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ORIGINAL ARTICLE

Phytochemical profiling and antimicrobial activity of ginger (*Zingiber officinale*) essential oils against important phytopathogens

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Abstract The objectives of this study were to profile ginger essential oils (EOs) phytochemical constituents and antimicrobial activity against important phytopathogens. Ginger EOs was extracted using a modified Clevenger-type apparatus by hydro-distillation then followed by GCMS and headspace analysis of its phytochemical constituents. The phytoconstituents identified were monoterpenes and sesquiterpene hydrocarbons. Food poisoned and disc diffusion techniques were applied to determine the percentage inhibition of fungal mycelial and bacterial growth respectively. The EOs produced mycelial growth inhibition in all the test fungal pathogens after five days of incubation. The MIC and MFC of the EOs on the tested fungi were in the range of 1 µl/ml and 5–6 µl/ml,

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respectively. The bacterial growth of all the tested isolates was also affected by EOs at 100–500 µl/ml, from weak to strong antibacterial activity. The EOs affected the *Xanthomonas oryzae* pv. *oryzae*-strain A isolate most at a higher concentration of 400–500 µl/ml with mean inhibition of 20.66 mm and 22.66 mm respectively, which are found to be effective. The MIC values on the bacterial pathogens were at100 µl/ml. The inhibition zone of positive control (streptomycin) at 15 µg/disc was 25.00 mm and appeared to be efficient. Metabolomics analysis to concurrently quantify variability among multiple compounds in the data sets and identify such compounds responsible for the *X. oryzae* pv. *oryzae*-strain-A inhibition were determined. The cross-validated PLS model has shown a strong correlation between ginger EOs and bioactivity. The action of ginger EOs on the cell structure was fully identified using SEM by observing the changes in morphology and integrity of *X. oryzae* pv. *oryzae*-strain-A cells. The DMSO treatment (control) showed a normal rod shape cell, while treatment with the ginger EOs showed irregular shape with sunken surfaces, and treatment with antibiotics display abnormal growth of the cells. These findings can, therefore, propose that the ginger EOs could be used as a new antimicrobial agent in suppressing the growth of phytopathogens and as possible new alternatives to synthetic fungicides and bactericides.

1. Introduction

Phytopathogens are the main cause of plant diseases that result in significant crop losses, especially in tropics. Prevention or reduction of plant disease infestation is a major concern of farmers. In spite of the advances in production agriculture, effective management of plant disease remains a challenge because of the side effects of existing pesticides as most of them chemically synthetic (bactericides, fungicides, among others) (Shaheen and Issa, 2020). Excessive used of synthetic pesticides has been implicated in their negative effects on the environment such as soil and water pollutions, long periods of degradation, residual accumulation in the food chain, and less control efficacy against pathogenic microbes with long-term usage (Ghormade et al., 2011; Nega, 2014; Bhavaniramya et al., 2019). Globally, the use of some of the existing antibiotics in agriculture is being banned because of the adverse effects of the antibiotics such as severe or extreme pathogens resistance, higher cost of production, prolonged cycles of chemicals degradation, and environmental pollutions (Hajano et al., 2012; Sundin et al., 2016; Buttimer et al., 2017). In terms disease resistance, streptomycin which is the main antibiotic that is used to control bacterial diseases in plants is no longer effective because of the resistant of bacterial to streptomycin (Xu et al., 2010). The limitations aforementioned limitations are posing a serious challenge for achieving sustainable management of plant diseases in many farming systems (Juroszek and Von Tiedemann, 2011; Dara, 2019). In particular, disease management systems are of the major concern because there is a strong need for natural antimicrobial agents or biopesticides that are effective, non-toxic, and ecologically safe to control plant diseases. Research on the alternative pesticides and antimicrobials, including natural plant products such as ginger essential oils is essential. The introduction of new generation/innovation of biopesticides could provide solutions for the pathogenic microbes that developed resistance to synthetic chemicals (Saha et al., 2016).

