AFRICAN JOURNAL OF SCIENCE, TECHNOLOGY AND SOCIAL SCIENCES

Journal website: https://journals.must.ac.ke

A Publication of Meru University of Science and Technology

Inhibition of *Ralstonia solanacearum* by *Warburgia ugandensis* stem bark and leaf crude extracts obtained using organic solvents

Oliver Libese Lideke[1*], Eric G. Mworia[1] and Cynthia Mugo Mwenda[2]

[1]*Department of Agriculture, Meru University of Science and Technology*
[2]*Department of Biological Sciences, Meru University of Science and Technology*

ARTICLE INFO

KEY WORDS

Ralstonia solanacearum,

Crude extracts,

Warburgia ugandensis,

minimum inhibitory concentration

ABSTRACT

Globally, *Ralstonia solanacearum* is a soil-borne bacterial pathogen that poses significant threat to the *Solanaceae* family and other crops. It causes widespread bacterial wilt, a devastating disease that affects the plant's water transport system, leading to wilting and death. Numerous chemical agents and treatment methods have been employed in attempts to control *R. solanacearum*, but are ineffective. The study aimed to determine the in vitro efficacy of *W. ugandensis* stem bark and leaf crude extracts against *R. solanacearum*. *W. ugandensis* stem bark and leaf crude extracts were obtained using organic solvents viz. methanol, ethanol, dichloromethane and hexane. In vitro, antagonistic activities against *R. solanacearum* of all organic crude extracts of *W. ugandensis* were determined by standard agar well diffusion assay on Kelman's 2, 3, 5- triphenyl tetrazolium chloride medium in triplicates. Two-way analysis of variance (ANOVA) was used in the statistical analysis of the mean diameter inhibition zones. All the organic solvents crude extracts of *W. ugandensis* were inhibitive against *R. solanacearum*. However, the stem bark crude extracts exhibited significantly higher efficacy against R. solanacearum compared to the leaf crude extracts. The crude extracts were subjected to a serial dilution to determine the minimum inhibitory concentration (MIC). *W. ugandensis* stem bark dichloromethane crude extracts had the lowest MIC of 1 mg/ml. *W. ugandensis* stem bark dichloromethane crude extracts were most effective against *R. solanacearum*. Further research is important to determine the bioactive compounds against R. solanacearum in *W. ugandensis* stem bark dichloromethane crude extracts.

Introduction

Ralstonia solanacearum is a lethal vascular soil-borne plant pathogen that causes bacterial wilt disease (Benti, 2023). García et al., (2019) reported that *R. solanacearum* is widely distributed in tropics, and warm temperate regions globally and affect crops in more than 450 species and 50 families. Bacterial wilt symptoms are easily detectable and appear in the plants at the mid-production stage. Symptoms range from a flaccid appearance

Corresponding author: Oliver Libese Lideke Email: libeseoliver@gmail.com

https://doi.org/10.58506/ajstss.v2i2.172

AFRICAN JOURNAL OF SCIENCE, TECHNOLOGY AND SOCIAL SCIENCES ISSN :2958-0560

on the young leaves usually at the warmest time of the day (Jiang et al., 2017) and subsequent wilting of the whole plant. The appearance of a cross-section of the stem exhibit a brown discoloration, and drops of white or yellow bacterial ooze may be visible(Prameela & Suseela Bhai, 2020). Bacterial wilt still remains a menace to the Solanaceae family and other crops production. Control against bacterial wilt involve cultural, chemical, biological, host resistance or combination of these strategies to form an integrated disease management strategy (Schaad et al., 2003). However, information on use of plant crude extracts to control *R. solanacearum* is scanty.

W. ugandensis is evergreen tree found in Eastern and Southern Africa. Studies conducted using its stem bark and leaf have reported presence of diverse active biochemical with antimicrobial properties against disease pathogens (Kamau et al., 2019). A series of unique sesquiterpentine 1-4 dialdehydes isolated from *W. ugandensis* have shown a broad antibacterial and antifungal activities (Kamau et al., 2021). Polygodial, warbuganal and muzigadial obtained from this plant show similar antibacterial spectra(Opiyo, 2020). Okello & Kang, (2021) opined that crude extracts obtained from *W. ugandensis* have been used in traditional medicine in several African countries to treat ailments such as toothaches, constipation and fever among others. The effectiveness of crude extracts from *W. ugandensis* obtained using various organic solvents against *R. solanacearum* has not been widely studied.

At the start of this millenium, the global use of synthetic pesticides exceeded 2.5 million tons per year (Goudie, 2018). Nevertheless, the indiscriminate use of these chemicals has led to detrimental environmental impacts, disruption in the ecological systems, health-related safety concerns and the emergence of resistant pathogens. Residues from pesticide interrupt natural processes and ecosystems, posing danger to human and animal health (Alengebawy et al., 2021). With mounting public concerns over environmental issues, there is growing demand for alternative disease management systems that reduce dependency on pesticides and rely on naturally occurring compounds (Lengai et al., 2020). Plant extracts with potent bioactive compounds are considered more benign compared to synthetic chemicals (Pavela, 2016). However, the potential of plant extracts in controlling bacterial wilt remains uncertain. The study assessed the in vitro effectiveness of *W. ugandensis* stem bark and leaf crude organic extracts against *R. solanacearum.*

Materials and Methods

Sample collection

W. ugandensis trees were identified at Meru University of Science and Technology (MUST) which is situated at 0.05° North latitude, 37.65° East longitude and 1,582 metres above sea level. Leaves and stem barks were collected from the identified trees. Debarking was done using a machete for stem bark which was cut into small pieces and leaves were plucked then washed under running tap water and then with sterile distilled water. Leaves and stem barks were dried under shade for three weeks and milled into a fine powder using an electrical blender and subjected to the extraction protocol.

