

# Exploring In Vitro Inhibition of the Causal agent of Banana Xanthomonas Wilt by Medicinal Plant Extracts sourced from North Kivu province, Eastern Democratic Republic of Congo.

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## Research Article

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# Abstract

Banana Wilt caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*), has emerged as a significant threat to food security in eastern DR 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 Kivu in inhibiting of *Xvm*. Three in vitro experiments were conducted at Uganda's National Agriculture Research Organization (NARO) laboratories and the International Institute of Tropical Agriculture (IITA) in Kivu. The bacterial samples were collected in banana cultivated in Kivu and isolated on Yeast Extract Peptone Agar (YPGA). Pure *Xvm* colonies were used for identification via Polymerase Chain Reaction (PCR) and greenhouse inoculation trials. A completely randomized design was used for the inhibition tests (1) on Mueller Hinton Agar (MHA) using disc diffusion with 10 extracts, (2) in liquid YPG Broth, and (3) on MHA using disc diffusion with 19 extracts. The first two trials used ten plant species extracts diluted in petroleum ether, while the third trial used 19 plant extracts diluted in methanol. After maceration, filtration, and solvent evaporation, 10mg of extract was diluted in 80 µl of distilled water + 10µl 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.

## 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 DR Congo alone, yielding 5.1 million tons [4]. Unfortunately, banana production has decreased by 20 to 60% in the last few decades in the DR Congo [5], this due to abiotic and biotic constraints (pests and diseases) [6]. The biotic constraints primarily include nematodes and weevils as pests, xanthomonas wilt, banana bunch top disease, 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 was also reported in central Uganda and eastern DR Congo. By 2010, Rwanda, Kenya, and Burundi had also reported cases [12, 13, 14, 15, 16].

[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%.

The responsible of banana bacterial wilt, also known as *Xanthomonas vasicola* pv. *musacearum* (*Xcm*), is a serious threat to the food security of the Great Lakes region [16, 18, 19]. 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 KOH, and does not reduce nitrate [20]. To date, the molecular characterization of the bacterium primarily uses the Primer coding for the general secretory protein D (GspDm), amplifying the 265-bp fragment of *XvmgspD* [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, 23, 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. [26, 27, 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 the bacterium responsible for bacterial wilt in Ensete.

There is great potential for plant extract-based treatments, as shown in ethnobotanical research conducted in DR Congo [29, 30], e.g., in Kinshasa and Kasangulu [30, 31, 32], and in North and South Kivu [33, 34, 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 properties [36, 37, 38]. These effects are attributed to the presence of an estimated 104 chemical components, including glycosidic, phenolic acids, coumarins, monoterpenoids, triterpenoids, diterpenoids, flavonoids, and 4 $\alpha$ -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 species commonly used by traditional practitioners in Eastern Democratic Republic of Congo.

## 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, while the second was the laboratory at the International Institute of Tropical Agriculture (IITA) Olusegun Obasanjo campus in Kalambo/Bukavu, eastern DR Congo.

### Preparation of Inoculum

To isolate the bacterium, a portion of the pseudostem was collected from a diseased plant showing characteristic symptoms of bacterial wilt [40]. The collected organ was cleaned with 70% ethanol and a smaller 4 cm<sup>2</sup> 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 hours. 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<sup>8</sup> CFU Colony-Forming Units per ml (CFU/ml).

#### Extraction of Desoxyribose Nucleic Acid (DNA)

As per [43] protocol, the DNA extraction process involved purifying bacterial cells through centrifugation of colonies in a 1 ml tube at 6000 rpm for 10 minutes in TES buffer: Tris, Ethylene-Diamine-Tetraacetic 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 250µl of 7.5% concentrated ammonium acetate solution to the mixture, followed by keeping it on ice for 10 minutes and then centrifuging it at 13,000 rpm for 10 minutes. 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).

