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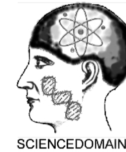

Effect of Solvent Extracts of Some Plants on *Ralstonia solanacearum*

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Authors' contributions

This work was carried out in collaboration between all authors. Author TES conceived, designed the study, proof read and corrected the draft while author AAO coordinated the laboratory work and drafted the manuscript. Both authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To study the efficacy of different solvent extracts (chloroform, ethanol, methanol and hexane) of ten plants on *Ralstonia solanacearum* the causal organism of bacterial wilt of tomato.
Place and Duration of Study: Departments of Crop Production, Soil and Environmental Management and Biological Sciences, Bowen University, Iwo, Nigeria from August 2011 to April 2012.
Methodology: Ten plants namely *Ocimum gratissimum*, *Vernonia amygdalina, Allium sativum, Zingiber officinale, Cymbopogon citratus, Azadirachta indica, Jatropha curcas, Senna obtusifolia, Senna occidentalis* and *Senna alata* were collected from Iwo, air dried and pulverized. Chloroform, ethanol, methanol and hexane were used to extract active ingredients from the ten plants. The solvent extracts were tested against *R. solanacearum* the causal organism of bacterial wilt of tomato and other plants using the disc diffusion method on Mueller Hinton agar. The minimum inhibitory concentration (MIC) of the effective extracts was determined.
Results: The plant extracts from chloroform were the most active and this was followed by methanol and ethanol, the lowest activity was recorded from the hexane extracts. The chloroform extracts of *J. curcas* had the widest zone of inhibition of 15mm followed by *O. gratissimum* (13mm). All the solvent extracts of *A. sativum* were active except the hexane

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extract. The means and standard error of triplicate tests were recorded. The MIC of the active extracts were studied, the MIC of the *A. sativum* ethanolic extract was 0.25 mg/ml while it was 0.5mg/ml for the *V. amygdalina* ethanol extract. The MIC of the *A. sativum* chloroform extract was 0.25mg/ml; *J. curcas* chloroform extract MIC was 0.125mg/ml, and the MIC for methanolic extract of both extracts were 0.5mg/ml and 0.25mg/ml respectively.
Conclusion: The activities of the different solvent extracts are remarkable when compared with the water extracts. Hence, solvent extracts will enhance the efficacy of these phytochemicals in the management of *R. solanacearum* infections as opposed to water extracts.

Keywords: Ralstonia solanacearum; plant extracts; crop protection; disc diffusion.

ABBREVIATIONS

MIC: Minimum inhibitory concentration; TTC: Triphenyl tetrazolium chloride; NIHORT: National institute for horticultural research and training.

1. INTRODUCTION

Ralstonia solanacearum, formerly known as *Pseudomonas solanacearum* and *Burkholderia solanacearum* is the causal agent of bacterial wilt of tomato [1]. *R. solanacearum* is an aerobic non-sporing, Gram negative plant pathogenic bacterium. It is soil borne and motile with a polar flagellar tuft and sometimes 1 to 4 polar flagella. It colonizes the xylem, causing bacterial wilt in a wide range of potential host plants [2]. This soil borne vascular pathogen is widely distributed in tropical and subtropical climates and affects an unusually broad range of crops including monocots and dicot plants [3,4]. Many affected hosts are of economic importance in developing countries because of their strategic importance as cash crops or as subsistence foods like potato (*Solanum tuberosum*), tomato (*S. lycopersicum*), egg plant (*S. melongena)*, banana (*Musa* spp) and peanuts (*Arachis hypogea*) [5]. *R. solanacearum* can survive for a long time in water (up to 40 years at 20-25ºC in pure water) and the bacterial population is reduced in extreme conditions of temperature, pH, salts etc. Infected land sometimes cannot be used again for susceptible crops for several years. *R. solanacearum* can also survive in cool weather and enter a state of being viable but not culturable [6]. Large numbers of *R. solanacearum* can be shed from roots of symptomatic and asymptomatic plants. Bacteria ooze on plant surfaces and can enter the surrounding soil or water, contaminating farming equipment or may be acquired by insect vectors. In addition, this pathogen can be spread out by contaminated flood water, irrigation, contaminated tools or infected seeds [4]. Aqueous extracts of some plants that have been shown to have medicinal properties did not have inhibitory effects on *Ralstonia solanacearum* [7], hence the need to study the effects of different solvent extracts other than water to repress the growth of *R. solanacearum.*