Organic solvents extraction

Fifty grams of *W. ugandensis* leaf and stem bark powder were transferred into separate one litre conical flasks. Sequential extraction was done with 80% organic solvents viz. dichloromethane, methanol, hexane and ethanol for each plant powder. Two hundred millilitres of each organic solvent was added into the conical flasks containing either leaf or stem bark powder. The conical flasks were placed on an orbital shaker for 72 hours with continuous swirling. The samples were filtered using Whatman filter paper (No. 1). Then the filtrates were concentrated using a rotary evaporator at their respective organic solvents boiling points. The concentrates were sterilized using a 0.45 µm nitrocellulose membrane by vacuum filtration. The crude extracts obtained were transferred into sterile falcon tubes, labelled, and

caped tightly. The samples were stored at 4°C until further analysis.

Identification of R. solanacearum

A survey was done at MUST demonstration farm in Meru County on Solanaceae family crops to identify affected tomato plants that presented bacterial wilt symptoms. The preliminary test of observing wilting of the plants was done for two weeks. The soil in the rhizosphere of affected tomato plants were collected and taken to MUST biological laboratory for isolation of *R. solanacearum.*

Isolation of R. solanacearum

Soil collected in the vicinity of affected tomato plants was used for the isolation of *R. solanacearum.* Ten grams of dry soil was placed in a beaker and 100 ml of sterilized distilled water was added. The sample was agitated for 20 minutes and serial dilutions were then carried out by adding 1 ml of the sample to 9 ml of sterilized distilled water to a dilution of 10^{-9}. One hundred microlitre of the solution was spread on the Kelman's 2, 3, 5- Triphenyl tetrazolium chloride (TZC) medium that was dispensed in a Petri plate and incubated at 28°C for between 18hours.

Sub culturing to obtain a pure culture

The single colony technique was adopted to obtain pure cultures and identified according to their morphological, cultural and physiological characteristic as a stated in Bergey's Manual of Systematic Bacteriology (Abo-Elyousr & Asran, 2009; Sneath et al., 1986). The microorganisms of interest were picked by sterilized inoculating wire loop from a mixed culture streaked on a Petri plate containing Kelman's 2, 3, 5-TZC medium and incubated at 28 °C. The observation was made after 12 hours to 48 hours, restreaking on fresh Petri plates containing 2, 3, 5-TZC medium was done to ensure purity. *R. solanacearum* was suspended and stored in distilled water at room temperature and sub-cultured every six months to maintain virulence(Champoiseau et al., 2009).

Confirmatory tests for R. solanacearum

The following tests below were carried out to determine the presence of *R. solanacearum.*

Gram staining

A loop full of the young pure cultures (18 h) was spread on a glass slide and fixed by heating on a very low flame. Aqueous crystal violet solution (0.5%) was spread over the smear for 30 seconds and then washed with running tap water for one minute. It was then flooded with iodine for one minute, rinsed in tap water and decolorized with 95% ethanol until colourless runoff. After washing the specimen was counter-stained with safranin for approximately 10 seconds, washed with water, dried and observed microscopically at 10X, 40X and 100X using oil (Khasabulli et al., 2017).

Catalase oxidase

Young agar cultures (18 hrs) and 3% hydrogen peroxide (H_2O_2) were used to observe the production of gas bubbles. A loop full of bacterial culture was mixed with a drop of H_2O_2 on a glass slide and observed for the production of gas bubbles with naked eye and under a dissecting magnification of 25X(Khasabulli et al., 2017).

Potassium hydroxide

Bacteria were aseptically removed from Petri plates with an inoculating wire loop, placed on a glass slide in a drop of 3% KOH solution, stirred for 10 seconds and observed for the formation of slime threads(Khasabulli et al., 2017).

Preparation of bacterial inoculum

Inoculum of the *R. solanacearum* was prepared by culturing it on Kelman's 2, 3, 5-TZC medium (1% 1 g of Casamino acids, 10 g of peptone, 5 g of glucose in 1000 ml of distilled water) (Kelman, 1954). Cultures were suspended in sterile distilled water and were adjusted to 1×10^{8} CFU ml^{-1} (colony forming unit) using 0.5 Mac Farland solution.