#### Polymerase Chain Reaction (PCR)

The purified nucleic acid was used to identify the *Xcm* strain using the method described by [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 10min; (ii) 25 cycles of denaturation at 94 °C for 30s, annealing at 60 °C for 90s, elongation at 72 °C for 90 s; then (iii) a final extension step at 60 °C for 30min. 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.

#### Inoculation of Shoots

For confirmation of *Xvm* identity, 1 ml of a solution containing 10<sup>8</sup> CFU/ml was injected into the main vein of the leaf of 60-days-old banana plants of the AAA-EAH cooking banana “Mbuzirume”. 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 hours, 10 days, and thereafter every 15 days after inoculation [16].

#### Inhibition Test

The inhibition test of *Xcm* was performed using perforated discs of sterile Whatman filter paper. These discs were soaked, for an hour, with 10µl of a solution containing 10 mg of plant extract, 10µl of Dimethyl Sulfoxide (DMSO), and 80 µl of sterile distilled water. Preparation of plant extracts involved diluting 62.5 g of dried plant powder in 250 ml of solvent (petroleum ether or methanol), followed by maceration, filtration, and solvent evaporation [44]. The viscous extract obtained was weighed and stored in sterile plastic bottles of 50 ml volume in the refrigerator at 4°C or immediately used for inhibition.

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 [45].

### In vitro Experiments

Two in vitro trials were conducted at the NARO to test 10 Petroleum Ether extracts of medicinal plants that are traditionally used in Kivu's medicine for human bacterial infections. These 10 plant species were collected between 9 a.m. and 3 p.m. in the vicinity of the Kalambo IITA research center in D.R.Congo after the disappearance of dew. The species were: *Allium sativum* (Liliaceae) AS, *Aloe Vera* (Xanthorrhoeaceae) AV, *Azadiracta indica* (Meliaceae) AI, *Bidens pilosa* (Asteraceae) BP, *Capsicum frutescens* (Solanaceae) CF, *Carica papaya* (Caricaceae) CP, *Persea americana* (Lauraceae) PA, *Ricinus communis* (Euphorbiaceae) RC, *Solanum lycopersicum* (Solanaceae) SL, *Tetradenia riparia* (Lamiaceae) TR [46, 47].

These plant species were sent to the research center in Lwiro, South Kivu, for species identification. In the initial 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) was 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, 1 tetracycline, and 1 distilled water + DMSO) in 3 replications. The second in vitro experiment carried out at the NARO, Uganda 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). 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].

At the IITA Kalambo research facility an additional trial was conducted to test inhibition of a list of plants known for their antibiotic properties collected in South Kivu [40, 41]. The test involved 19 methanolic extracts of the following plant species: *Bidens pilosa* (Asteraceae) BP, *Citrus limon* (Rutaceae) CL, *Conyza sumatrensis* (Asteraceae) CS, *Eucalyptus eugenoides* (Myrtaceae) EE, *Euphorbia heterophylla* (Euphorbiaceae) EH, *Euphorbia hirta* (Euphorbiaceae) EHi, Flower of *Ageratum conyzoides* (Asteraceae) ACf, Fruit of *Ricinus communis* (Euphorbiaceae) RCf, *Gymnathemum amygdalinum* (Vernionia

*amygdalina*: Ndole) (Asteraceae) VA, *Lantana camara* (Verbenaceae) LC, *Lantana trifolia* (Verbenaceae) LT, Leaves of *Ageratum conyzoides* (Asteraceae) ACI, Leaves of *Ricinus communis* (Euphorbiaceae) RCI, *Phyllanthus niruri* (Euphorbiaceae) PN, *Piper nigrum* (Piperaceae) PN<sub>i</sub>, *Psidium guajava* (Myrtaceae) PG, *Solanum aculeastrum* (Solanaceae) SA, *Tephrosia vogelii* H. (Fabaceae) TV, rootstock of *Zingiber officinale* (Zingiberaceae) ZO [46, 47]. The experiment was conducted using a completely randomized design with 21 treatments, which included 19 plant extracts, 1 tetracycline, and 1 distilled water + DMSO. 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.