2. MATERIALS AND METHODS

2.1 Sample Collection

Ten local plants were used in the study. The parts of the plants that were used in this study are the most popular and most readily available. They were obtained from Iwo, Osun State Nigeria. They are shown in the Table 1 below.

2.2 Sample Preparation

The various plant parts were collected and dried at room temperature. The dried plants were pulverized. Ten (10) grams of each ground plant material was weighed into glass containers. One hundred (100) milliliters of the extracting solvents i.e. ethanol, methanol chloroform and hexane were added and labeled accordingly. The samples were shaken constantly and filtered using Whatmann No 1 filter paper after 24h. The filtrate was put in a rotary evaporator until a thick pomace remained. The thickened extract was kept in the refrigerator.

Table 1. Plants sampled and parts used

Botanical name	Name	Part of plant used
1. *Ocimum gratissimum*	Basil	Leaf
2. *Vernonia amygdalina*	Bitter leaf	Leaf
3. *Allium sativum*	Garlic	Bulb
4. *Zingiber officinale*	Ginger	Rhizome
5. *Cymbopogon citrates*	Lemon grass	Leaf
6. *Azadirachta indica*	Neem	Leaf
7. *Jatropha curcas*	Physic nut	Leaf
8. *Senna obtusifolia*	Sickle *Senna*	Leaf
9. *Senna occidentalis*	Stink weed	Leaf
10 Senna alata	Candle bush	Leaf

2.3 Test Bacterium

The test bacterium *Ralstonia solanacearum* was collected from National Institute for Horticultural Research and Training (NIHORT) Ibadan, Nigeria. The bacterium was grown on Triphenyl Tetrazolium chloride (TTC) agar prior to inoculation and maintained on nutrient agar for long term culture storage [7].

2.4 Effects of Plant Extract on Test Bacteria

Antibacterial activity of the extracts was determined by the disc diffusion method on Mueller Hinton agar [8]. An overnight culture of the bacterium was diluted to 10^5 cells/ml using a spectrophotometer (Jenway 6305, UK) at a wavelength of 625nm. One milliliter of the bacterial suspension was introduced into sterile Petri dishes and 20 ml of Mueller Hinton agar at 40ºC was poured into the inoculated dishes. The plates were allowed to cool and solidify. A sterile filter disc (Whatmann No. 9) soaked in the different extracts of with a concentration of 1mg/ml was picked with sterile forceps and placed on the surface of a solid inoculated agar plates. The plates were incubated at 37ºC for 24h. This was carried out in triplicates. The Petri dishes were then assessed for antimicrobial activities [8,9]. The control consisted of the solvents alone and served as the negative control.

2.5 Minimum Inhibitory Concentrations (MIC) of the Active Extracts

This was determined by adding 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml of each extract into test tubes containing sterile nutrient broth. One milliliter of 10^5 cells/ml of *R. solanacearum* (as determined above) was introduced in the nutrient broths containing solvent extracts at different concentrations that showed susceptibility to *Ralstonia* in the previous test. The tubes were then incubated for 24h at 37ºC. The MIC was taken as the

lowest concentration of the extracts that did not permit any visible growth of *Ralstonia* [10, 11].

2.6 Antibiotic Sensitivity of *Ralstonia solanacearum*

Different antibiotics were tested against the growth of *R. solanacearum* using the antibiotic sensitivity multidisc (Abtek Biologicals Ltd) containing 8 antibiotics. The antibiotics were gentamycin (10µg), nitrofurantoin (200µg), co-trimoxazole (25µg), amoxycylin (10µg), tetracycline (10µg) augmentin (30µg), ofloxacin (5µg) and nalidixic acid (30µg). The bacterial suspension (1 ml of 10^5 cells/ml) was inoculated on Mueller Hinton agar. The multidisc was placed on the surface using sterile forceps. The plates were incubated for 24h and zones of inhibition were observed and recorded [7,12]. This served as the positive control.