In vitro determination of the efficacy of W. ugandensis stem bark and leaf extracts

Standard agar well diffusion assay was used to determine the efficacy of *W. ugandensis* stem

bark and leaf organic crude extracts against *R. solanacearum*. Petri dishes containing Kelman's 2, 3, 5-TZC medium were inoculated with 100 µl inoculum of *R. solanacearum* (1×10^8 CFU ml^{-1}). Wells of 5 mm diameter were cut into solidified agar media with the help of a sterilized cork borer. Aliquot 60 µl of each stem bark or leaf crude extract was introduced to the respective well and the Petri dishes were incubated at 28 ± 2 for 18h. Dimethyl sulfoxide (DMSO), in which was used for dilution to make up different concentrations (150 , 50 , 25 and 5 mg ml^{-1}) of the crude extracts, DMSO was used as negative control while 3% NaOCl solution was used as a positive control. The experiment was performed in triplicate with three repeats under aseptic conditions. The zones of inhibition for each of the crude extract was measured with a Vernier callipers, and was expressed in terms of the mean diameter (mm) inhibition zone produced by the respective crude extracts at the end of the incubation period (Yassin et al., 2021).

Minimum Inhibitory Concentration (MIC) Assay

W. ugandensis stem bark and leaf organic crude extracts were subjected to serial dilution (2.5 to 0.5 mg ml^{-1}). In a Petri dish 100 µl of *R. solanacearum* were inoculated on Kelman's 2, 3, 5-TZC medium and 60 µl of selected plant crude extracts were loaded in wells and incubated at 28 ± 2°C for 18 hours. The lowest concentration dilution of the plant crude extracts that retained its inhibitory effect resulting in no growth of *R. solanacearum* was recorded as the MIC value of the extract.

Data analysis

Two-way analysis of variance (ANOVA) was done to check whether there is a significant difference in different levels of concentrations. The LSD -test was done to compare the mean diameter of the zone of inhibition between the stem bark and leaf crude extracts.

Results

Colony Morphology of R. solanacearum

R. solanacearum was isolated from the soil in the vicinity of wilted tomato plant samples collected from Meru County during field survey. Virulent isolates grown on 2, 3, 5-TZC medium were highly fluidal, white coloured with a light pink centre and round to irregular margin. The isolated colonies were picked and sub cultured for purification of *R. solanacearum*.

Biochemical Confirmatory test for R. solanacearum

Morphological observations revealed that cells of *R. solanacearum* were straight rod-shaped, with circular ends, cells emerged singly or in pairs, red colouration, and encased when viewed under a compound microscope at 100X magnification with oil immersion. The isolated *R. solanacearum* bacteria were pink in colour in Gram staining reaction under the compound microscope at 100X magnification (Fig. 2A), confirming that they were Gram-negative. *R. solanacearum* showed a positive response to the catalase oxidase test, evidenced by the generation of air bubbles upon inoculation to the medium (Fig. 2B). Additionally, the bacterial culture of *R. solanacearum* produced a string-like viscous material on a glass slide in the KOH test which further confirmed its Gram-negative characteristics. Based on the morphological and biochemical traits we observed, the bacteria isolated was *R. solanacearum*.

Bioassay screening W. ugandensis of extracts

In the screening test, *R. solanacearum* showed different sensitivity patterns when exposed to the plant crude extracts, in terms of zone of inhibition around the well. Sodium hypochlorite (positive control) gave a mean diameter inhibition zone of 25.64 mm while dimethyl sulfoxide (negative control) showed no inhibition.

W. ugandensis methanolic crude extracts from both stem bark and leaves showed similar inhibition patterns against *R. solanacearum* (Figure 3B & 4). The methanolic stem bark crude extracts at 250 mg/mL had a mean diameter inhibition of 16.11 mm that decreased gradually to 8.67 mm at 5 mg/mL, whereas methanolic leaf crude extracts had a mean inhibition diameter of 14.11

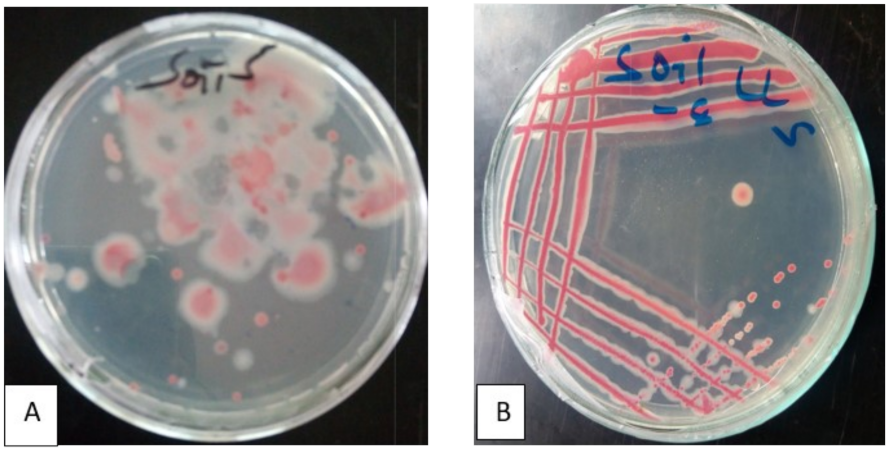

Figure 1: *Colony morphology of R. solanacearum.*

(A) Represents colony morphology of R. solanacearum isolated cultures grown on 2, 3, 5-TZC medium at incubator for 18 hours and (B) represents colony morphology of R. solanacearum subcultures grown on 2, 3, 5-TZC medium at incubator for 18 h.

