

Original Article

Chemical Composition and Antibacterial Activity of Essential Oil Extracted from *Citrus sinensis* L., *Citrus limon* L. and *Zingiber officinale* Roscoe

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ABSTRACT

Postharvest loss is a serious problem in Nigeria and a major drawback towards food security. Hence, discovery of potential agents capable of minimizing postharvest loss is urgently needed. This research is directed at investigating the *in vivo* antimicrobial activity of essential oils against bacterial pathogens isolated from *Ipomoea batatas* Lam. *Citrus sinensis* and *Citrus limon* peels and *Zingiber officinale* essential oils were extracted using hydro-distillation and their chemical constituents determined by gas chromatography mass spectrometry (GC-MS). Morphological and biochemical methods were employed for bacterial identification. A total of 22 terpenes were identified in *Citrus sinensis* comprising 13 monoterpenes, 8 sesquiterpenes and 1 diterpene. *Citrus limon* had 31 terpenes 15 of which are monoterpenes, 15 sesquiterpenes and 1 diterpene while a total of 30 terpenes were identified with *Zingiber officinale* comprising of 12 monoterpenes and 18 sesquiterpenes. Three of the four isolated bacteria identified from Hannah sweet potato were pathogenic: *Erwinia carotovora*, *Bacillus licheniformis* and *Pseudomonas fluorescens*. *Pseudomonas fluorescens* was more sensitive to the essential oils at ($p=0.05$). *Citrus sinensis* (64.29%, 9.0 ± 0.00 mm) was three fold more active than *Citrus limon* (21.43%, 3.0 ± 0.00 mm), while *Zingiber officinale* (53.57%, 7.5 ± 0.71 mm) was two and a half fold more active than *Citrus limon*. Results obtained in this study showed that essential oils extracted from *Citrus sinensis* and *Zingiber officinale* exhibited substantial antimicrobial activity against bacterial induced sweet potato rot. Therefore, essential oils extracted from *Zingiber officinale* and *Citrus sinensis* could be used to control postharvest bacteria induced rots in injured sweet potatoes.

Keywords: Postharvest losses, Pathogens, Bacteria isolates, Antibacterial activity, Sweet potato rot

INTRODUCTION

Sweet potato (*Ipomoea batatas* Lam.) is an important staple crop in 130 countries, ranking fourth in production after rice, maize, and wheat with Nigeria being the fourth biggest producer in Sub Saharan Africa.^{1, 2} Sweet potato is the third most important root tuber crop after cassava (*Manihot esculenta*) and yam (*Discorea spp*), and Nigeria produces about 0.2% of the world sweet potato.³ The starchy tuberous roots are the major source of food, but the leaves are also useful green vegetable in some countries.³

The production of sweet potato in Nigeria is constrained by several factors among which storage rot is one of the most significant.² It is estimated that 10% and 40% of loss of agricultural products worldwide are due to post harvest losses.^{4, 5} It has been reported that of all losses caused by plant pathogens, those occurring after harvest are of greater economic effects.⁴

Intolerable levels of post harvest loss of agricultural products has continually threatens the millennial goal of achieving food sustainability.⁶ Large amount of post harvest losses have been attributed to fungal elements,⁶ however, bacteria pathogens are also implicated.^{5, 7} The bacteria and fungi including, bacterial soft rot (*Erwinia chrysanthemi*), soft rot (*Rhizopus stolonifer*), fusarium rot (*Fusarium oxysporum*), black rot (*Ceratocystis fimbriata*), stem rot (*Fusarium solani*), foot rot (*Plenodomus destruens*), blue mold rot (*Penicillium sp*) dry rot (*Aspergillus fumigatus* and *Aspergillus niger*) are reported to be associated with sweet potato rot.³ These bacteria and fungi create local discoloration of the surrounding tissues of infected tubers, resulting in changes in appearance, deterioration of texture and possibly loss of flavour or taste, reduction in the

market value and misfortune to farmers.⁶

Natural plant products and their analogues have been reported to be effective against plant pests and disease pathogens.^{2, 3, 8} Aromatic plants produce secondary metabolites that provide chemical barriers against pests, animals and microbial pathogens. Examples of such plants include; rose, ginger, citrus, lemon grass, citrus lemon. These chemicals including essential oil possess activity against bacteria, fungi and viruses.³ Essential oils belong to a group of compounds called the terpenes, they contain basically mono- and sesquiterpenes.