### Phytochemical Screening

For Alkaloids test, 20 mg was dissolved in 2% HCl and filtered with 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. Brown solution was an indication for the presence of alkaloids [28, 48, 49, 50]. For flavonoids screening, 0.5 g was taken from plant extract and placed in test tubes. 10 ml of distilled water was added to test tube and mixed by shaking and filtered. Then 3 ml of the aqueous filtrate was mixed with 5 ml of ammonia (10%) added with 1 ml of concentrated sulphuric acid, which resulted in the formation of a yellow color for a positive test of flavonoids [48, 51]. To quantify phenolic compounds 2 ml of the extract were mixed with 3–4 drops of 5% Ferric Chloride (FeCl<sub>3</sub>) solution. The formation of black color indicated the presence of phenols [28, 48, 51].

For terpenoids screening, 0.5 g of plant powder was filled in test tubes, the 10 ml of methanol was added and centrifuged. 5 ml of the supernatant was mixed with 2 ml of chloroform added with 3 ml of sulfuric acid. The formation of the brown layer between the two solutions was confirmed the presence of terpenoids [48, 52]. Test for Saponins was completed with 1 gram of extract boiled in 5 ml of distilled water, and shaken vigorously for five minutes. The persistence of the frothing confirmed the presence of saponin [48, 53]. For Tannins screening, 0.2 grams was dissolved in 5 ml of distilled water, heated in a water bath and filtered. Then 2 ml of 5% ferric chloride solution was added to 1 ml of the filtrate. The blue color meant positive test of tannins [48, 53].

## Results and Discussion

### The PCR Results

The PCR results as illustrated 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 [54], indicating the positive presence of *Xvm* in these samples. Thus, the bacterium used in these 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 bacterial wilt [55]. 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 [56].

The first disease symptoms appeared 7 days after inoculation, with yellowing of the first/inoculated leaf as illustrated in Figure 2b. The second leaf showed symptoms after 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, symptoms appeared earlier on the plantlets compared to other studies. This could be due to the fact that the *Xvm* bacterium was more active in the high-altitude environment (1463 meters above sea level) with temperatures between 14° and 25°C as noted in [57] where the experiment was conducted. The bacterium only took 24 hours to grow in vitro on YPGA at Kalambo, whereas it took 72 hours at Kawanda in Uganda with temperatures are between 17° and 31°C as noted in Climate to Travel, [58]. The timing of symptom appearance was similar to the results of [54], who found that the first symptoms appeared as early as 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 development of bacterial wilt, which occurred from day 8 to day 47 in this experiment, could be explained by the fact that the tested variety was the susceptible highland cooking banana genotype ‘Mbwazirume’ (*Musa* AAA-EAH).

Plant extract yield by selected plant species.

In the first and second experiments, utilizing petroleum ether as a solvent, as shown in Figure 3, *Aloe vera* (9.44%), *Capsicum frutescens* (8.64%), and leaves of *Ricinus communis* (6.88%) exhibited high yields in plant extracts, while in the third experiment, using methanol as a solvent, illustrated in Figure 4, *Psidium guajava* (12.09%), leaves of *Ricinus communis* (10.72%), and *Phyllanthus niruri* (9.12%) demonstrated high productivity in plant extracts. The yield of plant extracts is influenced by various factors, including the type of solvent used and the duration of extraction [59]. 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% (Figures 3 and 4) for all species studied when using a single maceration process. This was mainly due to the lack of specialized equipment for solvent recycling after evaporation. On the other hand, when a solvent recycling apparatus like Soxhlet was used, the extraction process was optimized, which took several days, and the extract yield increased to over 40% [60]. It is important to note that the yield of plants varies due to the age of leaves and plants, as well as environmental factors [61].

Inhibition and absorbance of plant extracts with petroleum ether at NARO Kawanda, Uganda.