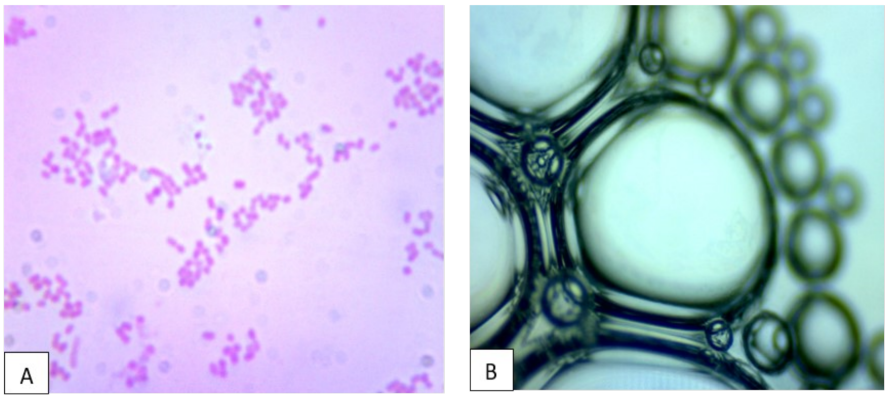

Figure 2: *Gram staining and Catalase Oxidase tests.*
(A) shows Gram staining of R. solanacearum. Pink colour indicates rod shaped cells of R. solanacearum at 100X magnification, and (B) shows production of air bubbles when a loop full of R. solanacearum bacterial culture was mixed with a drop of H_2O_2.

mm and 8.2 mm at 250 mg/mL and 5 mg/mL respectively (Figure 3B). At all tested concentrations, the methanolic stem bark crude extracts exhibited a significant response against *R. solanacearum* when compared to the methanolic leaf crude extracts (Figure 3B).

W. ugandensis dichloromethane stem bark crude extracts showed similar observations as the ethanolic and methanolic crude extracts, where the zones of inhibition decreased with as the concentrations decreased (Figure 3C & 4). Dichloromethane leaf crude extracts zone of inhibitions increased gradually with the highest zone of inhibition of 16.5 mm at 50 mg/mL (Figure 3C).

Contrary to observations made for *W. ugandensis* ethanolic and methanolic crude extracts, hexanoic stem bark crude extract showed the highest mean inhibition zone of 13.11 mm, followed by 12.22 mm at 50 mg/mL and 250 mg/mL respectively (Figure 3D & 4). However, the hexanoic stem bark extracts inhibition zones decreased at concentrations of 25 and 5 mg/mL. Hexanoic leaf crude extracts mean inhibition zones increased with decreasing concentration at concentrations of 250, 150, 50 and 25 mg/mL, then decreased at 5 mg/mL (Figure 3D).

Determination of minimum inhibitory concentrations of W. ugandensis crude extracts obtained by different organic solvents from leaf and stem barks

Minimum inhibitory concentrations of different active *W. ugandensis* stem bark and leaf crude extracts had been demonstrated in Table 1.

Dichloromethane stem bark crude extracts exhibited the least MIC of 1 mg/mL against *R. solanacearum* (Table 1). Generally, the results for screening and MIC for *W. ugandensis* stem bark and leaf crude extracts, all showed antagonistic activity against tested *R. solanacearum*.

Discussion

The efficiency of organic solvents in extracting crude bioactive compounds

Methanol and ethanol stem bark and leaf crude extracts showed similar inhibition patterns. From