Pathogenic bacteria responsible for rot of Hannah variety of *Ipomea batatas* have not been previously reported, and there is paucity of research into the use of plant-derived agents as inhibitors of these pathogens. Therefore, this study was designed to investigate the efficacy of plant-based chemical entities as preservatives to decrease post harvest losses caused by microbial pathogens isolated from sweet potato samples collected from Vandeikya Local Government of Benue State, Nigeria.

MATERIALS AND METHODS

Sample collection

Thirty (30) healthy sweet potato samples (*Ipomoea batatas*) were collect from Vandeikya Local Government Area of Benue State in sterile polyethene bags. Vandeikya Local Government council is located on latitude 7°5' and 7°15' North of the equator and longitude 9° and 9°6' East of Greenwich. The sweet orange (*Citrus sinensis*), lemon (*Citrus limon*) were collected at Makurdi local Government Area of Benue State located on the coordinates, 7°43'50" N 8°32'10"E. Ginger (*Zingiber officinale*) was collected from Kaduna south with the coordinate 10°20'N

7°45'E.⁸ The samples were identified at the Department of Plant Science and Biotechnology, Benue State University Makurdi, Benue State, Nigeria.

Sample preparation and extraction of essential oil

Reasonable quantities of fresh *Citrus sinensis* and *Citrus limon* peels and *Zingiber officinale*, were collected and separately size reduced to enhance efficient extraction of the essential oil by hydro-distillation. Briefly, each freshly blended sample was added into biomass flask separately and allowed to stand for about 3 hours. Subsequently, 150mL of distilled water was added to the flask [about 1/2 or 2/3 full] and left to boil for about 3 hours.⁹ Condensed steam with essential oil was then collected via a receiving flask and the essential oil separated by syringing. The separated oil was dried over anhydrous sodium sulphate and stored at 4°C in a sealed container until used.¹²

Preparation of culture media

The nutrient broth and agar (Oxoid) was prepared according to the manufacturer's instructions. The sterile molten medium was allowed to cool to 60°C and 20 mL aseptically dispensed into separate petri dishes and allowed to set.

Bacteria isolation and identification

Bacterial cultures were isolated using modification of methods described by Umunna and Anselem.¹ Three diseased sweet potato tubers were randomly selected from the collected samples and labelled as Vandeikya 1, 2 and 3 coded as VK1, VK2 and VK3 respectively. The entire surface of the diseased tubers were disinfected using swabs moistened with 1% sodium hypochlorite solution.¹ The tuber was excised with flamed knife to reveal boundary area between diseased and healthy portion. The diseased portion

was cut out and teased out aseptically in 10 mL of sterile normal saline in a beaker and used to inoculate (1:10) sterile nutrient broth of the inoculums and incubated overnight at 37°C. A loopful of the overnight bacterial culture from the nutrient broth was aseptically inoculated by streaking onto sterile nutrient agar plates and incubated at 37 °C for 24 hours.^{11, 12} Pure cultures obtained were then used for preliminary identification and subsequent tests.^{1,13} Bacterial isolates were identified by observing the colony characteristics in terms of extent of growth, colour, nature of colony edge, elevation. Gram staining was carried out.¹³ To further establish the identity of the isolated bacteria, biochemical tests such as catalase production and oxidase, mannitol, lactose, xylose, urease, citrate utilization, indole and Voges Proskauer were performed on the isolates.^{14,15,16}

