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Phytochemical Composition and Antibacterial Activity of Fruit Extract of *Solanum incanum* L. against *Ralstonia solanacearum*

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Authors' contributions

We declare that this work was done by the authors named in this article. Author LNK performed the experiment, collected data and wrote the first draft of the manuscript. Authors IOKO, PTW and RCR helped in designing and proofreading the manuscript and suggested necessary changes. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the phytochemical composition and antibacterial activity of *Solanum incanum* fruits against *Ralstonia solanacearum.*
Study Design: Experimental design involving completely randomized design
Place and Duration of Study: The study was conducted at department of Chemistry and Biochemistry, School of Sciences and Aerospace studies, Moi University, Kenya, between January and June 2021.

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Methodology: Extraction was done by maceration using ethanol as the extracting solvent. Phytochemical screening was done following standard procedures. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined using the Folin–Ciocalteu colorimetric method and aluminum chloride colorimetric assay respectively. The extract was further analyzed using Gas Chromatography Mass spectroscopy (GC-MS) and Fourier transformed Infrared (FT-IR). *In vitro* antibacterial activity was determined using disc diffusion method while *in vivo* studies was done under greenhouse conditions.
Results: Phytochemical analysis showed presence of alkaloids, glycosides, steroids, tannins, flavonoids, phenols, saponins and terpenoids. The TPC and TFC were found to be 84.997 ± 0.2 mg GAE/g and 20.535 ± 0.2 mg/g QE of dried sample respectively. GC-MS analysis revealed the presence of 15 compounds, (9E)-1-Methoxy-9-Octadecene (26.85%), 9-Octadecenamide (Z) (21.43%), E-15-Heptadecenal (7.28%), E-14-Hexadecenal (6.28%), 2,4-Di-tert-butylphenol (4.96%) among others. FT-IR analysis revealed presence of OH, C-H, N-H, CO functional groups at wavenumbers 3348 cm^{-1}, 2931 cm^{-1}, 1589 cm^{-1}, and 1218 cm^{-1} respectively. The antibacterial activity for *in vitro* studies at concentrations 0.01, 0.05, 0.10, and 0.15 g/10 mL, the diameters of zone of inhibition were 20.75 ± 1.3, 25.75 ± 0.5, 27.25 ± 0.5, and 30.75 ± 0.5 mm respectively. This was comparable (*P*= .02) to that of ampicillin (positive control) which had zones of inhibition of 26.75 ± 0.5, 28.75 ± 0.5, 31.75 ± 0.4, and 35.00 ± 0.0 mm at the concentrations respectively. For the *in vivo* studies the plant extract and ampicillin delayed the development of the disease by eight and ten days post-inoculation respectively while symptoms of bacterial wilt for water treatment (negative control) were observed four days post-inoculation.
Conclusion: The plant extract had remarkable antibacterial activity and can be used to make viable formulations to control the devastating bacterial wilt disease.

Keywords: Bacterial wilt; Post-inoculation; Ampicillin; in vitro; in vivo.

1. INTRODUCTION

The bacterium *Ralstonia solanacearum* is a serious soil-borne pathogen belonging to class β-proteobacteria [1]. It's responsible for bacterial wilt on more than 450 species representing 54 botanical families [2], lethal to Solanaceous species, including *Solanum melongena, Capsicum anuum*, *Solanum tuberosum*, *Solanum lycopersicum*, *Capsicum frutescens,* and *Nicotiana tabacum* [3]. The bacterium enters plants through wounds and lateral root emergence points and proliferate in the vascular system, causing clogging of xylem vessels which block xylematic flow, leading to yellowing of the leaves, general wilting and finally death of the plant [4]. Due to its greater capability to survive in soil as well as broad host range, it's very hard to eliminate once established in the soil [5]. Control of bacterial wilt disease majorly depends on use of chemical fumigants [6], nevertheless, this chemical fumigant is undesirable since they lead to development of resistant pathogen [7], they persist and accrue in the environment [8], increases human health risks [9] as well as high and hidden cost incurred during their use [10]. There is need therefore for more effective and eco-friendly fumigants.

Naturally, plants produce bioactive constituents which appears to be a viable and appealing remedy of the problems. Numerous researchers are pursuing to ascertain natural products that can be used in place of synthetic pesticides. Plants produces phytochemicals that enable them to adapt in the environment and as defense against pathogens and pests [11]. Bio pesticides are non-toxic to humans and ecofriendly as compared to synthetic chemicals [12]. Plant extracts have been found effective in control of bacterial wilt. The extract of *Eichhorina crassipes* significantly reduced bacterial wilt diseases index by 91% in tomato plants [13]. The current study aimed at assessing the phytochemical composition and antibacterial activity of *Solanum incanum* fruits extracts against *Ralstonia solanacearum* a common tomato wilt causing pathogen.

2. MATERIALS AND METHODS

2.1 Materials

Solanum incanum fruits, analytical grade (Sigma Aldrich USA) ethanol, chloroform, Folin-Ciocalteus assay (FCR), gallic acid, and Quercetin, HCL, H2SO4, FeCl3, NaOH, Mayer's reagent, Na_2CO_3, and distilled water.

2.2 Study Location

The study was conducted at Chemistry laboratory (0˚17′17.4"N 35˚17′17.6"E), department of Chemistry and Biochemistry, school of Sciences and Aerospace Studies, Moi University, Kenya, between January and June 2021.

