved the final version of the manuscript.

Competing interests The authors declare no competing interests.

References

1. Marimo P, Caron C, Van den Bergh I, Crichton R, Weltzien E, Ortiz R, Tumuhimbise R. Gender and trait preferences for banana cultivation and use in Sub-Saharan Africa: a literature review. Afr Econ Bot. 2020;74(2):226–41.
2. Karamura DA, Karamura EB, TinzaaraW,. Banana cultivar names, synonyms and their usage in Eastern Africa. Kampala: Bioversity International; 2012.
3. Bezerra JA, Roewer SP, Andrade KL. Avaliação Do Conhecimento Sobre O Uso Da Banana Prata (*Musa* Spp.) Na HidrataçãoCapilar. REI. 2020;12(1):1–12.
4. FAOstat 2019. Crops. http://www.fao.org/faostat/en/#data/QC. Accessed 16 June 2020.
5. Mobambo P, Staver C, Hauser S, Dheda B, Vangu G. An innovation capacity analys is to identify strategies for improving plantain and banana (*Musa* spp.) productivity and value addition in the Democratic Republic of Congo. Acta Hortic. 2010;879:821–8.
6. Tinzaara W, Ssekiwoko F, Kikulwe E, Karamura E. Effectiveness of learning and experimentation approaches for farmers as a community-based strategy for banana Xanthomonas wilt management. J Agric Ext Rural Dev. 2019;11(7):128–38.
7. Blomme G, Ocimati W, Amato S, zum Felde A, Kamira M, Bumba M, Bahati L, Amini D, Ntamwira J. Banana pestris assessment a long banana trade axes running from low to high altitude sites, in the Eastern DR Congo and in Burundi. Afr J Agric Res. 2020;16(9):1253–69.
8. Blomme G, Dita M, Jacobsen KS, Pérez Vicente L, Molina A, Ocimati W, Poussier S, Prior P. Bacterial diseases of bananas and enset: current state of knowledge and integrated approaches toward sustainable management. Front Plant Sci. 2020;8:1290. https://doi.org/10.3389/fpls.2017.01290.
9. Ocimati W. Sustainability of banana-based agroecosystems affected by Xanthomonas wilt disease of banana, Thesis, Wageningen University and Research. 2019. p. 240.
10. Yirgou D, Bradbury JF. Bacterial wilt of Enset (*Ensete ventricosum*) incited by Xanthomonas musacearum sp. Phytopathology. 1968;58:111–2.
11. Yirgou D, Bradbury JF. A note on wilt of banana caused by the enset wilt organism *Xanthomonas musacearum*. East Afr Agr For J. 1974;40:111–4.
12. Tushemereirwe W, Kangire A, Smith J, Ssekiwoko F, Nakyanzi M, Kataama D, Musiitwa C, Karyeija R. An outbreak of bacterial wilt on banana in Uganda. Info Musa. 2003;13:44–5.
13. Tushemereirwe W, Kangire A, Ssekiwoko F, Offord LC, CrozierJ BE, Rutherford M, Smith J. First report of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. Plant Pathol. 2004;53:802.

14. Ndungo V, Bakelana K, Eden-Green S, Blomme G. An outbreak of banana Xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum*) in the Democratic Republic of Congo. Info Musa. 2004;13:43–4.
15. Carter BA, Reeder R, Mgenzi SR, Kinyua ZM, Mbaka JN, Doyle K, Nakato V, Mwangi M, Beed F, Aritua V, Lewis-Ivey ML, Miller SA, Smith JJ. Identification of Xanthomonas vasicola (formerly *X. campestris* pv *musacearum*), causative organism of banana Xanthomonas wilt, in Tanzania, Kenya and Burundi. Plant Pathol. 2010;59:403–403.
16. Nakato V, Mahuku G, Coutinho T. Xanthomonas campestris pv musacearum:a major constraint to banana, plantain and enset production in central and east Africa over the past decade. Mol Plant Pathol. 2018;19(3):525–36.
17. Petsakos A, Kozicka M, Blomme G, Nakakawa JN, Ocimati W, Gotor E. The potential impact of banana Xanthomonas wilt on food systems in Africa: modeling scenarios of policy response and disease control measures. Front Sustain Food Syst. 2023;7:1207913. https://doi.org/10.3389/fsufs.2023.1207913.
18. Tripathi L, Mwangi M, Aritua V, Tushemereirwe W, Abele S, Bandyopadhyay R. Xanthomonas wilt, a threat to Banana Production in East and Central Africa. Plant Dis. 2009;93(5):440–51.