Pathogenicity test

Pathogenicity test was carried out using a modification of the method described by Agu *et al.*² The sweet potato tubers were surface sterilized with a 1% calcium hypochlorite solution swab. Sterile cork borer was aseptically used to bore a hole on the tuber and inoculated with approximately 0.5 mL of overnight culture of bacteria isolate. The hole was covered by replacing the removed fleshy core and the site sealed up with sterile petroleum jelly.^{2,17} The infected tubers were labelled accordingly and kept at room temperature. Sterility control was set up. One week after inoculation, the tubers were aseptically examined by cutting open transversely along the line of inoculation to reveal the extent of rot inside. Bacteria isolate which caused clearly visible rot was considered pathogenic when viewed against the control. Pathogenic strains were further used as test organisms for treatment with essential oil extracts.^{1,2,18}

Investigation of inhibition of sweet potato rot *in vivo* by essential oil extracts

Sweet potato tubers were surface disinfected by mopping the surface with a 1% calcium hypochlorite solution swab. About 3 mm deep excisions were made with sterile cork borer dipped in extracted essential oil. The removed core was replaced after point inoculation with the pathogen and sealed with sterile petroleum gel.¹ A negative control was set up in each. The setups were incubated at room temperature for 7 days, after which the potato tubers were cut open along the point of inoculation. The extent of rot in each case was measured with a pair of callipers. Percentage (%) growth inhibition was obtained using the expression:

$$\% \text{ inhibition} = \frac{\text{length of rot in control} - \text{length of rot in treated}}{\text{length of rot control}} \times 100\%$$

Data obtained was subjected to Analysis of Variance (ANOVA) at 5% level of significance using Duncan *post-hoc* test.

GC-MS analysis of essential oils

Identification of compounds in the essential oils was done using a GC-MS machine Model; 7890A GC System, 5675C Inert MSD with triple axis; The column model is Agilent 19091-433HP-5Ms 5% Phenyl methyl silox with triple axis detector equipped with an auto injector (10 µL syringe) equipped with a fused silica capillary column of 30 m length, 0.25 mm diameter, and 250 µm film thickness treated with phenyl methyl silox. The ion source temperature (EI) was 250 °C while the interface temperature was 300 °C, pressure 16.2 psi, out time 1.8 mm, 1 µL injector in split mode with split ratio 1:50 with injection temperature of 300 °C. Helium gas was used as a carrier gas. The column temperature started at 35 °C

for 5 minutes and changed to 150 °C at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 minutes. The total elution time was 47.5 minutes. The compounds in the extracted essential oils were identified by comparing their retention indices and patterns of mass spectra with reference to NIST library (NIST II).

RESULTS

In this study, four different bacteria with features presumptive of *Erwinia carotovora*, *Bacillus licheniformis*, *Pseudomonas fluorescens* and *Micrococcus luteus* were isolated from Hannah sweet potato and characterised (Table 1). Result from this study differs from those reported by Banso,⁷ who isolated and characterized organisms causing postharvest rot and spoilage in the sweet potato *Ipomea spp.*

Pathogenicity is the ability of an organism to cause disease in healthy sweet potato tubers. Of the four bacteria species isolated from sweet potato in this study, three, *Erwinia carotovora*, *Bacillus licheniformis*, *Pseudomonas fluorescens* were found to be pathogenic and cause rot on healthy sweet potato tuber as shown in Table 2.

Results of bacteria sensitivity to the essential oils shows that *Pseudomonas fluorescens* significantly ($p < 0.05$) more sensitivity to the essential oils with percentage inhibition of 64.3% (9/14) for *Citrus sinensis*, 54.6% (7.5/14) for *Zingiber officinale* and 21.4% (3/14) for *Citrus limon*. However, the bacteria *Erwinia carotovora* was least sensitive with percentage sensitivities of *Citrus limon* 9.9% (1/14.5) *Citrus sinensis* 17.2% and *Zingiber officinale*, 31.0% (4.5/14.5).

Table 3, 4 and 5 shows the chromatogram of hydro-distilled essential oils extracted from peels of *Citrus*

sinensis and *Citrus lemon* and roots of *Zingiber officinale* as analyzed by GC-MS. Results obtained showed that a total of 22 terpenes were identified with *Citrus sinensis* comprising of 13 monoterpenes, 8 sesquiterpenes and 1 diterpene detected in high percentage (>4%) of the monoterpenes; linalool, camphene, terpinen-4-ol, m-mentha-4,8-diene,(1S,3S), geraniol, -citral and citral (Table 3). D-limonene and citronellal were detected, but at lesser quantities. Of the eight sesquiterpenes detected, only four were observed to be above 1% (-ylangene, caryophyllene, -copaene and bicyclogermacrene) of the total percentage of the terpenes detected. Phytol, the only diterpene detected was <1% of percentage total (Table 3).