Table 1: Zone of inhibition and absorbance (mm) for plant extracts assessed at NARO in Uganda.

| Treatments                              | Inhibition zone (mm) | Absorbance OD <sub>600</sub> |     |
|-----------------------------------------|----------------------|------------------------------|-----|
| Negative control                        | 7.00 a               | 3.35                         | d   |
| <i>Allium sativum</i> <b>AS</b> ,       | 7.00 a               | 3.30                         | d   |
| <i>Aloe Vera</i> <b>AV</b> ,            | 7.00 a               | 3.30                         | d   |
| <i>Azadiractaindica</i> <b>AI</b> ,     | 7.00 a               | 3.01                         | c d |
| <i>Bidens pilosa</i> <b>BP</b> ,        | 7.00 a               | 3.11                         | c d |
| <i>Capsicum frutescens</i> <b>CF</b> ,  | 7.00 a               | 3.05                         | c d |
| <i>Caricapapaya</i> <b>CP</b> ,         | 7.00 a               | 3.27                         | d   |
| <i>Perseaamericana</i> <b>PA</b> ,      | 7.00 a               | 3.15                         | c d |
| <i>Solanum lycopersicum</i> <b>SL</b> , | 7.00 a               | 3.26                         | d   |
| <i>Tetradeniariparia</i> <b>TR</b>      | 7.00 a               | 3.22                         | d   |
| <i>Ricinus communis</i> <b>RC</b> ,     | 16.33 b              | 2.12                         | b   |
| Amoxiciline                             | 19.33 c              | 1.65                         | b   |
| Tetracycline                            | 33.67 d              | 0.14                         | a   |

The means followed by the same letter are not different according to the Tukey test at 0.05.

The above results in table 1, indicate that only the extract of *Ricinus communis*'s leaves had antibacterial activity. The other tested species did not show any antibacterial activity. According to [28], the phytochemical groups present in *Ricinus communis* allow it to act differently on gram-negative bacteria species like *Xcm*. 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. [28] observed an inhibition zone ranging from approximately 9 to 21 mm in leaves of *R. communis* on the same bacterium in Ethiopia, depending on the different concentrations used. However, as reported by [27], antibiotics are more effective than leaves of *R. communis* in inhibiting *Xvm* Bacteria.

Inhibition of the 19 methanolic extracts at IITA Kalambo in eastern DR Congo.

Table 2 : Zone of inhibition and absorbance (mm) for methanolic extracts.

| Treatments                                        | Inhibition zone (mm) |
|---------------------------------------------------|----------------------|
| Negative control                                  | 7.00 a               |
| <i>Conyza sumatrensis</i> <b>CS</b>               | 7.00 a               |
| <i>Solanum aculeastrum</i> <b>SA</b> ,            | 7.00 a               |
| <i>Bidens pilosa</i> <b>BP</b> ,                  | 7.33 a               |
| Leaves of <i>Ageratum conyzoides</i> <b>ACI</b>   | 7.00 a               |
| <i>Lantana camara</i> <b>LC</b>                   | 9.33 a b             |
| <i>Vernonia amygdalina</i> <b>VA</b>              | 9.67 a b c           |
| <i>Euphorbia hirta</i> <b>EHi</b>                 | 10.7 a b c           |
| <i>Lantana trifolia</i> <b>LT</b>                 | 12.0 b c             |
| <i>Citrus limon</i> <b>CL</b>                     | 13.00 b c d          |
| <i>Eucalyptus eugenoides</i> <b>EE</b>            | 13.00 b c d          |
| <i>Psidium guajava</i> <b>PG</b>                  | 13.33 c d            |
| <i>Phyllatusniruri</i> <b>PN</b>                  | 13.33 c d            |
| Flower of <i>Ageratum conyzoides</i> <b>ACf</b> , | 16.00 d e            |
| <i>Piper nigrum</i> <b>PNi</b>                    | 19.00 e f            |
| Fruit of <i>Ricinus communis</i> <b>RCf</b>       | 19.33 e f            |
| <i>Tephrosia vogelii</i> <b>TV</b>                | 21.67 f g            |
| <i>Euphorbia heterophylla</i> <b>EH</b>           | 21.67 f g            |
| Leaves of <i>Ricinus communis</i> <b>RCI</b>      | 22.00 f g            |
| Rootstock of <i>Zingiber officinale</i> <b>ZO</b> | 23.33 g              |
| Tetracycline                                      | 31.67 h              |

The means followed by the same letter are not different according to the Tukey test at 0.05.