2.3 Sampling Procedure

Fruits of *Solanum incanum* were collected from Moi University farm (0˚17'05.3" N 35˚17'16.8"E) Eldoret. Purposive and simple random sampling were used where the first plant of *S. incanum* was purposively selected and the fruits were selected randomly from *S. incanum* plants. The plant of *S. incanum was* harvested by hand picking and transported to the Biology laboratory at Biological department Moi University where the plant was identified by a Taxonomist. Fresh fruits of approximate weight of 9.73 grams were harvested by hand picking, stored in a clean sealable cotton bag and later transported to Chemistry laboratory for preparation, extraction, and analysis.

2.4 Sample Preparation

The fruits of *S. incanum* were washed using tap water and rinsed with distilled water. The samples were sliced into pieces of equal size and placed in an oven at 40℃ to dry for 5 days until a constant weight was obtained. Finally, the dried sample was pulverized into fine powder using an electric blender. The fine powders were kept in dark airtight container in the laboratory freezer at 4 ℃ until used [14].

2.5 Extraction of Crude Extract from Fruits Using Maceration Method

Crude plant extract was obtained through maceration using ethanol as the solvent. About 50 g of powdered sample was macerated using 200 mL of ethanol for three days with occasional shaking and later filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 45 ± 2℃ and further concentrated to dryness on a water bath. The dried extract was stored at 4℃ prior to use [15].

2.6 Phytochemical Screening

Chemical tests were carried out using the extracts from plants following standard procedures to identify the phytochemicals present in the extract. Standard procedures were obtained from [16].

2.6.1 Tests for alkaloids- Mayer's test

About 2 mL of the filtrate was put in a test tube and a drop of Mayer's reagent was added by the side of the test tube. Formation of creamy or white precipitate indicated the test is positive.

2.6.2 Test for saponins -Froth test

About 5 mL of the sample was added into a graduated cylinder and diluted with distilled water to 20 mL. The suspension was shaken for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

2.6.3 Tests for flavonoids- alkaline reagent test

A total of 5 mL of the filtrate was put in a beaker and treated with 2 drops of sodium hydroxide solution. Intense yellow color was formed. On addition of 3 drops of HCl, the mixture turned colorless. This indicated presence of flavonoids.

2.6.4 Tests for terpenoids-Salhowski test

About 5 mL of the filtrate was put into a test tube and mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid to form a layer. Formation of reddish brown coloration of the interface indicated presence of terpenoids.

2.6.5 Test for phenolic compounds-Ferric chloride test

Exactly 2 mL of the filtrate was put into a test tube and diluted to 5 mL with distilled water. To this a 3 drops of neutral 5 % ferric chloride solution was added. Formation of dark green color indicated presence of phenolic compounds.

2.6.6 Test for tannins

About 0.5 mg of dried powdered samples was boiled in 20 mL of water in test tubes then filtered. Three drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

2.6.7 Test for steroids

Crude plant extract (1 mg) was placed into a test tube and dissolved with 10 mL chloroform. Equal

volume of concentrated sulphuric acid was added to the mixture by the side of the test tube. The upper layer in the test tube turned into red and sulphuric acid layer showed yellow with green fluorescence. This indicated presences of steroids.

2.6.8 Test for glycosides

Exactly 2 mL of the plant extract was placed in a test tube. Three mL of chloroform and 10% ammonia solution was added. Formation of pink color indicated presence of glycosides.

2.7 Quantitative Analysis of the Phytochemical Constituents

2.7.1 Determination of total phenolic content

Preparation of standard gallic acid for calibration: The total phenol content (TPC) was determined with the Folin- Ciocalteu's assay using gallic acid as standard. To prepare standard gallic acid stock solution, 0.25 g of analytical standard gallic acid was weighed using analytical balance (Mettler Toledo) and dissolved using 250 mL of methanol in a 250 mL volumetric flask. Various concentrations of gallic acid solutions in methanol (10, 20, 40 and 80mg/mL) were prepared from the standard solution. To each concentration, 5mL of 10% Folin–Ciocalteu reagent (FCR) and 4mL of 7% Na_2CO_3 were added making a final volume of 10 mL. The obtained blue colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Absorbance was measured at 760 nm against blank. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve [17].

Preparation of the sample for determination of phenolic content: About 0.01 g of dry sample was weighed using analytical balance and dissolved in 10 mL of methanol. The procedure as described for standard gallic acid was followed, and absorbance of the plant extracts was recorded. The sample was prepared in triplicate and the average value of absorbance was used to calculate the concentration of extract using the regression equation of the calibration curve. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in the samples was calculated by using the formula:

$C = Df.c\ v/m$

Where Df= dilution factor, C = total phenolic content mg GAE/g dry extract, c= concentration of gallic acid obtained from calibration curve in mg/mL, v= volume of extract in mL and m = mass of extract in gram.

Values were expressed in mean ± standard deviation in terms of phenol content per g of dry weight.

2.7.2 Determination of total flavonoid content

Preparation of standard quercetin for calibration curve: Total flavonoid contents in the extracts were determined by aluminum chloride assay using quercetin as the standard. Various concentrations of quercetin solutions in methanol (10, 20, 40, 80 and 160 mg/mL) were prepared from the standard solution. One mL quercetin of each concentration was added to the test tube containing 4 mL of distilled water. At the same time, 0.3 mL of 5% $NaNO_2$ was added to the test tube. After 5 min 0.3 mL of 10% $AlCl_3$ was added. Six minutes later, 2mL of 1M NaOH was added to the mixture. The volume of the mixture was made 10mL by immediately adding 4.4 mL of distilled water. Absorbance was measured at 420 nm against blank. The total flavonoids content was expressed as quercetin equivalents using the linear equation based on the calibration curve [18].