GC-MS analysis of *Citrus limon* showed thirty one terpenes (Table 4). Uneven distribution of monoterpenes showing 7-propylidene-bicyclo[4.1.0] heptanes with the highest percentage abundance of 8%, followed by eucalyptol (5.6%). Camphene and 2-methyl-trans-3a,4,7,7a-tetrahydroindane were detected at 4.8 and 4.7% respectively. Other monoterpenes including citral and limonene oxide were detected at percentage concentration ranging from 0.39 – 3.0%. Fifteen sesquiterpenes were detected including -curcumene, however, only -bisabolene, was seen to be >4%. Again -springene the only diterpene detected was only 0.3% of the total essential oils found in *Citrus limon*. GC-MS analysis of *Zingiber officinale* recorded eleven monoterpenes and eighteen sesquiterpenes. Except the monoterpene, citral (5.2%) all other monoterpenes including citronellal, citronellol, geraniol and Dlimonene were 2% of the total terpene composition. However, the sesquiterpenes -curcumene, -curcumene, -cedrene, -cadinene and -eudesmol were in detected in abundance (3.5%). The sesquiterpenes -zingiberene was also detected in

low amount of 0.32% of the total terpenes in *Zingiber officinale* (Table 5).

Table 1: Colony features and biochemical characteristics of bacteria isolated from sweet potato

Isolate code	Colony characteristics	Gram stain	Catalase	Oxidase	Citrate	Indole	H ₂ S	Mannitol	Lactose	Xylose	Urease	VP	Presumptive identity
VK1a	Colonies are circular, raised to convex, white, smooth and shiny	-	-	+	+	-	+	+	+	+	-	-	<i>Erwinia carotovora</i>
VK1b1	Colonies are yellow, transparent and shiny, irregular, with smooth to wavy margins and raised elevation	-	+	+	+	-	+	+	-	+	+	+	<i>Pseudomonas fluorescens</i>
VK1c	Colonies are dry, flat and irregular with lobate margin and appear waxy	+	+	+	-	-	+	-	+	+	-	-	<i>Bacillus licheniformis</i>
VK1d	Colonies are orange yellow, translucent, flat with irregular edges	+	+	+	-	-	-	+	-	+	+	+	<i>Micrococcus luteus</i>
VK2a	Colonies are circular, raised to convex, white, smooth and shiny	-	-	+	+	-	+	+	+	+	-	-	<i>Erwinia carotovora</i>
VK2b	Colonies are yellow, transparent and shiny, irregular, with smooth to wavy margins and raised elevation	-	+	+	+	-	+	+	-	+	+	+	<i>Pseudomonas fluorescens</i>
VK2c	Colonies are dry, flat and irregular with lobate margin and appear waxy	+	+	+	-	-	+	-	+	+	-	-	<i>Bacillus licheniformis</i>
VK2d1	Colonies are orange yellow, translucent, flat with irregular edges	+	+	+	-	-	-	+	-	+	+	+	<i>Micrococcus luteus</i>
VK3a2	Colonies are circular, raised to convex, white, smooth and shiny	-	-	+	+	-	+	+	+	+	-	-	<i>Erwinia carotovora</i>
VK3b	Colonies are yellow, transparent and shiny, irregular, with smooth to wavy margins and raised elevation	-	+	+	+	-	+	+	-	+	+	+	<i>Pseudomonas fluorescens</i>
VK3c1	Colonies are dry, flat and irregular with lobate margin and appear waxy	+	+	+	-	-	+	-	+	+	-	-	<i>Bacillus licheniformis</i>
VK3d	Colonies are orange yellow, translucent, flat with irregular edges	+	+	+	-	-	-	+	-	+	+	+	<i>Micrococcus luteus</i>