19. Studholme D, Wicker E, Abrare S, Aspin A, Broders K, Dubrow Z, Grant M, Jones J, Karamura G, Lang J, Leach J, Mahuku G, Nakato G, Coutinho T, Bull C. Transfer of *Xanthomonas campestris* pv. *arecae* and *X. campestris* pv. *musacearum* to *X. vasicola* (Vauterin) as *X. vasicola* pv. *arecaecomb*. nov. and *X. vasicola* pv. *musacearumcomb*. nov. and description of *X. vasicola* pv. *vasculorum* pv. nov. Phytopathology. 2018;110(6):1153–60.
20. Haile B, Adugna G, Handoro F. Physiological characteristics, and pathogenicity of *Xanthomonas campestris* pv. *musacearum* strains collected from enset and banana in Southwest Ethiopia. Afr J Biotechnol. 2014;13(24):2425–34.
21. Adriko J, Aritua V, Mortensen N, Tushemereirwe W, Kubiriba J, Lund S. Multiplex PCR for specific and robust detection of *Xanthomonas campestri* spv. musacearum in pure culture and infected plant material. Plant Pathol. 2012;61:489497.
22. Biruma M, Pillay M, Tripathi L, et al. Banana Xanthomonas wilt: a review of the disease, management strategies and future research directions. Afr J Biotech. 2007;6:953–62.
23. Tahat MM, Sijam K. *Ralstonia solanacearum*: the bacterial wilt causal agent. Asian J Plant Sci. 2010;9(7):385–93.
24. Aytenfsu M, Haile B. Distribution and management of bacteria l Wilt (*Xanthomonas campestris* pv. *musacearum*) of Ensete (*Ensete ventricosum*) in Ethiopia: a review. Int J Res Agric For. 2020;7(2):40–53.
25. Blomme G, Dusingizimana P, Ntamwira J, Kearsley E, Gaidashova S, Rietveld A, Schagen BV, Ocimati W. Comparing effectiveness, cost-and time-efficiency of control options for Xanthomonas wilt of banana under Rwandan agro-ecological conditions. Eur J Plant Pathol. 2021;160:487–501.
26. Yemata G, Fetene M. In vitro evaluation of the antibacterial activity of some medicinal plant extract sagainst *Xanthomonas campestri* spv. *musacearum*. Ethiop J Sci Technol. 2017;10(1):14–32.
27. Yemata G, Fetene M.*In vitro* antibacterial activity of some antibiotics against *Xanthomonas campestris* pv *musacearum*:*Ensete ventricosum* (Welw) Cheesman Pathogen Journal of Phytopathology and Pest Management 2017b; 4 (1) : 48–57.
28. Yemata G, Desta B, Fetene M. In vitro antibacterial activity of traditionally used medicinal plants against *Xanthomonas campestris* pv. *musacearum* in Ethiopia. Biodiversitas. 2019;20(2):555–61. https://doi.org/10.13057/biodiv/d200235.
29. Shalukoma C, Bogaert J, Duez P, Stévigny C, Pongombo C, Visser M. Les plantes médicinales de la région montagneuse de Kahuzi-Biega en République démocratique du Congo : utilisation, accessibilité et consensus des tradipraticiens. Bois Et For Des Trop. 2015;326(4):43–55.
30. Masengo CA, Ngbolua JP, Butoto B, Gbolo B, Inkoto C, Mpiana P. Ethno-botanical survey of medicinal plants species traditionally used for treatment of diseases in Kasangulu Territory, DRCongo, Moroccan. J Agric Sci. 2023;4(2):76–85.
31. Konieczynski P, Zarkov A, Viapiana A, Chrubczynska EM, Wesolowski M. Studies on the chemical composition of plants used in traditional medicine in Congo. Open Chem. 2022;20:370–8.
32. Fundiko FC, Biduaya FM, Mwassa A, Mokoso M. Antibacterial activities of 13 medicinal plants used against infectious and parasitic diseases in Kinshasa and its surroundings, D.R. Congo. Int J Biol Pharm Sci Arch. 2022;3(2):39–47.
33. Bashige C, Bakari A, Okusa N, Kahumba B, Duez P, Lumbu S. Ethnobotanical study of plants used as antimalarial in traditional medecine in Bagira in Easter R.D. Congo. J Pharmacogn Phytochem. 2020;9(4):01–14.
34. Kaboyi G, Imani B, Ndabaga C, Gendusa P, Kang JW. Quantitative ethnomedicinal study of plants used by the population in Middle plateaux of Uvira territory (D.R. Congo). For Sci Technol. 2021;17(3):144–54. https://doi.org/10.21203/rs.3.rs-291478/v1.