The experiment conducted at IITA Kalambo using plant species collected in the Kivu region has expanded the list of medicinal plants from Kivu that are effective in controlling *Xvm*. Previously, only one extract, leaves of *Ricinus communis* RC as shown in Table 1, was known to be effective, but now 13 plants have been found to be effective (Table 2). Results in Table 2, indicate that the most effective plant was the extract of rootstock of *Zingiber officinale* ZO, which is locally known as Ginger. According to the Tukey test at 0.05, it was observed that the species of *R. communis* for leaves, *T. vogelii* and *E. heterophylla* exhibited a similar inhibition zone comparable to that of ZO (Table 2). Additionally, Three

other plant species, namely *Tephrosia vogelii* and *Ricinus communis* (for its Fruit), displayed an inhibition zone similar to RCI, but distinct from that of ZO (Table 2). The rhizomes of this ZO plant are commonly used in the region as part of therapeutic beverages [62]. According to [63], *Zingiber officinale* (ginger) is as effective as the antibiotic streptomycin in inhibiting *Xanthomonas oryzae* *pv.* *Oryzae* [64] also found that ginger is more effective in inhibiting certain pathogenic bacteria (30mm diameter of inhibition) compared to antibiotics and could be used for developing broad-spectrum antibiotics. 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 [65]. 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 [66].

#### Phytochemical screening of the five most effective plant species

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

| Plant extract                                     | Alkaloids | Flavonoides | Phenolic | Terpenoids | Saponins | Tannins |
|---------------------------------------------------|-----------|-------------|----------|------------|----------|---------|
| <i>Flower of A.conyzoides</i> <b>ACf</b>          | +++       | +           | +++      | +++        | +++      | +       |
| <i>Piper nigrum</i> <b>PNi</b>                    | +         | +           | +++      | +          | +++      | +++     |
| <i>Frut of Ricinus communis</i> <b>RCf</b>        | +++       | +           | +++      | +          | +        | +++     |
| <i>Euphorbia heterophylla</i> <b>EH</b>           | -         | -           | +++      | +          | +++      | +       |
| <i>Tephrosia vogelii</i> <b>TV</b>                | -         | •           | +++      | +++        | -        | +++     |
| <i>Leaves of Ricinus communis</i> <b>RCI</b>      | +         | +++         | +++      | +          | +        | +++     |
| <i>Rootstock of Zingiber officinale</i> <b>ZO</b> | +         | +           | +++      | +          | -        | +++     |

+++ : Abundant ; + Moderate ; - Absent

Results presented in Table 3, indicate that all effective plant species found in this research share a rich amount of molecules from the phenol group, while molecules from the tannin and terpenoid groups are present in varying quantities. Phenol is a polar compound with antibacterial properties. Its mechanism of action involves denaturing bacterial cell proteins, which halts all protein-catalyzed metabolic activities [67]. Given that the antibiotic tetracycline used effectively inhibited this bacterium by also targeting proteins, it seems likely that phenol is one of the major compounds involved in this inhibition. Similar results to this research were obtained by [65] for *Z. officinale*; [28] for *R. communis*; [49] for *E. heterophylla*; [50] for *T. vogelii*; [68] for *P. nigrum*, and [69] for the flowers of *A. conyzoides*

## Conclusion

The study underscores the promising antibacterial potential of 6 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*. It's noteworthy that all of the plants 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. to sterilize garden tools.