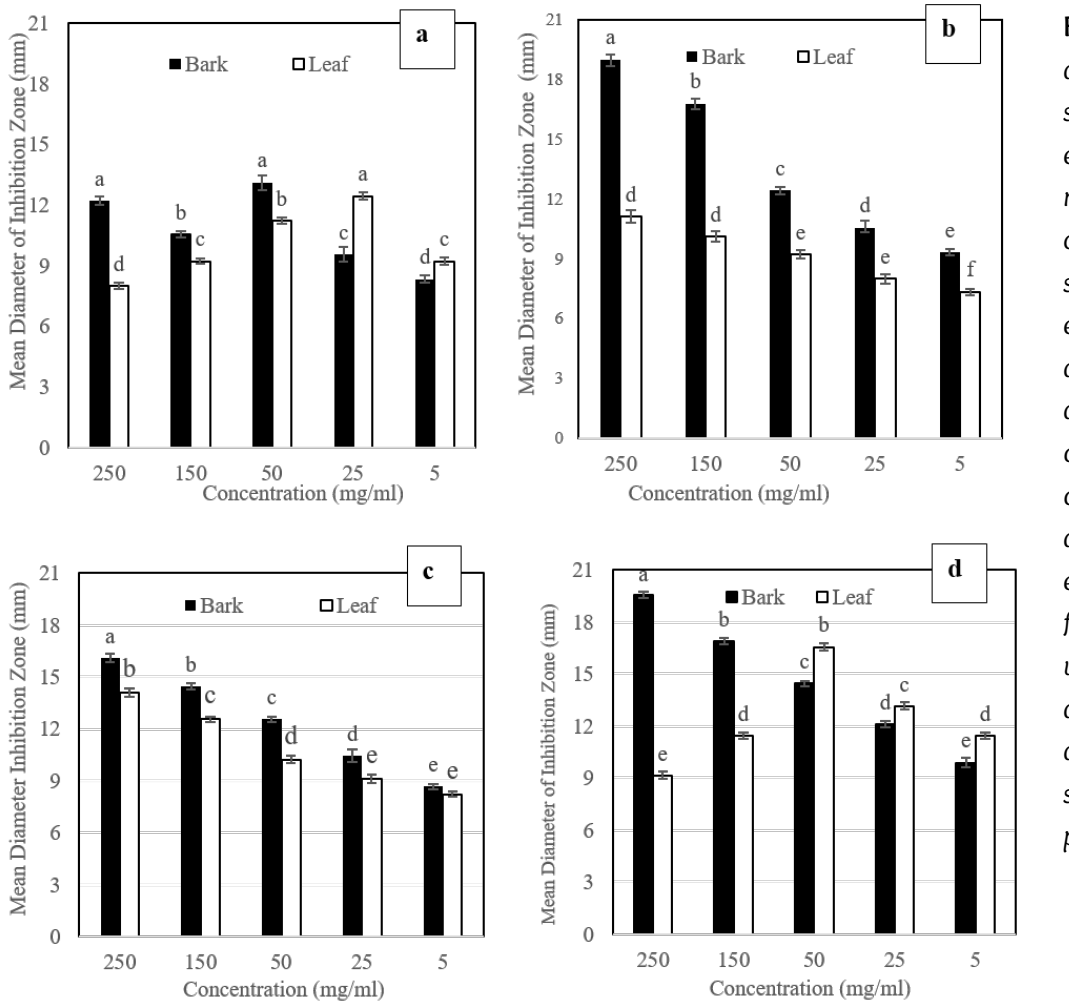

Figure 3: *Antagonistic activity of W. ugandensis stem bark and leaf crude extracts against R. solanacearum. W. ugandensis obtained using four organic solvents (a-hexane, b-ethanol, c-methanol and d-dichloromethane) were applied at five different concentrations. Agar plate containing R. solanacerum and wells of W. ugandensis extracts incubated at 28°C for 18 hours. In each column letters are identical and dissimilar letters are differed significantly. The statistical significance is $p \leq 0.05$.*

the pattern of inhibition, both organic solvents may not be extracting different active compounds but rather varying concentrations. For instance, methanolic leaf crude extract seems to be more effective in inhibition while ethanolic stem bark crude extract showed higher inhibition efficiency suggesting that methanol has higher extracting efficacy of leaf bioactive compounds while ethanol has higher extracting efficacy of stem bark bioactive compounds. Based on their relative ionic acceptor numbers, both solvents are classified as polar thus their bioactive extraction capacity are relatively similar(Nguyen, 2016). This may explain why crude extracts obtained from both stem bark and leaf showed similar inhibition activity patterns.

Inhibition efficacy of crude extracts obtained from methanol and ethanol appeared to reduce gradually with decreasing concentrations suggesting that those crude extracts may have similar biochemical characteristics. These findings concur with Aloo et al., (2019) reported that the inhibitory activities of the tested plant crude extracts against *Fusarium oxysporum* increased with a corresponding increase in their concentrations suggesting that effectiveness of plant crude extracts were dependent on the quantity of bioactive components and their nature. Similar to a study conducted by (Jha & Sit, 2022), these study demonstrates that different organic solvents have different extracting capacity and mechanisms and subsequently yields different biochemical components from different plant tissues.

Stem bark extracts obtained with dichloromethane showed a similar pattern of inhibition to those of ethanol and methanol. Additionally, dichloromethane at 250 mg/mL showed high mean diameter inhibition zone of 19.5mm similar to observation made under ethanol obtained stem bark crude extracts inhibition suggesting that relative