VK= Vandeikya; +=Positive test; - = Negative test; VP= Voges-Proskauer

Table 2 : Pathogenicity of isolated organisms

Organism	Result
<i>Erwinia carotovora</i>	+
<i>Pseudomonas fluorescens</i>	+
<i>Bacillus licheniformis</i>	+
<i>Micrococcus luteus</i>	-

+ = positive, - = negative

Table 3: Chemical composition of hydro-distilled *Citrus sinensis* peel essential oil

S/N	Retention time (min)	Percentage total (%)	M/z (g/mol)	Molecular formula	Compounds present
1	3.368	0.431	136.234	C ₁₀ H ₁₆	D-limonene
2	3.525	8.686	154.244	C ₁₀ H ₁₈ O	Linalool
3	3.587	4.482	136.234	C ₁₀ H ₁₆	Camphene
4	3.681	1.220	152.233	C ₁₀ H ₁₆ O	Trans-p-mentha-2,8-dienol
5	3.812	1.936	154.244	C ₁₀ H ₁₀ O	Citronellal
6	4.031	4.228	154.244	C ₁₀ H ₁₈ O	Terpinen-4-ol
7	4.138	9.317	136.234	C ₁₀ H ₁₆ O	m-mentha-4,8-diene, (1S,3S)
8	4.313	5.912	154.249	C ₁₀ H ₁₈ O	Geraniol
9	4.419	4.220	152.233	C ₁₀ H ₁₆ O	-citral
10	4.463	4.786	150.218	C ₁₀ H ₁₄ O	Carvone
11	4.601	5.750	152.233	C ₁₀ H ₁₆ O	Citral
12	4.676	2.624	150.218	C ₁₀ H ₁₄ O	L-perillaldehyde
13	4.751	2.538	152.233	C ₁₀ H ₁₆ O	P-mentha-1(7), 8(10)-dien-9-ol
14	5.326	1.954	204.351	C ₁₅ H ₂₄	-ylangene
15	5.626	1.113	204.351	C ₁₅ H ₂₄	Caryophyllene
16	5.676	1.065	204.351	C ₁₅ H ₂₄	-copaene
17	5.752	0.526	204.351	C ₁₅ H ₂₄	(E)- -farnesene
18	5.845	0.928	204.351	C ₁₅ H ₂₄	Humulene
19	6.077	0.973	204.351	C ₁₅ H ₂₄	-farnesene
20	6.102	1.566	204.351	C ₁₅ H ₂₄	Bicyclogermacrene
21	6.677	0.389	204.351	C ₁₅ H ₂₄	Isocaryophyllene
22	8.911	0.570	296.531	C ₂₀ H ₄₀ O	Phytol

Table 4: Chemical composition of hydro-distilled *Citrus limon* peel essential oil

S/N	Retention time (min)	Percentage total (%)	M/z (g/mol)	Molecular formula	Compounds present
1	3.431	0.386	154.244	C ₁₀ H ₁₈ O	Sabinene hydrate
2	3.531	4.805	136.234	C ₁₀ H ₁₆	Camphene
3	3.656	1.222	152.233	C ₁₀ H ₁₆ O	trans-p-Mentha-2,8-dienol
4	3.750	2.708	152.233	C ₁₀ H ₁₆ O	Limonene oxide
5	4.050	5.696	154.249	C ₁₀ H ₁₈	Eucalyptol
6	4.150	4.745	136.238	C ₁₀ H ₁₆	2-Methyl-trans-3a,4,7,7a-tetrahydroindane
7	4.181	1.714	152.233	C ₁₀ H ₁₆ O	(E)-3(10)-Caren-4-ol
8	4.213	0.618	154.249	C ₁₀ H ₁₈ O	Cis-piperitol
9	4.400	3.004	152.233	C ₁₀ H ₁₆ O	Carveol
10	4.588	2.074	152.233	C ₁₀ H ₁₆ O	Citral
11	4.669	0.977	150.218	C ₁₀ H ₁₄ O	L-perillaldehyde
12	4.988	0.739	168.233	C ₁₀ H ₁₆ O ₂	(1R,4R)-p-Mentha-2,8-diene, 1-hydroperoxide
13	5.082	2.357	222.366	C ₁₅ H ₂₆ O	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane
14	5.389	8.000	136.238	C ₁₀ H ₁₆	7-Propylidene-bicyclo[4.1.0]heptanes
15	5.501	0.648	152.233	C ₁₀ H ₁₆ O	2H-Inden-2-one, octahydro -3a-methyl-, cis
16	5.701	3.041	204.351	C ₁₅ H ₂₄	-bergamotene
17	5.745	0.654	204.351	C ₁₅ H ₂₄	-elemene
18	5.852	1.201	150.218	C ₁₀ H ₁₄ O	1(2H)-Pentalenone,hexahydro-5-methyl-4-methylene-
19	5.970	1.300	202.335	C ₁₅ H ₂₂	-curcumene
20	6.008	0.485	204.351	C ₁₅ H ₂₄	-sesquiphellandrene