35. Syamasamba M, Kapiri M, Muhesi K, Mbayahi K, Mavinga M. Ethnobotanical study of plants used by traditherapists for the treatment of malaria in city of Butembo, North Kivu, East of the Democratic Republic of Congo. IJIAS. 2022;2(3):219–33.
36. Kasrina K, Zukmadini AY. Ethnobotany study of medicinal plants in Bengkulu as medium of student learning: the Euphorbiaceae family. J Phys: Conf Ser. 2021;1731:1–9.
37. Sharma M, Shama M, Bithel N, Sharma M. Ethnobotany, phytochemistry, pharmacology and nutritional potential of medicinal plants from Asteraceae family. J Mountain. 2022;17(2):67–83.
38. Saber RF, Munekata PE, Rizwan K, El-Nashar H, Fahmy NM, Aly SH, El-Shazly M, Bouyahya A, Lorenzo JM. Family Myrtaceae: the treasure hidden in the complex/diverse composition. Crit Rev Food Sci Nutr. 2023. https://doi.org/10.1080/10408398.2023.2173720.
39. Smail A, Mourad A, Miloudia S, Mohamed E. Traditional uses, pharmacological, and phytochemical studies of Euphorbia: a review. Curr Top Med Chem. 2022;22(19):1553–70. https://doi.org/10.2174/1568026622666220713143436.
40. Nakakawa J, Mugisha J, Karamura E. Banana Xanthomonas wilt dynamics with mixed cultivars in a periodic environment. Int J Biomath. 2020;13(1):2050005.
41. Haile B, Fininsa C, Terefe H, Chala A, Hussen S. Evaluation of enset (*Ensete ventricosum*) clones for resistance reaction against pathogenic *Xanthomonas campestris* pv. *musacearum* isolates from southwester Ethiopia. Food Agric. 2020;6:1773094.
42. Sutton S. Accuracy of platecounts. J Valid Technol. 2011;17(3):42–6.
43. Mahuku GS. A simple extraction method suitable for PCR-based analysis, fungal and bacterial DNA. Plant Mol Biol Rep. 2004;22:71–81.
44. Balagizi I, Vayire K, Rati E. Les plantes médicinales du Kivu. Gênes: Associzione San Francesco; 2021.
45. Korangi V, Kubindana G, Fingu-Mabola J, Sulu A, Kasereka G, Matamba A, Ndindir J. Utilisation des biopesticides pour une agriculture durable en République Démocratique du Congo (Synthèse bibliographique). Revue Afr Environ Agric. 2021;02:53–67.

46. Chuma GB, Mondo JM, Ndeko AB, Bagula EM, Lucungu PB, Bora FS, Karume K, Mushagalusa GN, Schmitz S, Bielders C. Farmers' knowledge and practices of soil conservation techniques in smallholder farming systems of northern Kabare. Environ Chall. 2022. https://doi.org/10.1016/j.envc.2022.100516.
47. Bashagaluke J, Pieter P, Walangululu J, Shepherd K, Mochoge B, Mugwe J, Boeckx P. Application of infrared technique in soil properties characterization in South Kivu prince of D.R. of Congo. Afr J Food Sci Technol. 2015;6(2):2141–5455.
48. Shrestha T, Lamichhane J. Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of *Tinospora cordifolia* (Gurjo), Nepal. J Biotechnol. 2021;9(1):18–23. https://doi.org/10.3126/njb.v9i1.38646.
49. Nasser K. Utilization of fungal and bacterial endophytes for the management of banana nematode *Radopholus similis* in East African Highland banana, thesis, Makerere University. 2023.
50. Harbone JB. Phytochemical methods. London: Chapman and Hall; 1983.
51. Okeniyi SO, Adedoyin BJ, Garba S. Phytochemical screening, cytotoxicity. Antioxidant and antimicrobial activities of stem and leave extracts of euphorbia Heterophylla. J Biol Life Sci. 2013;4:87.
52. Patle T, Shrivas K, Kurrey R, Upadhyay S, Jangde R, Chauhan R. Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna*using UV–vis and FTIR spectroscopy. Spectrochim Acta Part A Mol Biomol Spectr. 2020;242:118717. https://doi.org/10.1016/j.saa.2020.118717.
53. Sharma Y, Velamuri R, Fagan J, Schaefer J, Streicher C, Stimson J. Identification and characterization of polyphenols and volatile terpenoid compounds in different extracts of garden sage (*Salvia officinalis* L.). Pharmacogn Res. 2020;12(2):149–57.