3. RESULTS AND DISCUSSION

Crude extracts prepared from different plants using different solvents were tested against *R. solanacearum*. These extracts had varying antibacterial effects on the test bacterium.

Table 2 gives the diameters of zones of inhibition of the solvent extracts on *R. solanacearum*. The highest zone of inhibition was from the *J. curcas* chloroform extract (15mm) followed by *O. gratissimum* chloroform extract (13mm). The chloroform extracts were more active followed by the methanol and ethanol extracts. Least activity was recorded by the hexane extracts. All *A. sativum* solvent extracts were very active, with the exception of its hexane extract. All *Z. officinale* and *O. gratissimum* solvent extracts were as well very active except their ethanol extracts (Table 2) while none of the *A. indica* extracts were active in inhibiting *Ralstonia* growth .

The MIC of the active extracts was studied and results are reported in Fig. 1. The MIC of the *A. sativum* ethanoholic extract was 0.25 mg/ml while it was 0.5mg/ml for the *V. amygdalina* ethanol extract. The MIC of the *A. sativum*, *O.gratissimum* and *Z. officinale* chloroform extracts was 0.25 mg/ml; *J. curcas* chloroform extract MIC was 0.125mg/ml. The MIC for methanolic extract of both *A. sativum* and *Z. officinale* was 0.5 mg/ml while for *J. curcas* and *O. gratissimum* was 0.25 mg/ml respectively (Fig. 1).

Table 2. Effects of Solvent extracts on *R. Solanacearum*

	Plant	Diameter of zone of inhibition (mm*)				
		Ethanol	**Chloroform**	**Hexane**	**Methanol**	**Control**
1.	*O. gratissimum*	-	13	8	11	-
2.	*V. amygdalina*	8	7	-	-	-
3.	*A. sativum*	12.5	10	-	10	-
4.	*Z. officinale*	-	12	9	11	-
5.	*C. citratus*	-	8	-	-	-
6.	*A. indica*	-	-	-	-	-
7.	*J. curcas*	-	15	-	12	-
8.	*S. obtusifolia*	-	-	-	9	-
9.	*S. occidentalis*	-	-	-	9	-
10.	*S. alata*	-	10	-	-	-

**mean ± SEM of triplicates; - no inhibition.*

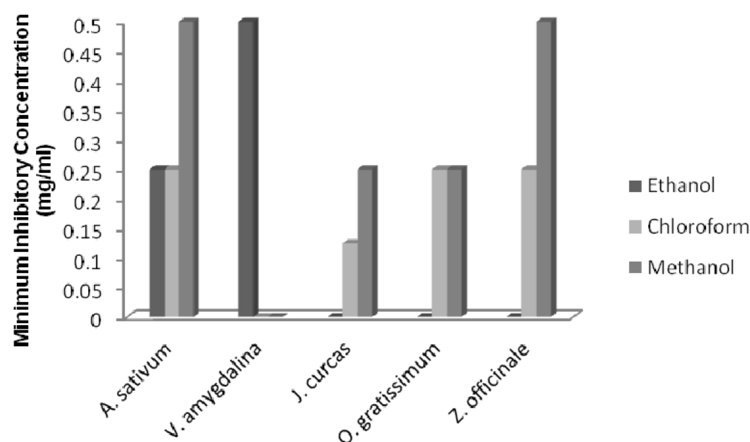

Fig. 1. Minimum inhibitory concentration of effective extracts

The sensitivity of the test bacterium, *R. solanacearum* to antibiotics was very low. The highest zone of inhibition was obtained with augmentin (14mm) and this was followed by amoxycilin (12mm) and gentamycin (11mm). Such results are reported in Table 3.