Ginger essential oils (EOs) are one of the natural products that could be an alternative class of natural antimicrobials with a wide range of metabolite spectrum to pave the way for new and more effective compounds in controlling plant pathogens. It is becoming clear that these natural products have the ability to influence the modern agrochemical solutions for their biological and antimicrobial activities (Abdel-Kader et al., 2015) as well as economic viability and low toxicity (Brusotti et al., 2014). They are also well known for their antibacterial, antiviral, antifungal activities, insect repellents (Venkateshwarlu, 2014; Sendanayake et al., 2017; Azhari et al., 2017), biodegradable, and generally embraced by many societies. Thus, it is a new solution for protecting plants from being attacked by pathogens (Lim et al., 2012; Lanzotti et al., 2013).

Considering the economic importance of the plant diseases caused by the bacterial and fungal pathogens besides the toxic effects of synthetic chemicals, it is important to explore an alternative way that is eco-friendly for the management of plant diseases. To date, the knowledge on the use of ginger EOs constituents in plant disease control is limited. The current research efforts are directed towards to profile ginger EOs phytochemical constituents so as to determine antimicrobial activity against selected major phytopathogens. The outcomes of this study may serve as one of the new control alternatives for controlling plant diseases in the tropics. This is considered as effective and sustainable solution to synthetic chemicals by exploring the untapped potentials of ginger EOs (natural products) for its antimicrobial activity against phytopathogenic bacteria and fungi.

2. Materials and methods

2.1. Collection and preservation of plant sample

The plant materials used were the rhizomes of wild and domestic gingers. To reduce variations between samples, fresh ginger rhizomes raised in the same environmental and growth conditions were obtained in February 2019 from local producers in Bentong, Pahang (GPS coordinates: 3.8126°N, 103.3256°E). The matured rhizomes were divided into 3 kg of six experimental groups after which the ginger rhizomes were kept in an ice chest and transported to the laboratory for extracting EOs.

2.2. Extraction of the essential oils

The EOs were extracted from the ginger rhizomes using standard procedures. The rhizomes were washed with tap water to remove dirt followed by washing them with distilled water after which they were cut into pieces and ground using a blender. The extractions were performed using hydro-distillation (Clevenger-type apparatus). The device is a custom-sized machine coupled with an electric boiler as well as condenser and glass decanter to separate oil from water condensate. The ratio of 3:5 of pretreated ginger rhizomes with solvent (distilled water) was used. The process was maintained for five hours. The ground ginger rhizomes were put directly inside the 10 L flask and boiled. The hot steam enables the aromatic compounds (essential oils) in the plant parts to be released. Thereafter, the molecules of these volatile oils escaped from the plant material and evaporated into the vapor within the system. The steam temperature was carefully regulated. The sample was boiled at 100 °C for 20 min after which the temperature of the sample was reduced to 45 °C for 5 h. This was to ensure that the EOs were extracted. Afterwards, the samples with the EOs were condensed and separated (Mesomo et al., 2013). The yield OEs was computed using the below formula after which the samples were stored at − 20 °C in Bacteriology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia, until when it was required for the antimicrobial bioassays.

$$\text{The Oil Yield } (\%) = \text{Weight of Oil (g)} / \text{Weight of sample (g)} \times 100.$$

2.3. Analysis of the chemical contents of domestic and wild ginger essential oils using GCMS

The GCMS analysis was carried out at the Institute of Bioscience (IBS), Universiti Putra Malaysia. The analysis was conducted to determine the volatile compounds and their amounts in the ginger EOs. The approach used by Bhattarai et al. (2018) to analyze the volatile compounds was adopted using Shimadzu QP-2010 GCMS system that consists of gas chromatograph interfaced with a mass spectrometer and equipped with a Zebron ZB5-MS capillary fused silica column (30 m × 0.25 nm I.D. × 0.25 m film thickness). The initial temperature of the oven was set at 70 °C (isothermal for 3 min) with a 10 °C/min after which it was increased to 240 °C followed by increasing it to 300 °C for 10 min isothermal. A scan interval of 0.5 s and fragments of 45 to 480 Da were taken at 70 ev. Total GC running time was 45 mins for the domestic ginger and 51 mins for the wild ginger. Carrier gas, helium (99.999%) with a flow rate of 1 ml/min; injection volume 1 µl with a split ratio of 10:1 and injector temperature 250 °C were used in this present study. The mass spectrum of the obscured constituents of both gingers was identified by comparing their retention times (RT), similarity index (SI), and mass spectral data with those from FFNSC1.3.lib, NIST11.lib, and WILEY229. Lib mass spectral databases, as well as with related literature (Wei et al., 2010; Nagappan and Palaniveloo, 2012; Trimanto et al., 2018). The composition of EOs was expressed as a percentage of total peak area. The chemical compound's names, percentages, retention time, and molecular formula were determined.