## Statements and Declarations

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.

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### 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

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## Figures



**Figure 1**

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

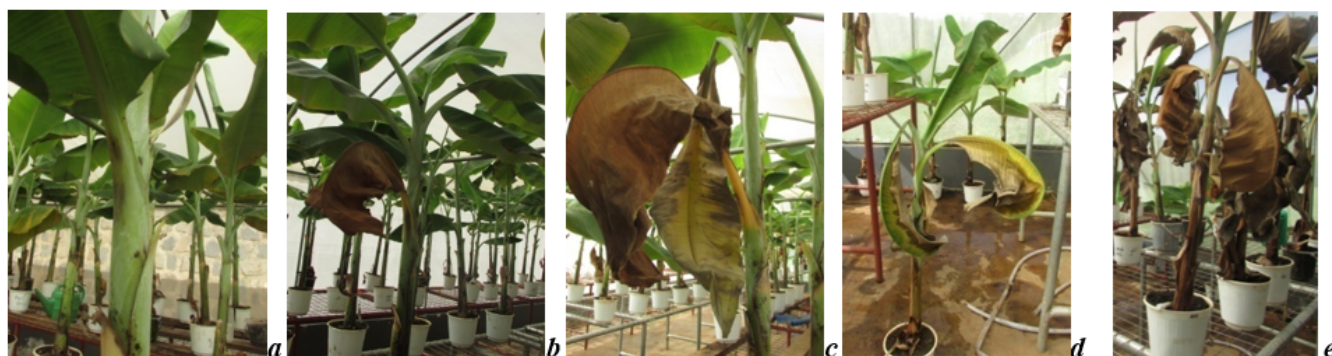


**Figure 2**

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.

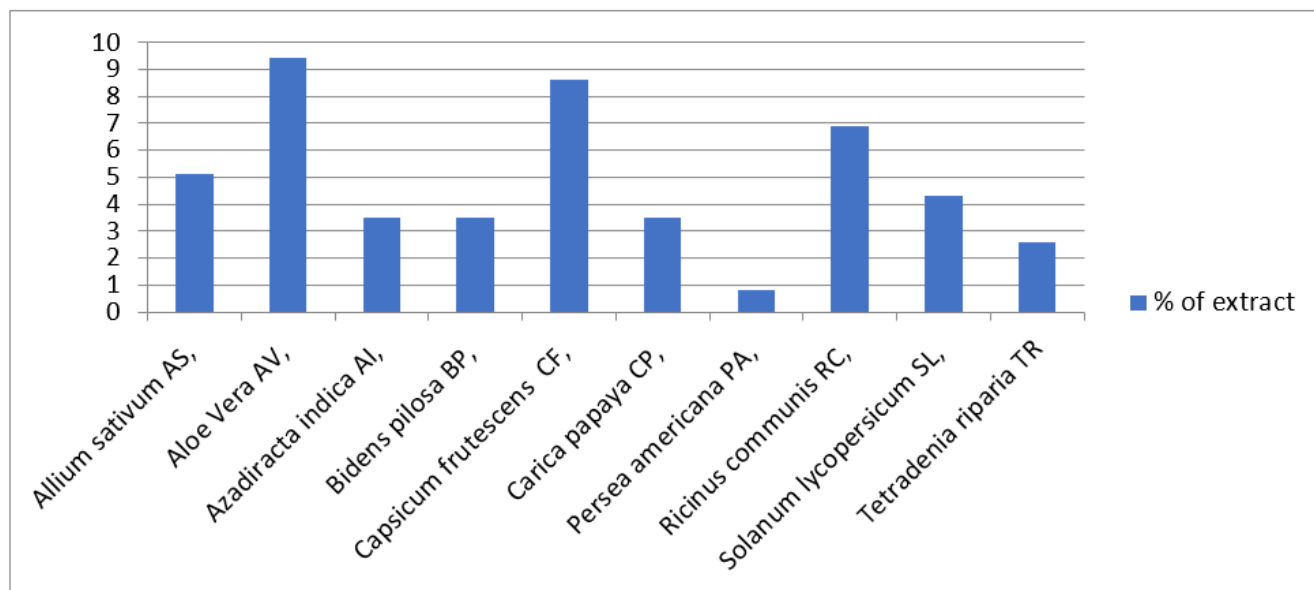


**Figure 3**

Yield in Petroleum Ether Extract at Kawanda NARO in Uganda.