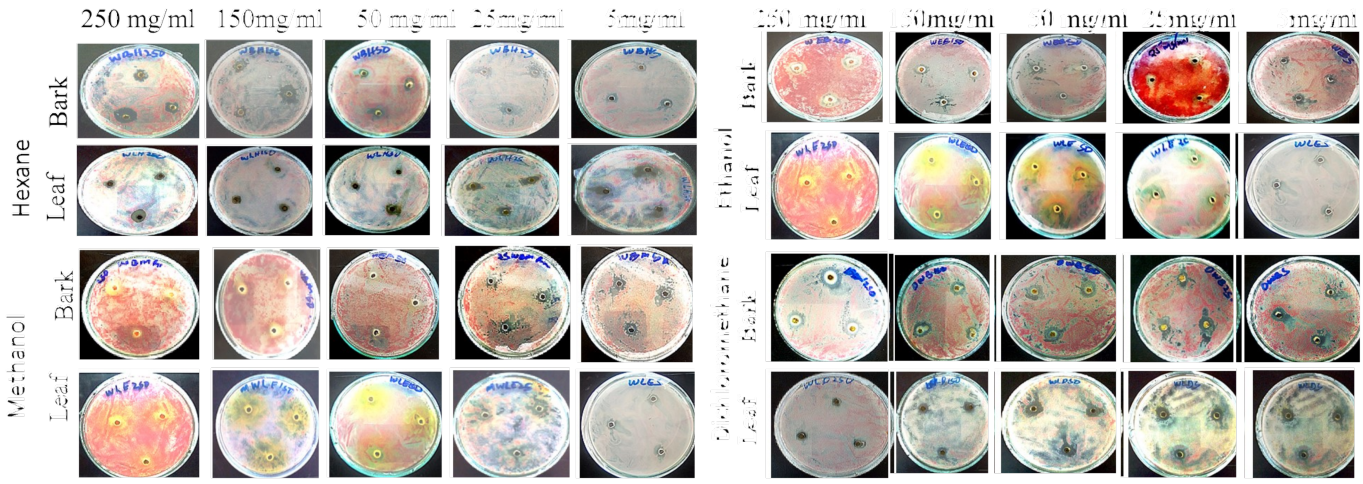

Figure 4: *R. solanacearum (bacterial wilt) cultured on Kelman's 2, 3, 5-tetrazolium chloride agar medium. W. ugandensis stem bark and leaf hexane, ethanol, methanol and dichloromethane crude extracts of five (5) concentrations were placed on a 5mm wells made on agar media and incubated for 18 hours at 280C. A distinct area around the well indicates inhibition zone*

polarity did not have a significant impact on obtained crude extract components as previously reported (Al-Salt, 2012).

Leaf crude extracts obtained using hexane and dichloromethane had the lowest mean diameter inhibition zones. This indicates that these crude extracts were more effective at MIC and less effective at high concentration. Al-Salt, (2012) reported that water extracts of *Arum discoridis* with MIC had the highest inhibition. Both suggested a potentially higher solubility with water obtained crude extracts perhaps a possible occurrence with solvents used in this study. These observations agree with previous studies that concluded biochemicals at lower concentrations are more effective(Nwakiban et al., 2019).

Concentration efficacy of crude extracts obtained from W. ugandensis stem back and leaf

Observation made in this study shows that the efficacy of stem bark obtained crude extracts by all solvents except hexane reduced with decreasing concentration. Relative polarity of dichloromethane exists at a transitional level thus while stem bark crude extracts showed similar properties to those of methanol and ethanol, leaf obtained dichloromethane crude extracts inhibition properties were significantly different and only their pattern were similar to those of hexane.

Extraction efficiency of solvents is highly dependent on various factors such as rotary evaporation and boiling temperatures of solvents used to obtain crude extracts. Different organic solvents

	Organic solvents							
	Methanol		Ethanol		Dichloromethane		Hexane	
Concentration	Leaf	Bark stem	Leaf	Bark stem	Leaf	Bark stem	Leaf	Bark stem
2.5 mg/ml	+	+	+	+	+	+	+	+
1.75 mg/ml	+	+	+	+	+	+	-	+
1 mg/ml	-	-	-	-	-	+	-	-
0.5 mg/ml	-	-	-	-	-	-	-	-

Table 1 *Minimum inhibitory concentrations for leaf and stem bark extracts*

Key: + Present inhibition - No inhibition

differ in their boiling points which determine the antibacterial properties of crude extracts (Ibrahim & Kebede, 2020). Previous studies have reported that properties of bioactive crude extracts depend on type of the plant, solvent used, time of harvest, age of the plant, extraction method, drying and processing of the material (Al Ubeed et al., 2022).

W. ugandensis leaf ethanol and methanol obtained leaf crude extracts showed that the mean diameter zone of inhibition decreased with a reduction in the concentration while *W. ugandensis* hexane and dichloromethane obtained leaf crude extracts showed an increase in the mean diameter of inhibition zones of 12.44mm at 25mg/ml and 16.55mm at 50mg/ml respectively. Hexane and dichloromethane solvents being non polar solvents their efficacy increases with the decrease in their concentrations. It has been reported that polar solvents obtained plant crude extracts efficacy is highest when the concentration is high (Aloo et al., 2019).Oliveira et al., (2013) also opined that to an extent, efficacy of crude extracts obtained using nonpolar solvents increases with decreasing concentration.

Inhibition response to crude extracts obtained from different plant tissues using different organic solvents

This study showed that extraction of bioactive compounds by specific solvents is tissue dependent. Both stem bark and leaf showed varying inhibition properties under different extraction organic solvents. Previous studies by Abuto & Morono, (2018) showed that antibacterial and antifungal effects was dependent of site where extraction materials were obtained from, type of organic solvent used and plant part used for extraction. Previously, differences observed in antibacterial and antifungal activity has been linked to synergistic or antagonistic actions of various secondary metabolites such as fatty acids, flavonoids and sesquiterpenoids present.(Manguro et al., 2003)

W. ugandensis stem bark methanol and ethanol obtained crude extracts showed higher mean diameter inhibition zones suggesting higher concentration of bioactive components against *R. solanacearum* than the leaf organic solvent obtained crude extracts. These results agree with those of Linner et al., (2017) reported that *W. ugandensis* leaf obtained crude extracts had least inhibitive effect against *Phomopsis theae* growth. The stem bark of plant is known as the storage and translocation vessel of water and photosynthetic products and may therefore contain a myriad of biochemical components (Kagawa & Battipaglia, 2022) while the leaf part of the plant is a photosynthetic and gaseous tissue(Magwaza et al., 2020).