2.4. Analysis of the chemical contents of domestic and wild ginger by headspace

The analysis was carried out using the procedure of Yang et al., (2009). A 0.5 g amount of ginger rhizome particle was hermetically sealed in a 4 ml screw-top amber vial. The sealed vial was exposed to the extraction of the ginger component by immediately inserting it into the GC injector and the fiber thermally desorbed. A desorption time of 2 min at 250 °C was used in splitless mode with a valve oven temperature at 110 °C and transfer line temperature of 120 °C. Thereafter, the above stated GCMS condition was established using a Shimadzu QP-2010 GCMS system. The chemical compounds names, percentages, retention time, and molecular formula were recorded.

2.5. Description and retrieval of fungal and bacterial pathogens

The phytopathogenic bacteria and fungi were obtained from the Culture Collections Unit, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. Isolates of five plant pathogenic fungi and bacteria were *Fusarium oxysporum, Pyricularia oryzae*, *Colletotrichum falcatum*, *Ganoderma boninense*, and *Rigidoporus microporus* as well as *Xanthomonas oryzae* pv. *oryzae*- strain A, *X. oryzae* pv. *oryzae*- strain B, *Ralstonia solanacearum*, *Bacillus* sp. and *Klebsiella* sp. Confirmation of the identity of the resultant cultures was based on their morphological and molecular characteristics (Table 1).

Table 1 The Description of Phytopathogenic Bacteria and Fungi.

Fungal and Bacterial Pathogens	Disease	Host	Reference
Fusarium oxysporum (*Foc-TR4*)	Fusarium wilt of banana	Isolated from infected roots of banana	(Ahmad et al., 2020; Wong et al., 2019)
Pyricularia oryzae	Rice blast	Isolated from infected rice leaves	(Awla et al., 2016)
Colletotrichum falcatum	Red rot of sugarcane	Isolated from infected stalks of sugarcane	(Hossain et al., 2020)
Ganoderma boninense	Basal stem rot (BSR) of oil palm	Isolated from infected root of oil palm	(Rakib et al., 2017)
Rigidoporus microporus	White root rot of rubber	Isolated from infected root of rubber	Un-published
Xanthomonas oryzae pv. *oryzae*- strain A and B	Bacterial leaf blight (BLB) of rice	Isolated from infected rice leaves	(Azman et al., 2017; Chibuike et al., 2019)
Ralstonia solanacearum	Bacterial wilt of eggplant	Isolated from infected root of eggplant	Un-published
Bacillus sp.	–	Isolated from agriculture soil	–
Klebsiella sp.	–	Isolated from agriculture soil	–

2.6. Determination of antifungal activity of ginger essential oils

The ginger EOs were screened against the fungal pathogens using the food poison technique (Talibi et al., 2012). The test concentrations 1, 2, 3, 4, 5, and 6 µl/ml were prepared by adding an appropriate amount of ginger EOs containing 0.5% (v/v) of Tween 80 to the cooled molten PDA (45 °C) followed by manual rotation in a sterile Erlenmeyer flask to disperse the oil in the medium and thoroughly mixed before solidification. After the samples had solidified, 6 mm bits of fungus culture was cut from a seven day old culture with sterilized cork borer after which it then placed at the center of Petri dish plates with a sterilized inoculation needle in three replications of each treatment. All Petri dishes were sealed with sterile laboratory parafilm to prevent the EOs from evaporating. The Petri dish plates were incubated at 25 ± 2 °C. Negative control was maintained in the medium that was not mixed with anything but inoculated with the pathogen whereas the positive control was mixed with 60 µl/ml of Azoxystrobin/Difenoconazole. Data were collected when the control petri plates were fully grown with mycelium. The data of the radial growth of the fungal colony was measured in millimeters. The percentage inhibition over control was calculated by the below formula (Aman and Rai, 2015):

$$\text{Percent inhibition over control} = [(C - T)/T] \times 100$$

Where C = Growth of fungus in control T = Growth of fungus in treatment.