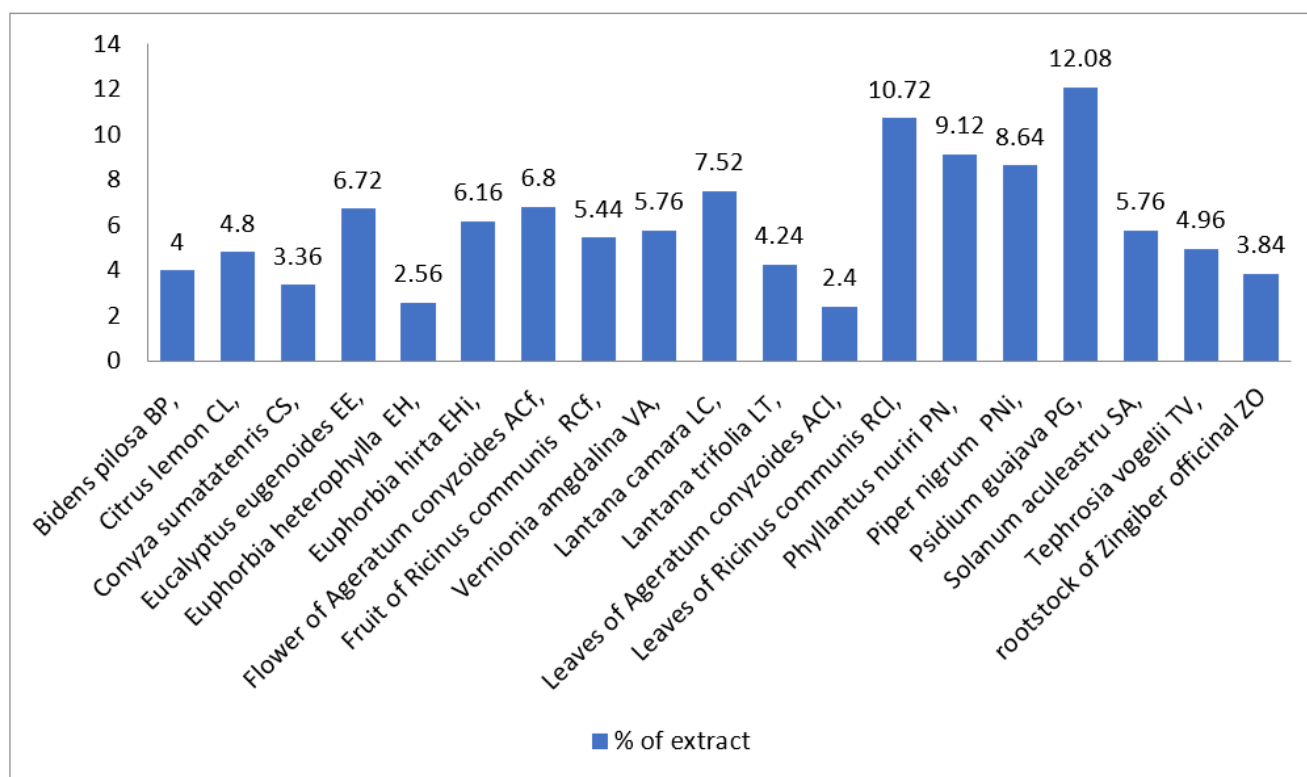


**Figure 4**

Percentage Yield of Methanol Extract atITTA Kalamboin eastern DR Congo.