Table 3. Sensitivity of *R. solanacearum* to antibiotics

	*Tet	Amo	Aug	Ofl	Cot	Nit	Nal	Gen
Zone of Inhibition (mm)	8.5	12.5	14	11	10	-	-	11

**Tet- Tetracycline; Amo- Amoxycilin; Aug- Augmentin; Ofl- ofloxacin; Cot- Co-trimoxazole; Nit-Nitrofurantoin; Nal-Nalidixic acid; Gen-Gentamycin.*

It should be noted that the concentration of the antibiotics used for this particular test was doubled to compare with the results of our earlier study [7]. It was observed that even with the doubled concentration, sensitivity of *R. solanacearum* to these antibiotics was still low with none having above 14mm as diameter of inhibition zone.

Ralstonia solanacearum is classified as one of the world's most important phytopathogenic bacteria due to its lethality, persistence, wide host range and broad geographic distribution. *R. solanacearum* is of economic importance because it infects over 250 plant species in over 50 families [13]. It causes a wilt disease in several important agricultural crops such as potato, tomato, tobacco, banana, pepper and eggplant [14]. Many more dicots suffer from the disease than monocots. Among the monocot host, the order *Zingiberales* dominate with 55.6% of families being affected by this bacterium. The reason why some families are more susceptible to bacterial wilt is unknown [13]. Based on the results presented in this study, the antimicrobial effects of solvent extracts of different plants were tested on *R. solanacearum in vitro*. The *J. curcas* chloroform extract had the widest zone showing better inhibitory effects on the test bacterium. This was followed by *O. basilicum* chloroform extract. This indicates that the active antimicrobial agents contained in the plants and dissolved by chloroform are highly inhibitory to the test bacterium. The crude ethanolic extract of most of the plant parts used was not found to inhibit the growth of the test bacterium except the *Vernonia amygdalina* and *Allium sativum* extracts. The *A. sativum* ethanolic extracts had the widest zone of inhibition out of all the ethanolic extracts. Many of these plants have been shown to have antimicrobial properties against some other pathogens [15]. The *A. sativum* extract was

observed to possess bioactive effects attributed to the sulphur-containing molecules [16]. The widespread efficacy of garlic extract has been linked to the ease by which these molecules pass through cell membranes and react biologically at the low level of thiol bonds in amino acids [16]. *Zingiber officinale* for instance contain compounds that are active against a form of diarrhea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active ingredient against enterotoxigenic *Escherichia coli* heat labile enterotoxin induced diarrhea [17]. *Cymbopogon citratus* (lemon grass) oil is used as a pesticide and a preservative. The oil also contains anti-fungal properties [18]. Species of *Senna* have been used in the treatment of several infections. The leaves are useful in treating gonorrhea, oedema and is also used as a purgative. The ethanolic extracts of *Senna* species have been reported to show high activity against dermophytic fungi [8]. The roots of *Vernonia amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity.

All the solvent extracts of *A. indica* as well as the ethanolic extracts of *C. citratus, S. obtusifolia* and *S. occidentalis* were inefficient to inhibit the growth of the test bacterium as indicated by the results obtained in this study. This could be due to the fact that *Ralstonia* is a very difficult pathogen to inhibit and this may be a major contribution to its high occurrence and infectivity in soil, water, contaminated tools and infected seeds [6]. *A. indica* has been indicated in treatment of parasitic infections especially *Plasmodium falciparum*. Extracts of *A. indica* leaves is used as a malaria prophylaxis [19].

The efficacy of solvent extracts of *A. sativum¸ J. curcas, O. gratissimum* and *V. amygdalina* at various concentrations showed the potentials of their incorporation into effective management strategies of this important plant pathogen. These plants are commonly found in the environment and do not pose any threat to environmental safety as observed in many chemical pesticides. The antimicrobial properties that were not observed in the aqueous extracts of these plants [7] might be due to the fact that the active ingredients were not released in water or that they were not stable in water during extraction.