Preparation of the sample for determination of flavonoids content: About 0.01 g of the dry sample was weighed and dissolved in 10 mL of methanol. The procedure as described for standard quercetin was followed, and absorbance of the plant extracts was recorded. The sample was prepared in triplicate and the average value of absorbance was used to calculate the concentration of flavonoids in the extract using the regression equation of the calibration curve. The flavonoid content was expressed as quercetin equivalent (mg QE/g). Values were expressed in mean ± standard deviation in terms flavonoid content per g of dry weight.

2.8 GC-MS Analysis

The extracts of *S. incanum* was initially dissolved in HPLC grade methanol and filtered through 0.45 µM syringe filters. They were then transferred into auto-sampler vials for GC-MS (Shimadzu QP 2010) for further analysis. Carrier gas used was ultrapure Helium with the flow rate set at 1mL / minute. A BPX5 non-polar column,

30m; 0.25 mm ID; 0.25 µM film thickness, was used for separation.

The GC-MS machine was set and programmed as follows: temperature of 60 ˚C (1 minute). This was subsequently increased at a rate of 10 ˚C /min up to 270 ˚C (18 minutes) with the total run-time being exactly 30 minutes. 1 µL of the sample was injected into the GC at 200 ℃ in split mode. This were in split ratios of 10:1 with the interface temperatures set at 250 ℃. The Electron Ionization (E.I) ion source was set at 200 ℃. Mass analysis, was done in full scan mode within the ranges 50-500 m/z and the detected peaks auto-matched against the NIST libraries for possible identification. Both Fragmentation patterns and retention index, were used for matching.

2.9 FT-IR Analysis

A Shimadzu Fourier Transform Infrared spectrophotometer, Model FTS- 8000, was used to study FT-IR spectra with signals provided in wave numbers (cm-1) as usual in order to monitor the functional groups. The KBr pellets of samples were prepared by mixing 10 mg of samples, finely ground, with 250 mg KBr (FT-IR grade). The 13 mm KBr pellets were prepared in a standard device under a pressure of 75 kN cm–1 2 for 3 min. The spectral resolution was set at 4 cm–1 and the scanning range from 400 to 4000 cm–1.

2.10 *In vitro* Antibacterial Activity of the Plant Extract

Disc diffusion method was used for antibacterial activity on a nutrient agar. Plant extract was prepared by dissolving 0.01, 0.05 and 0.15 g in 10 mL of ethanol. 2 µL of each dissolved plant extract was impregnated into sterile, blank discs 6mm in diameter. The discs were allowed to dry before the next 2 µL was spotted to ensure precise impregnation. The positive control used was ampicillin antibiotic discs' treatment with same concentration as that of plant extract. Ethanol- loaded discs were used as negative controls. All discs were fully dried before the application on bacterial lawn. The plates were incubated in an upright position, at 28 °C for 48 hours. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs using a 30 cm ruler. The assay was repeated four times. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the fruits ethanol extract [19].

2.11 In Vivo Antibacterial Activity of the Plant Extract

An in vivo experiment was conducted in a greenhouse on the campus ground at Moi University to study the effects of *S. incanum* fruits extract on the development of bacterial wilt in tomatoes. Tomato seeds of the cultivar Rio Grande were sterilized using sodium hypochlorite and later rinsed with distilled water. They were later placed on moist filter paper in a petri dish and incubated at 28 ± 2 ℃ for seven days. After seven days they were transplanted into sterilized soil in 17 cm experimental pots. Each pot contained one kilogram of soil sterilized using Autooclave (Electric Model No. 25X) at 121 psi for 20 minutes.

After 5 weeks, each pot received a treatment of 0.15 g/10 mL of dried plant material of fruit extract of *S. incanum*. For comparison, two control treatments were used: one with 0.15g/10 mL of the standard antibiotic ampicillin, the other control treatment was 10 mL of distilled water that was applied simultaneously each day. Inoculations were performed by pouring 40 ml of a 1.07 x10^7 CFU/mL bacterial suspension on every pot after making four holes in the soil with a disposable 5 mL syringe. The greenhouse experiment was performed under a completely randomized design protocol with four replicates. All plants were watered as needed [19].

The disease incidence was recorded following the equation below.

Disease incidence= (Number of infected plants)/ (Total number of experimental subject) ×100

2.12 Statistical analysis

GC-MS/MS data files were analyzed with reference to web-based resources (e.g. Chemspider) and published research papers. ANOVA was used to statistically compare difference between different treatments used against the bacteria.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Determination of the percentage yield is very important as it is significant toward discovery of

drugs and bio-insecticides. A yield of 5.67 ± 0.2%was obtained in this study. Amount of yield extracted depends on methodology, solvent to solid ratio, extracting solvent, duration and particle size [20]. Higher solvent to solid ratio, longer duration, finer particles and polar solvents results to higher yield. Total quantity of the yield has great effect on the overall and selection for bio-prospecting and in the calculation of total antibacterial activity [21]. Higher yield means more phytochemicals have been extracted thus higher biocidal activity [22].