54. Muharrami L, Munawaroh F, Ersam T, Santoso M. Phytochemical screening of ethanolic extract: a preliminary test on five medicinal plants on Bangkalan. Jurnal Pena Sains. 2020;7(2):96–102.
55. Uwamahoro F, Bucagu C, Berlin A, Bylund H, Yuen J. Host Range and banana cultivars' susceptibility to *Xanthomonas campestris* pv. *musacearum* in Rwanda. Afr Crop Sci J. 2019;27(3):463–78. https://doi.org/10.4314/acsj.v27i3.10.
56. Nakato V, Wicker E, Coutinho A, Mahuku G, Studholme D. A highly specific tool for identification of *Xanthomonas vasicola* pv. *musacearum* based on five Xvm-specific coding sequences. Heliyon. 2018. https://doi.org/10.1016/j.heliyon.2018.e01080.
57. Hodgetts J, Hall J, Karamura G, Grant M, Studholme D, Boonham N, Karamura E, Smith J. Rapid, specific, simple, in-field detection of *Xanthomonas campestris* pv. *musacearum* by loop-mediated isothermal amplification. J Appl Microbial. 2015;119:1651e1658.
58. Oueslati S, Gharsalli W, Abdelkarim M, Ben Aissa-Fennira F, Ksour R. Biochemical evaluation and exploration of the antioxidant, antibacterial and anticancer potential of*Zingiber officinale*. J New Sci. 2018;54:3561–8.
59. Valle SB, Ribeiro RS, Silva V, Martins TS, Pereira SS. Avaliação Da Extração Por Solventes Para Obtenção Do Extrato De Espinafre (Tetragonia Tetragonioides), Congresso Brasileiro de engenharia quimica eminiciçao cientifica, Uberlandia. 2019.
60. Chen XM, Ma Z, Kitts DD. Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves. Food Chem. 2018;249:143–53.
61. Muhesi K, Betti L, Din N, Kapiri M, Afiong H, Billong P. Ethnobotanical knowledge of *Prunus africana* (Hook f.) Kalkman (Rosaceae) by people living in community forests in North Kivu, Eastern Democratic Republic of Congo. Ethnobot Res Appl. 2023;26(12):1.
62. Sebiomo A, Awosanya A, Awofodu A. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. J Microbiol Antimicrob. 2011;3(1):18–22.
63. Ranjan RK, Bimala R, Jha PK. In vitro screening of plant extract to control bacterial leaf blight of rice disease caused by *Xhanthomonas oryzae* pv *oryzae*. Environ Ecol. 2017;35(2):1348–53.
64. Yousfi F, Abrigach F, Petrovic JD, Sokovic M, Ramdani M. Phytochemical screening and evaluation of the antioxidant and antibacterial potential of *Zingiber officinale* extracts. S Afr J Bot. 2021;2021(142):433–40.
65. Alkandarhi M, Kusumati A, Fikayuniar,. Antibacterial Activity of *Zingiber officinale* Rhizome. Int J Psychol Rehabil. 2020;24(7):3701–6.
66. Mabhiza D, Chitemerere T, Mukanganyama S. Antibacterial properties of alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Int J Med Chem. 2016;1(8):1.
67. Ewané CA, Lepoivre P, De Lapeyre de Bellaire L, Lassois L. Involvement of phenolic compounds in the susceptibility of bananas to crown rot, a review. Biotechnol Agron Soc Environ. 2012;16(3):393–404.
68. Lassois L, Frettinger P, De Lapeyre L, Lepoivre P, Jijakli H. Identification of genes involved in the response of banana to crown rot disease. Mol Plant Microbe Interact. 2011;24(1):143–53.
69. Juariah S, Bakar FI, Bakar MF, Endrini S, Kartini S, Mohamad A, Hanafi AF. Antibacterial activity of red ginger (*Zingiber officinale* var. *rubrum*) and black turmeric (*Curcuma caesia*) extracts as growth inhibitors of *Klebsiella pneumonia*. Trop J Nat Prod Res. 2023;7(6):3658–65.
70. Anitha G, Sudarsanam G. Studies on antimicrobial activity of *Tephrosia calophylla* against *Xanthomonas citri* and *Salmonella typhimurium*. Int J Appl Biol Pharm Technol. 2013;4(1):339.
71. Nahak G, Sahu R. Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. J Appl Pharm Sci. 2011;1(08):153–7.
72. Amadi BA, Duru MK, Agomuo EN. Chemical profiles of leaf, stem, root and flower of *Ageratum conyzoides*. Asian J Plant Sci Res. 2012;2(4):428–32.