4. CONCLUSION

The activities of the different solvent extracts are remarkable when compared with the water extracts. Hence, solvent extracts will enhance the efficacy of these phytochemicals in the management of *R. solanacearum* infections as opposed to water extracts. Pesticide companies may also use the findings as a baseline study for formulation of phyto based “green technology” for the management of bacterial wilt of tomatoes and other members of the Solanaceae family that are often infected by *R. solanacearum.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia solanacearum* (Smith, 1986) comb. Nov. and *Ralstonia eutropha* (Davis, 1969) comb. Nov. Microbiol Immunol. 1995;39:897–904.

2. Agrios GN. Plant pathology. Amsterdam: Elsevier Academic press. 2008;647-649.
3. Hayward AC. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 1964;27:265-277.
4. Elphinstone JG. The current bacterial wilt situation: A global overview. In: Allen C, Prior P, Hayward AC, editors. Bacterial Wilt: The Disease and the *Ralstonia solanacearum* species complex. American Phytopathological Society, St. Paul, Mn, USA: APS Press 2005;9-28.
5. Remenant B, Coupat-Goutaland B, Guidot A, Cellier G, Wicker E, Allen C, Fegan M, Pruvost O, Elbaz M, Calteau A, Salvignol G, Mornico D, Mangenot S, Barbe V, Medigue C, Prior P. Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence. BMC Genomics. 2010; 11:379. PMID 20550686.
6. Andersona RC, Gardener DE. An evaluation of the wilt causing bacterium *Ralstonia solanacearum* as a potential biological control agent for the alien Kahili Ginger (*Hedychium gardnerianum*) in Hawaiian forests. Biol. Cont. 1999;15(2):89-96.
7. Sangoyomi TE, Owoseni AA, Adebayo OS, Omilani OA. Evaluation of some botanicals against bacterial wilt of tomatoes. Int. Res. J. Microbiol. 2011;2(9):365-369.
8. Ibrahim D, Osman H. Antimicrobial activity of *Cassia alata* from Malaysia. J. Ethnopharmacol. 1995;45(3):151–156.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a Standardized single disk method. Am. J. Clin. Path. 1966;45:439-496.
10. Owoseni AA, Ajayi A. Antimicrobial properties of ethanolic and aqueous extracts of *Cymbopogon citratus* on selected bacteria and fungi. J Med Appl Biosci. 2010;2:65-73
11. National committee for clinical laboratory standards (NCCLS). Methods for dilution of antimicrobial susceptibility tests of bacteria that grow aerobically. Approved standard M100- S12. Wayne, PA; 2002.
12. Owoseni AA, Ayanbamiji TA, Ajayi YO, Ewegbenro IB. Antimicrobial and phytochemical analysis of leaves & bark extract from *Bridelia ferruginea* African J of Biotechnology. 2010;9(7):1031-1036.
13. Milling A, Meng F, Denny T, Allen C. Interactions with hosts at cool temperatures, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* race 3 biovar 2. Phytopathol. 2009;99:1127-1134.
14. Janse JD, Beld HE, Elphinstone J, Simpkins S, Tjou-Tamsin NAA, Vaerenbergh JV. Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale* cuttings . J. Plant Pathol. 2004;86:147–155.
15. Thomson M, Ali M. Garlic (*Allium sativum*): a review of its potential use as an anticancer agent. Curr. Cancer Drugs Targets. 2003;3(1):67-81.
16. Lemar KM, Passa O, Aon MA. Allyl alcohol and garlic (*Allium sativum*) extract produce oxidative stress in *Candida albicans*. Microbiol. 2005;151:3257-3265.
17. Huang LJ, Chen JC, Wu SL, Kuo SC, Ho TY, Hsiang CY. Ginger and its bioactive component inhibit enterotoxigenic Escherichia coli heat- labile enterotoxin-induced diarrhea in Mice. J. Agric Food Chem. 2007;55(21):8390-8397.
18. Chaudhary FM, Shadab Q, Hanif M. Antifungal activity by lemon grass essential oils. Pak. J. Sci. Ind. Res. 1992;35:246-249.

19. Subapriya R, Nagini S. Medicinal properties of Neem leaves: A Review. Current Medicinal Chemistry-Anticancer Agents. 2005;5(2):149-156.

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