3.2 Phytochemical Analysis

Phytochemical analysis of S. incanum fruits ethanol extract tested positive for alkaloids, phenols, flavonoid, steroids, tannins, glycosides, terpenoids and saponins as given by Table 1. Several other researchers had previously reported about the presence of alkaloids, flavonoids, tannins, glycosides, saponins, and steroids in the plant [23]. In addition, the identified phytochemicals have been reported previously in the fruits extract of *Solanum nigrum* [24] and ethanolic extract of *S. melongena* [25] which are examples of plants belonging to family Solanaceae. Presence of the aforementioned phytochemicals in *Solanum incanum* ethanolic fruits extract implies that the plant can be used to control plants pests and pathogens. Phenols for example, from their simple structure they penetrate the microorganisms resulting to considerable damage to the cell metabolism [26]. On the other hand, flavonoids inhibit synthesis of nucleic acid, alters the permeability of membrane, inhibits the function of cytoplasmic membrane among other effects [27]. Similarly, alkaloids inhibit synthesis of nucleic acid and tannins destroys bacterial membrane and deters biofilm formation by its bacteriostatic properties [28]. It could then be considered from chemical composition of *S. incanum*, its ability to affect pests and pathogens.

3.3 Quantitative Analysis of Ethanolic Extract of *Solanum incanum* Fruits

3.3.1 Total phenolic content

Total phenolic content was calculated from regression equation of the calibration curve (Y = 0.0098x + 0.0177; R2 = 0.9967). The total phenolic content in ethanol fruits extract was found to be 84.997 ± 0.2 mg/g of the dried sample. Comparing the work of literature, Mongalo et al., [29] reported a total phenolic content (TPC) of 490.7 ± 0.02 mg/g GAE in *Solanum panduriforme*. This was six times higher compared to that reported in this study. On the other hand, Ghosal & Mandal [30] reported a total phenolic content of 2.306 ± 0.37 mg/g in fruits extract of *S. incanum*. This was 41 times lower compared to the TPC reported in this study. Further, Aryal et al., [31] found the TPC to be 97.96 ± 0.62 mg GAE/ g dry extract weight of *S. nigrum* fruits extract which was slightly higher compared to the one reported in the current study.

Phytochemicals are polar or non-polar in nature, thus the type of solvent used for extraction is accountable for dissolving the compounds of the plant. Phenolic compounds contains hydroxyl group making them more soluble in polar organic solvent [32]. Similarly, duration, abiotic environmental factors, geographical variation, methodology used for extraction can also cause difference in total phenolic content [33]. Environmental factors, such as ultraviolent radiation, rainfall, temperature and soil composition affects the concentration of phenolic compounds in the plant [34]. Availability of nutrients, water and light lead to preferential allocation of photo-synthetically absorbed carbon to the growth and development of the plant other than in concentration of secondary metabolites [35]. Geographical regions with severe climatic conditions and low humidity have been reported to contribute to increase in total phenolic content in the plants [36].

3.3.2 Total flavonoid content

Total Flavonoid content was calculated from regression equation of the calibration curve (Y = 0.0021x – 0.0055; R2 = 0.9998). The TFC content of the plant extract was found to be 20.535 ± 0.2 mg/g of dried sample. In a survey of previous literature work, Mongalo et al.,[29] reported a total flavonoid content (TPC) of 23.92 ± 0.92 mg/g QE dry weight of aqueous extract of *S. panduriforme*. These was slightly higher compared to the results of this study. In another study by Ghosal & Mandal [30] the total flavonoids content of methanolic fruits extract of *S. incanum* was 0.207 ± 0.09 mg/g QE dry weight. This was very much lower compared to the TFC reported in this study. Similarly, Nwanna et al., [37] reported low TFC content of 1.50 ± 0.17 mg QE/g of S. incanum aqueous fruits extract.

Table 1. Phytochemical compounds identified in *Solanum incanum* ethanol fruits extract

S/N	Phytochemicals	Type of test performed	Inferences
1	Alkaloids	Wagner's test	+++
2	Saponins	Froth test	++
3	Steroids	Salkowskis test	+
4	Phenolics	Ferric chloride test	+++
5	Tannins	Ferric chloride test	++
6	Glycosides	Keller Kelliani test	+
7	Flavonoids	Alkaline reagent test	+++
8	Terpenoids	Salkowski's test	+

Key +++ = Highly present, ++ = Moderately present + = Low amount present

Environmental stressors contribute to variation in amount of TFC in plant. For example, increased in UV-radiation, pathogen attack, nutrient deficiency and high light results to increased phenylpropanoids which is a general pathway in plant for flavonoid synthesis [38]. According to [39] seasonal changes such as, shifting of temperature from low to high using proper frequency as well as moderate drought are very beneficial for accumulation of flavonoids in plants. Low level of nitrogen and phosphorous contribute to increased content of flavonoid in plants which is correlated to reduced content of soluble protein [40]. Soil condition example nutrition stress and low amount water also contribute to difference in amount of flavonoids present the plant [34].

3.4 GC-MS Results of *Solanum incanum*

A Shimadzu QP 2010-SE GC-MS was used. The total run rime was 44 minutes. Several compounds were detected and their fragmentation patterns and retention indexes compared to NIST 2014, MS library. The GC chromatogram was as shown in Fig. 1 and summary of the compounds name, retention time, molecular weight, molecular formula, area, and area% are summarized in Table 2.