Table 5: Chemical composition of hydro-distilled *Zingiber officinale* essential oil

S/N	Retention time (min)	Percentage total (%)	M/z (g/mol)	Molecular formula	Compounds present
1	3.275	0.282	136.234	C ₁₀ H ₁₆	2-carene
2	3.325	1.318	154.249	C ₁₀ H ₁₈ O	Linalool
3	3.694	0.275	154.249	C ₁₀ H ₁₈ O	Citronellal
4	3.856	1.840	154.249	C ₁₀ H ₁₈ O	Endo-borneol
5	3.919	0.329	154.249	C ₁₀ H ₁₈ O	Terpinen-4-ol
6	4.006	1.506	154.249	C ₁₀ H ₁₈ O	-terpineol
7	4.225	1.925	156.265	C ₁₀ H ₁₈ O	Citronellol
8	4.426	2.142	154.249	C ₁₀ H ₁₈ O	Geraniol
9	4.457	0.325	152.233	C ₁₀ H ₁₆ O	cis-p-mentha-1(7),8-dien-2-ol
10	4.569	5.176	152.233	C ₁₀ H ₁₆ O	Citral
11	5.126	0.601	168.233	C ₁₀ H ₁₆ O ₂	Geranic acid
12	5.257	1.134	136.234	C ₁₀ H ₁₆	D-limonene
13	5.301	0.434	204.351	C ₁₅ H ₂₄	Copaene
14	5.389	0.697	204.351	C ₁₅ H ₂₄	-elemene
15	5.439	0.317	204.351	C ₁₅ H ₂₄	-zingiberene
16	5.652	0.413	204.351	C ₁₅ H ₂₄	-elemene
17	5.745	0.347	204.351	C ₁₅ H ₂₄	Cis- -farnesene
18	5.877	0.367	204.351	C ₁₅ H ₂₄	Allo-Aromadendrene
19	5.971	6.003	202.335	C ₁₅ H ₂₂	-curcumene
20	6.046	6.046	204.351	C ₁₅ H ₂₄	-curcumene
21	6.127	4.488	204.351	C ₁₅ H ₂₄	-cadinene
22	6.233	6.315	204.351	C ₁₅ H ₂₄	-cedrene
23	6.396	2.599	222.366	C ₁₅ H ₂₆ O	Elemol
24	6.796	1.525	204.351	C ₁₅ H ₂₄	-bisabolene
25	6.421	1.279	222.366	C ₁₅ H ₂₆ O	-nerolidol
26	6.634	1.059	222.366	C ₁₅ H ₂₆ O	7-epi-cis-sesquisabinene Hydrate
27	7.153	3.456	222.366	C ₁₅ H ₂₆ O	-Eudesmol
28	7.209	0.757	204.351	C ₁₅ H ₂₄	-santalene
29	7.591	0.898	222.366	C ₁₅ H ₂₆ O	Farnesol
30	8.160	0.606	220.351	C ₁₅ H ₂₆ O	Acorenone