Inhibition of R. solanacearum by W. ugandensis crude extracts

In vitro tests showed a significant difference in mean diameter inhibition zones of *R. solanacearum. W. ugandensis* stem bark ethanol, methanol and dichloromethane stem bark obtained crude extracts showed a progressive increase in mean diameter inhibition zones in a concentration-dependent manner except for hexane stem bark obtained crude extracts. Most of stem bark organic solvents obtained crude extracts were more effective compared to leaf organic solvents obtained from crude extracts.

According to the results, the crude extracts obtained from *W. ugandensis* stem bark and leaf have shown efficacy against tested *R. solanacearum* to certain concentrations. Abuto & Morono, (2018) and Kamau et al., (2021) reported also antibacterial and antifungal activities of crude extracts of *W. ugandensis* against *Staphylococcus aureus*, *Candida albican, Alternaria solani* and *Phytophthora infestans. W. ugandensis* crude extracts showed statistically significant differences in the inhibitory effects of the stem bark and leaf parts on antimicrobial properties. The disparity in antimicrobial activities of *W. ugandensis* stem bark and leaf crude extracts could be due to antagonistic action of different secondary metabolites (Abuto & Morono, 2018). Malhotra et al., (2022) asserted that the concentration of bioactive components varies in different plant parts and are sec-

ondary metabolites in all plant organs this could contribute to the inhibitory difference of *W. ugandensis* stem bark and leaf crude extracts.

Minimum inhibitory concentration

W. ugandensis stem bark crude dichloromethane extracts were more effective compared to the stem bark and leaf crude extracts obtained using ethanol, methanol and hexane. Dichloromethane has been known to have high extractive ability of biochemical compounds from plant tissues (Axiotis et al., 2020).

Conclusion

All the raw extracts derived from *W. ugandensis* demonstrated inhibition against *R. solanacearum*. Particularly, the organic crude extracts from the stem bark exhibited higher efficacy in vitro compared to the organic crude extracts from the leaves. Nonetheless, further investigations are needed to identify the specific bioactive compounds within these extracts responsible for their action against *R. solanacearum*.

References

Abo-Elyousr, K. A. M., & Asran, M. R. (2009). Antibacterial activity of certain plant extracts against bacterial wilt of tomato. *Archives of Phytopathology and Plant Protection*, *42*(6), 573–578.

Abuto, J. O., & Morono, D. A. (2018). Interaction effects of site, samples, plant parts and solvent types on antimicrobial activity of the Kenyan populations of Warburgia ugandensis (Sprague). *Journal of Pharmaceutical, Chemical and Biological Sciences*, *6*, 1–12.

Al Ubeed, H. M. S., Bhuyan, D. J., Alsherbiny, M. A., Basu, A., & Vuong, Q. V. (2022). A comprehensive review on the techniques for extraction of bioactive compounds from medicinal cannabis. *Molecules*, *27*(3), 604.

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, *9*(3), 42.

Aloo, B. N., Oluko, P., & Wanjohi, B. (2019). In vitro analysis of antimicrobial and phytochemical properties of crude extracts of selected plants against the tomato wilt disease. *African Crop Science Journal*, *27*(3), 479–499.

Al-Salt, J. (2012). Antimicrobial activity of crude extracts of some plant leaves. *Res J Microbiol*, *7*, 59–67.

Axiotis, E., Petrakis, E. A., Halabalaki, M., & Mitakou, S. (2020). Phytochemical profile and biological activity of endemic Sideritis sipylea Boiss. In North Aegean Greek islands. *Molecules*, *25*(9), 2022.

Benti, E. A. (2023). Control of Bacterial Wilt (Ralstonia solanacearum) and Reduction of Ginger Yield Loss through Integrated Management Methods in Southwestern Ethiopia. *The Open Agriculture Journal*, *17*(1). https://doi.org/10.2174/18743315-v17-e230109-2022-39

Champoiseau, P. G., Jones, J. B., & Allen, C. (2009). Ralstonia solanacearum race 3 biovar 2 causes tropical losses and temperate anxieties. *Plant Health Progress*, *10*(1), 35.

García, R. O., Kerns, J. P., & Thiessen, L. (2019). Ralstonia solanacearum species complex: A quick diagnostic guide. *Plant Health Progress*, *20*(1), 7–13.

Goudie, A. S. (2018). *Human impact on the natural environment*. John Wiley & Sons.

Ibrahim, N., & Kebede, A. (2020). In vitro antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. *Saudi Journal of Biological Sciences*, *27*(9), 2261–2268.

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, *119*, 579–591.