GC-MS results showed that the extract was a complex mixture of 15 bioactive compounds; with some being present in trace amount. On the other hand, 1-methoxy, 9-Octadecene (E) - (26.85%), 9-Octadecenamide, (Z) (21.43%), E-15-Heptadecenal (7.28%), E-14-Hexadecenal (6.28), n-Tetracosanol-1 (5.45%), 2,4-Di-tert-butylphenol (4.96%) were present in considerable amount. The structures of the identified compounds are given in Fig 2.

The compound 1-Dodecene is plant volatile compound which has been previously detected using GC-MS in *S. incanum,Oryza sativa*¸ *Ipomoea separia*, *Seneciocoincyi*, *S. congestus* among other plants [41-43]. This compound has been reported to have antimicrobial and antioxidant activity as well as used in manufacture of detergents [44,42]. 1-Tridecene is a plant metabolized fatty acid which has been previously reported to have remarkable antibacterial activity as well as use in treatment of respiratory irritation in human beings [45]. The compound 1-Tridecene has been identified using GC-MS in Solanaceae species such as *Solanum spirale* and *S. incanum* [46].

Phenol, 2,4-di-tert-butyl- (2,4-DTBP) is a natural occurring lipophilic phenol which has been reported in about 169 species of organisms [47] and in plants including *S. incanum*, *S. nigrum*, and *S. lycopersicum* where it's reported to enhance disease resistance [48-49]. The phenol has been reported to have antifungal, antibacterial, antioxidant, anti-Inflammatory and antimalarial activity [50-51]. E-14-Hexadecenal and E-15-Heptadecenal are aldehydes which have been previously reported in solanum species such as *S. xanthocarpum* [52], and have good antibacterial and antioxidant activity.

The compound n-Tetracosanol is a plant metabolite which is alcoholic in nature and has been reported to have good antibacterial activity [53]. n-Tetracosanol has been identified using GC-MS in *S. incanum* and *S. melongena* [54-56]. Thunbergol belong to group of terpens and has remarkable antioxidant activity. It has been previously identified in *Solanum dasyphyllum* using GC-MS [57]. 2,3-Dehydro-4-oxo-beta-ionol was previously reported in *Abutilion hirtum* [58]. 2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z)- has been identified using GC-MS in *S. tuberosum* [59], and has found application as precursor for organoleptic compounds especially for fragrance, flavor, masking agent as well as antimicrobial compound [60].

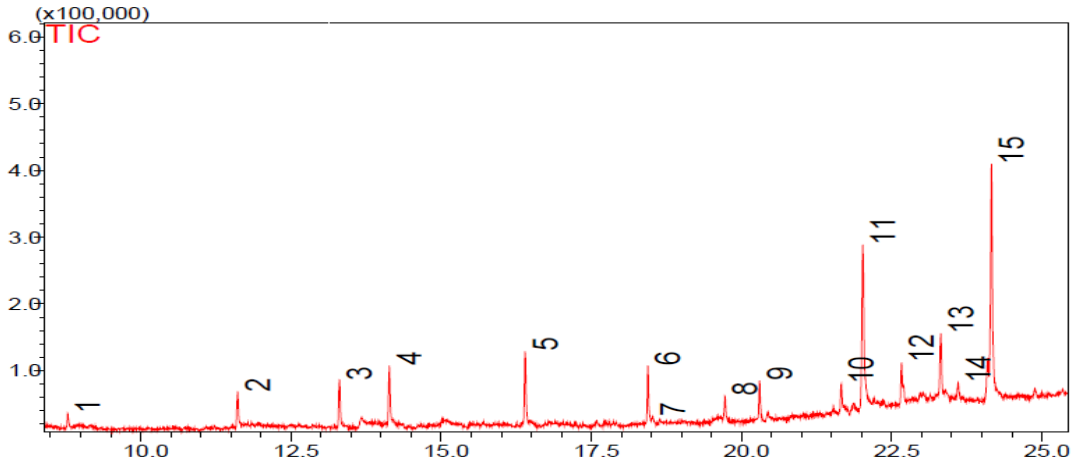

Fig. 1. GC chromatogram for *Solanum incanum* extract

9-Octadecenamide is an amide derived from fatty acid oleic acid and has a role as plant metabolite as well as human metabolite. The most recognized function of 9-Octadecenamide, (Z) is its sleep inducing effect as well as remedy for mood disorder and cannabinoid–regulated disorder [61]. The compound has also been reported to have antibacterial and anti-inflammatory activity [62]. 9-Octadecenamide (Z) has been reported to be present in Solanaceae plants such as *S. torvum*, *S. xanthocarpum*, *S. nigrum* and *S. incanum* using GC-MS [63,64]. (9E)-1-Methoxy-9-Octadecene is a plant hydrocarbon which has been previously identified using GCMS in plant such as *Terminalia catappa* and palm oil [65,66].

The identified compounds in *S. incanum* confirms the functional groups shown on the FT-IR spectra (Fig. 3). Presence of OH group could correspond to 2,4-Di-tert-butylphenol, 2,3-Dehydro-4-oxo-beta-ionol, n-Tetracosanol-1and Thunbergol. Presence of 1-Tridecene and 1-Dodecene correspond to the presence of alkene group. 2H-Pyran-2-one, tetrahydro-6-(2-pentenyl)-, (Z) and 9-Octadecene, 1-methoxy-, (E)- correspond to CO stretch and Alkyl substituted ether C-O stretch respectively. Butanoic acid, 4-formylphenyl ester correspond to carboxylic acid stretch. N-H stretch vibration may be due to presence of 9-Octadecenamide, (Z)-.