DISCUSSION

Discovery of natural plant products effective for control of sweet potato rot caused by different pathogens is crucial.^{7, 11} Three bacterial species *Erwinia carotovora*, *Bacillus licheniformis* and *Pseudomonas fluorescens* were found to be pathogenic and cause rot on healthy sweet potato (Hannah) tuber grown collected from Vandeikya in Benue State with *Pseudomonas fluorescens* being the most virulent. This result partly agrees with previous finding.^{15,16, 18} Suleiman and Falaiye⁶ reported bacteria and fungi pathogens as major causes of sweet potato rot. Other bacteria species, such as *Flavobacteria*, *Ralstonia*, *Arthrobacter woluwensis*, *Pseudomonas syringae*, *Arthrobacter nicotianae* *Staphylococcus spp.*, and *Lysinibacillus fusiformis* can also cause rot in sweet potato.^{1, 11} The differences in bacterial isolates from the different studies may be associated with the variety of sweet potato, the study area and soil conditions such as temperature and predominant soil microbes amongst other factors.^{6,11}

Extracts from plants have been widely reported to exhibit inhibitory activity against pathogens causing tuber rot.⁷ In this study, *Pseudomonas fluorescens* was sensitive to the essential oils a pathogenic bacterium in sweet potato in the order, *Citrus sinensis* > *Zingiber officinale* > *Citrus limon*. However, *Erwinia carotovora* was not sensitive to the inhibitory activity of the three essential oils used for this study. In this study, essential oil extracts of *Citrus sinensis* was more activity against tested bacterial, however, essential oil extracts of *Zingiber officinale* inhibited tested bacterial and also exhibited anti-fungal activity as previous study.⁷ This implies that essential oil of *Zingiber officinale* has a broad antimicrobial activity spectrum compared to *Citrus sinensis*.

To study association between inhibitions of bacterial

induced rot with antimicrobial activity of the active principles present in the essential oil extracts, it was therefore necessary to analyse the chemical constituents, hence GC-MS was employed. The method of GC-MS has been previously used to analyze chemical composition of essential oil was employed in this study to identify the chemical constituents of essential oils extracted from peels of *Citrus sinensis* and *Citrus lemon* and roots of *Zingiber officinale*.¹⁹ Terpenoids such as geraniol, linalool and citral are shown to have high antimicrobial activities compared to terpenes such as limonene and camphene detected in *Citrus limon*.¹⁹ The presence of the more active geraniol, linalool and citral in essential oils from *Citrus sinensis* and *Zingiber officinale* most likely than not, accounted for the greater anti- *Pseudomonas fluorescens* activity.

Essential oils are frequently used in traditional medicine as antimicrobial agents and in perfumery as fragrance.³ In this study percentage of geraniol, limonene and citral that accounted for the antibacterial activity of essential oil obtained from *Citrus sinensis* was consistent with the findings of Cholke *et al.*¹⁹ However, higher amounts were reported by other researchers.^{9, 20,21} The most abundant constituents of essential oil obtained from *Citrus limon* were -bisabolol, -nerolidol and -springen. Dosoky and Setzer,²⁰ reported higher percentage abundance (64.0-70.5%) of limonene compared to 2.1% observed in this study. More chemical constituents were detected in essential extract from *Zingiber officinale* in this study compared to fewer, but higher quantity observed by Sharma *et al.*²² The composition and amount of essential oils in this study and other studies may be largely due to environmental factors such as growing conditions, geography,

species and time of harvest previously reported to affect the composition of essential oils.^{21,23}

CONCLUSION

Isolated bacteria from Hannah variety of sweet potato characterized as pathogenic are *Erwinia carotovora*, *Bacillus licheniformis* and *Pseudomonas fluorescens*. Hydrodistilled essential oils from *Citrus sinensis* and *Zingiber officinale* that inhibited *Pseudomonas fluorescens* induced sweet potato rot are promising sources of effective natural preservatives to reduce bacteria induced postharvest losses in injured sweet potatoes.

Limitation(s)

Identification of bacteria isolated from diseased tubers of Hannah variety of sweet potatoes in this present study was carried out by morphological and biochemical methods. However, the isolates have been archived for further characterization with polymerase chain reaction and genome sequencing.

Conflicts of interest

The authors declare that there are no conflicts of interest

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