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., Macho, A. P., Ding, W., & Liao, B. (2017). Bacterial wilt in China: History, current status, and future perspectives. *Frontiers in Plant Science*, *8*, 1549.

Kagawa, A., & Battipaglia, G. (2022). Post-photosynthetic carbon, oxygen and hydrogen isotope signal transfer to tree rings—How timing of cell formations and turnover of stored carbohydrates affect intra-annual isotope variations. In *Stable isotopes in tree rings: Inferring physiological, climatic and environmental responses* (pp. 429–462). Springer.

Kamau, E. W., Mworia, E. G., Masinde, P. W., & Maingi, J. M. (2021). *Studies of Warburgia ugandensis lyophilized crude extract with Trichoderma asperellum on tomato blight pathogens*.

Kamau, E. W., Mworia, G., Maingi, J., & Masinde, P. W. (2019). *Screening of Warburgia Ugandensis Crude Extracts Obtained from Different Organic Solvents against Tomato Phytophthora Infestans and Alternaria Solani*.

Kelman, A. (1954). The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. *Phytopathology*, *44*(12). https://www.cabdirect.org/cabdirect/abstract/19551101405

Khasabulli, B. D., Musyimi, D. M., Miruka, D. M., Opande, G. T., & Jeruto, P. (2017). Isolation and characterisation of Ralstonia solanacearum strains of tomato wilt disease from Maseno, Kenya. *Journal of Asian Scientific Research*, *7* (9), 404–420.

Lengai, G. M., Muthomi, J. W., & Mbega, E. R. (2020). *Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific African, 7, e00239*.

LINNER, C. S., BIRGEN, J. K., & MAINGI, J. (2017). In vitro response of Phomopsis theae to the products of Azadirachta indica and extracts of Warburgia ugandensis. *Asian Journal of Tropical Biotechnology*, *14*(2), 37–46.

Magwaza, S. T., Magwaza, L. S., Odindo, A. O., Mashilo, J., Mditshwa, A., & Buckley, C. (2020). Evaluating the feasibility of human excreta-derived material for the production of hydroponically grown tomato plants-Part I: Photosynthetic efficiency, leaf gas exchange and tissue mineral content. *Agricultural Water Management*, *234*, 106114.

Malhotra, B., Kumar, P., & Bisht, N. C. (2022). Defense versus growth trade-offs: Insights from glucosinolates and their catabolites. *Plant, Cell & Environment*.

Manguro, L. O. A., Ugi, I., Lemmen, P., & Hermann, R. (2003). Flavonol glycosides of Warburgia ugandensis leaves. *Phytochemistry*, *64*(4), 891–896.

Nguyen, T. T. (2016). *Oxidative stress in C. elegans: Discovery of a mechanistic role for gamma-ketoaldehyde lipid peroxidation products in the Free Radical Theory of Aging* [PhD Thesis].

Nwakiban, A. P. A., Sokeng, A. J., Dell'Agli, M., Bossi, L., Beretta, G., Gelmini, F., Tchamgoue, A. D., Agbor, G. A., Kuiaté, J.-R., Daglia, M., & Magni, P. (2019). Hydroethanolic plant extracts from Cameroon positively modulate enzymes relevant to carbohydrate/lipid digestion and cardio-metabolic diseases. *Food & Function*, *10* (10), 6533–6542. https://doi.org/10.1039/C9FO01664C

Okello, D., & Kang, Y. (2021). Ethnopharmacological potentials of Warburgia ugandensis on antimicrobial activities. *Chinese Journal of Integrative Medicine*, *27*(8), 633–640.

Oliveira, D. A., Salvador, A. A., Smânia Jr, A., Smânia, E. F., Maraschin, M., & Ferreira, S. R. (2013). Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. *Journal of Biotechnology*, *164*(3), 423–432.

Opiyo, S. A. (2020). *Evaluation of Warburgia ugandensis extracts and compounds for crop protection against Prostephanus truncates*.

Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects – a review. *Plant Protection Science*, *52 (2016)*(No. 4), 229–241. https://doi.org/10.17221/31/2016-PPS

Prameela, T. P., & Suseela Bhai, R. (2020). Bacterial wilt of ginger (Zingiber officinale Rosc.) incited by Ralstonia pseudosolanacearum-A review based on pathogen diversity, diagnostics and

management. *Journal of Plant Pathology*, *102*, 709–719.

Schaad, N. W., Frederick, R. D., Shaw, J., Schneider, W. L., Hickson, R., Petrillo, M. D., & Luster, D. G. (2003). Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. *Annual Review of Phytopathology*, *41*(1), 305–324.

Sneath, P. H., Mair, N. S., Sharpe, M. E., & Holt, J. G. (1986). *Bergey's manual of systematic bacteriology. Volume 2*. Williams & Wilkins.

Yassin, M. T., Mostafa, A. A.-F., & Al-Askar, A. A. (2021). In vitro antagonistic activity of Trichoderma harzianum and T. viride strains compared to carbendazim fungicide against the fungal phytopathogens of Sorghum bicolor (L.) Moench. *Egyptian Journal of Biological Pest Control*, *31*(1), 118. https://doi.org/10.1186/s41938-021-00463-w