3.5 FT-IR Analysis of Crude Ethanol Extract of *Solanum incanum* Fruits

The FT-IR spectrum (Fig. 3) of the crude extract was used to identify the functional groups of the bioactive compounds present in *S. incanum* fruits. FT-IR analysis of the extract confirmed the presence of alcohol, alkanes, aromatic, carboxylic acid, alkyl halide, alkene, primary and secondary amines and ethers. The peak at 3348 cm^{-1} correspond to OH stretching in alcohol and phenol, 2931 cm^{-1} is attributed to C-H vibration of alkane groups. The peak at 1589 cm^{-1} is due to amides N-H characteristics of enzyme and protein, a peak at 1396 cm^{-1} represent C=C aromatics, a peak at 1218 cm^{-1} is attributed to CO stretch. A peak at 1110 cm^{-1} is assigned to alkyl substituted ether C-O stretch (C-O-C). The peak at 995.2 cm^{-1} is assigned to N-H stretch vibration, primary and secondary amines, peaks at 702.0 cm^{-1} and 601 cm^{-1} are attributed to alkyl halide and 524-493 cm^{-1} represent C-H out of plane bending alkene groups. These functional groups are integral parts of most bioactive compounds such as tannins, flavonoids, polyphenol, alkaloids and terpenoids [67]. Functional groups in S. incanum fruits makes them a potential source of therapeutic products such as for anti-cancer, anti-ulcers, headache, stomachache, and as source of antimicrobial compounds [68].

3.6 In vitro Antibacterial Activity of the Plant Extract

The ethanolic fruits extract of *Solanum incanum* produced auspicious results with the capability to deter the growth of *R. solanacearum*. Its activity was comparable (P =.02) to that of ampicillin. A clear zone around the agar disk was observed. The diameter of the zones of inhibition depicts the relative vulnerability of the bacterium toward the antimicrobial agent. The results on antibacterial activity against *R. solanacearum* is presented in Table 3.

Table 2. A summary of information obtained from GC-MS showing names, chemical formulas, molecular weights and possible compounds based on compared NIST 2014 entries. GC retention time (RT) is also provided

Peak #	R. time	Area	Area %	Molecular weight	Molecular formula	Name
1	8.782	36348	1.48	168	$C_{12}H_{24}$	1-Dodecene
2	11.611	115750	4.73	182	$C_{13}H_{26}$	1-Tridecene
3	13.307	121512	4.96	206	$C_{14}H_{22}O$	2-4-Di-tert-butylphenol
4	14.136	153861	6.28	238	$C_{16}H_{30}O$	E-14-Hexadecenal
5	16.397	178327	7.28	252	$C_{17}H_{32}O$	E-15-Heptadecenal
6	18.441	133601	5.45	354	$C_{24}H_{50}O$	n-Tetracosanol-1
7	18.519	6639	0.27	290	$C_{20}H_{34}O$	Thunbergol
8	19.722	57864	2.36	227	$C_{11}H_{17}NO_4$	4a-Hdroxy-7-methyl-4-nitrooctahydro-1-(2H)-naphthalenone
9	20.300	91291	3.73	354	$C_{24}H_{50}O$	n-Tetracosanol-1
10	21.659	48905	2.00	192	$C_{11}H_{12}O_3$	Butanoic acid, 4-formylphenyl ester
11	22.018	524948	21.43	281	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-
12	22.663	133784	5.46	206	$C_{13}H_{18}O_2$	2,3-Dehydro-4-oxo-beta-ionol
13	23.315	152683	6.23	168	$C_{10}H_{16}O_2$	2H-Pyran-2-one, tetrahdro-6-(2-pentenyl)-Z-
14	23.606	36236	1.48	354	$C_{24}H_{50}O$	n-Tetracosanol-1
15	24.160	657531	26.85	282	$C_{19}H_{38}O$	(9E)-1-Methoxy-9-octadecene
		2449280	100.00			

Table 3. Mean zones of inhibition (mm) of ethanolic fruits extract, ampicillin and ethanol

Treatment	Concentration	Mean zone of inhibition (mm)
Ethanolic fruits extract	0.01 g/10 mL	20.75 ± 1.3
	0.05 g/10 mL	25.75 ± 0.5
	0.10 g/10 mL	27.25 ± 0.5
	0.15 g/10 mL	30.75 ± 0.5
Ampicillin (positive control)	0.01 g/10 mL	26.75 ± 0.5
	0.05 g/10 mL	28.75 ± 0.5
	0.10 g/10 mL	31.75 ± 0.5
	0.15 g/10 mL	35.00 ± 0.0
Ethanol (negative control)		0.0

In a study by Aloo et al., [69] aqueous flower extract of S. incanum showed an inhibition zone of 9.3 ± 0.0, 10.0 ± 0.0, and 10.0 ± 0.6 mm at a concentration of 0.01, 0.05, 0.15 g l-1 against *R.solanaceraum* respectively. This was higher inhibition compared to that of positive control (amoxicillin) which had an inhibition zone of 9.9 ± 1.1. In the same study, the aqueous root extract of *S. incanum* showed an inhibition zone of 9.7 ± 0.6, 10.3 ± 0.5 and 10.3 ± 0.7 at the aforementioned concentrations respectively. The amoxicillin had an inhibition zone of 10.6 ± 3.8. Fruits extract of the plants were reported to have a diameter of inhibition of 13.2 mm against *Colletotrichum gloeosporoides* a fungus that affect pawpaw production in Kerio Valley region in Kenya [70]. *S. incanum* have also been reported to have antibacterial activity against clinical bacteria. Methanolic fruits extract had an inhibition zone of 10.1 ± 0.3 mm against *Staphylococcus aureus* [71]. In another study, 100 µg/ml of methanolic fruits extract of *S. incanum* had a diameter of inhibition zone of 39 ± 1.0 mm against E. coli [72].

Most of the secondary metabolites are soluble in ethanol which may have contributed to the profounding activity of the extract [73]. Major antimicrobial compounds i.e alkaloids, tannins, saponins, phenols, terpenoids were reported to

be present in the extract. GC-MS analysis identified presence of compounds such as E-15-Heptadecenal, 2,4-Di-tert-butylphenol, 9-Octadecenamide, (Z)-, Thunbergol, 1-Dodecene among other compounds which have been previously reported to have antibacterial activity. Flavonoids are phenolic compounds which have been reported to have the capacity of coagulating bacteria cell proteins and affecting enzymes which are responsible for synthesis of amino acids in the bacteria [74]. Tannins directly destroys the cell membrane resulting to death of the bacteria [75]. Steroidal saponins hinders bacteria growth by reacting with membrane sterol and halting membrane function [76]. Monoterpenoid alkaloids acts upon DNA intercalating agent which sometime causes poisoning [77]. Terpenoids act upon phospholipids bilayer of cells affecting process like protein translocation, electron transport, phosphorylation steps and enzyme dependent reaction which eventually disrupt the cell membrane thus hindering growth of the bacteria [78].

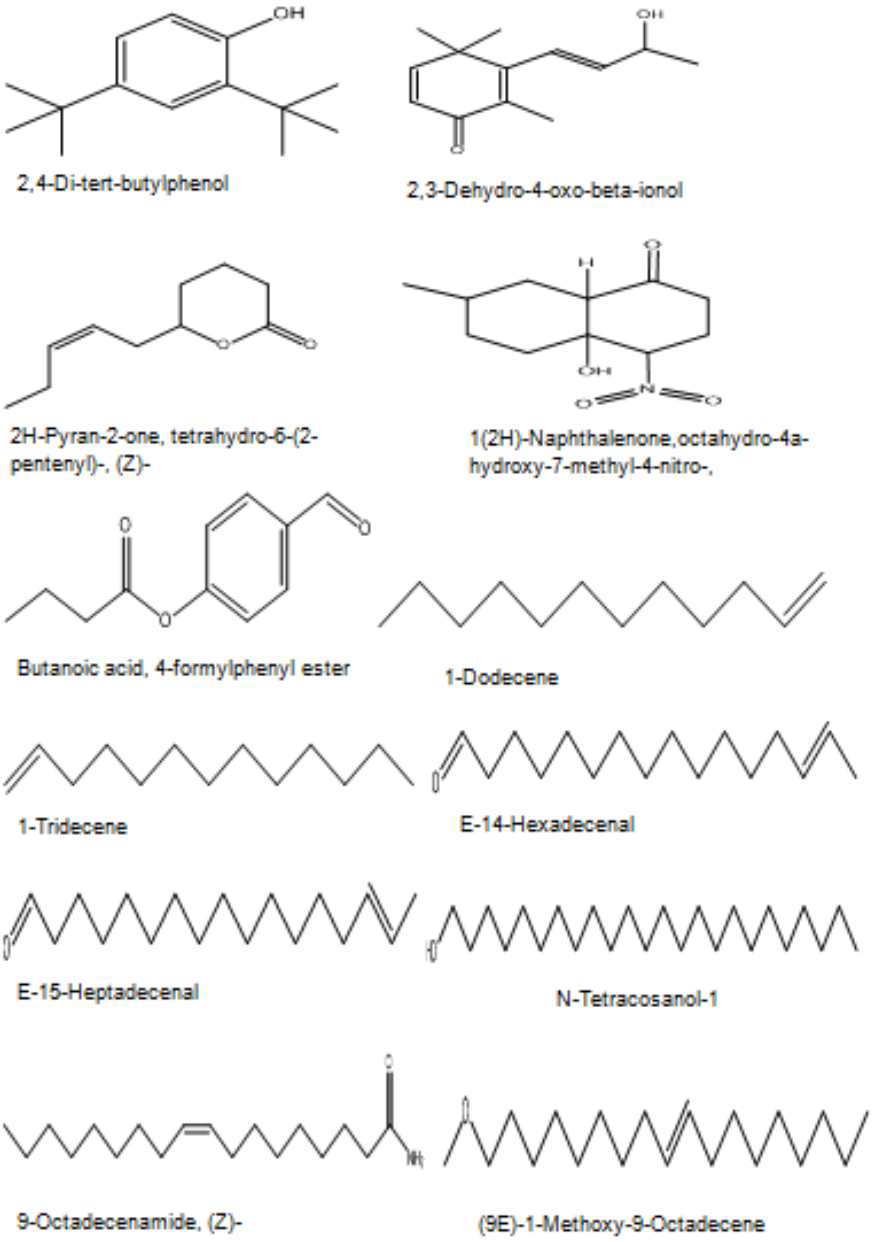

Fig. 2. Structures of compounds identified in *Solanum incanum fruits extract*

3.7 Effect of Plant Extract and Ampicillin on Disease Incidence

Bacterial wilt related symptoms were observed 4 days post inoculation in distilled water (negative control) treatment as seen in Fig. 4. In contrast, these symptoms were not observed in plant extract and ampicillin treatment. This depicts that plant extract and ampicillin delayed the development of the disease until after8 and 10days respectively. Even then, 8 days post inoculation the disease incidence in plant extract (0.15 g) treatment was 20% while no diseases incidence was observed in ampicillin treatment. Twelve days post inoculation the percentage disease incidence was 20.0 ± 0.0% in ampicillin treatment and 40.0 ± 6.7% in plant extract treatment. Highest percentage diseases incidence for plant extract treatment was observed 16 days post-inoculation which was 100.0 ± 0.0% while that of ampicillin was 60 ± 6.7%. Twenty-two days post-inoculation the disease incidence in ampicillin treatment was 80.0 ± 0.0%. Only one plant in each replicate survived in ampicillin treatment in the entire study period (90 days). These results complemented that of in vitro studies since the activity of ampicillin was also slightly higher compared to that of the plant extract. Similar results were reported by [79].

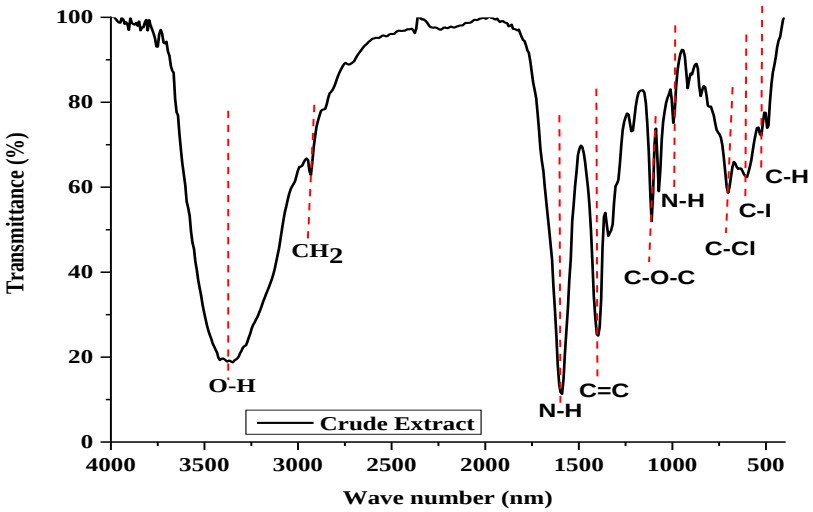

Fig. 3. FT-IR spectra of *S. incanum* crude extract

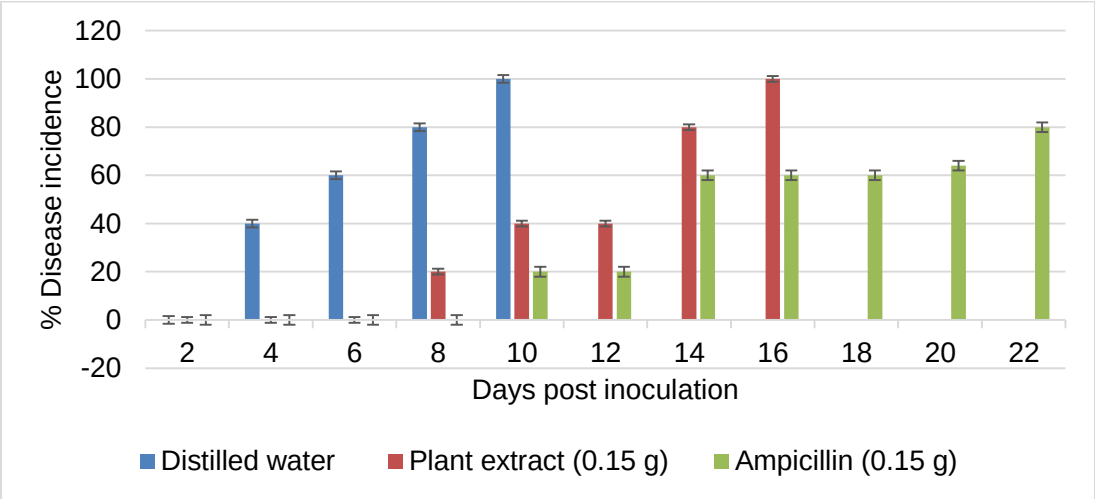

Fig. 4. Suppressive impact of plant extract and that of ampicillin

4. CONCLUSION

The results of the study revealed that the plant contains potential bioactive compounds such as 2,4-Di-tert-butylphenol, 1-Dodecene, E-14-Hexadecenal, E-15-Heptadecenal, n-Tetracosanol-1,9-Octadecenamide (Z) among others. It also depicted that there is a greater potential of getting effective compounds from natural sources, which can be of importance in control of plant pathogens. Purified compounds may even have higher potential of inhibiting microbe growth. More work on purification of individual constituent may disclose the precise capacity of the plant to control many plant pathogens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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