To determine whether the EOs have fungicidal effect on the test pathogens, a plug of 6 mm PDA from the plate with no growth or suppressed growth was transferred to un-amended PDA medium. The treatment in which the mycelial did not growth after additional seven days of incubation was considered fungicidal to the test pathogens (Talibi et al., 2012).

2.7. Determination of antibacterial activity of ginger essential oils

The analysis was done using the method of Rajip et al., (2016) but with modifications. A standard disc diffusion method was used to determine the antibacterial activity of the EOs against the bacterial pathogens in triplicate using 24–48 h grown bacterial species reseeded on nutrient media. The cultures were adjusted with saline water to obtain a suspension at concentration of 1×10^6 CFU/ml using spectrophotometer, then 100 µl of the suspension was spread on Muller Hinton (MH) agar media plates to obtain uniform microbial growth using a sterile glass rod. Sterile filter paper discs (Whatman's No. 6 mm in diameter) was impregnated with 10 µl of oils diluted in dimethyl sulfoxide (DMSO) and obtained different concentrations range from 50, 100, 200, 300, 400 and 500 µl/ml and finally placed on the surface of the agar test plate at intervals. The positive control discs were saturated with 10 µl of streptomycin (15 µg/ml/disc) and negative control was DMSO buffer. All Petri dishes were sealed with sterile laboratory parafilm to prevent the EOs from evaporating. The dishes were left for 30 min at room temperature to enable the oil to diffuse. Afterwards, the plates were incubated at 37 °C for 24 h. The appearance or absence of a zone of inhibition has been used as a means of identifying active or inactive concentrations of the EOs, they were determined by measuring the growth inhibition diameter in millimeter (mm).

2.8. Metabolomics' Profiling of chemical constituents of ginger essential oils

After GCMS analysis of domestic (DMT) and wild (WLD) ginger EOs, the chromatographic and spectral data were properly processed and analyzed by multivariate data analysis (MDA). The metabolomic analysis started by obtaining the comma-separated values (CSV) file, which enables data to be saved in a tabular format. The deconvolution was done to convert these data into a data matrix suitable for multivariate data analysis (MDA) using Microsoft Excel 2010. After these procedures, a multivariate data matrix containing information about sample identities (sample code), ion identities (RT and m/z values), and normalized peak intensities were introduced into the analytical software called SIMCA (MKS Umetrics, version 14.1.0.2047 (32-bit) whereby the generated normalized metabolite peaks were transformed into variables. The data were mean centered and Pareto scaled. The PCA was then used to see the discrimination among the different observations from different ginger samples, (DMT and WLD). A model containing PCs (PC1 and PC2) were established representing a portion of examined data set. Results were visualized in the score plot of the two main components (DMT and WLD), in which each point was a representative of an individual sample spectrum. The analysis was checked and validated by default seven-fold internal cross validation based on the values R2X and Q2 which are the fitness and goodness of prediction respectively. The PCA, which is an unsupervised analysis that was performed to evaluate summary of the possible differences or similarities between sample groups based on the score plots observation. The corresponding loading plot were created to show the metabolites leading to group separation.

The supervised partial least square (PLS) was subsequently conducted to classify the metabolites associated with bioactivity addition *via* the score plot, that is, PLS model was used to find the correlation between the antimicrobial activities to the metabolites identified in each of the two different ginger EOs. There was validation by determining R2Y and Q2 through permutation test. Thereafter, the most significant metabolites (VIP > 1.0) were then identified with the bioactivity and subsequently, the bi-plot gave the correlation of bioactivity in a single plot.

2.9. Determination of antibacterial activity against *Xanthomonas oryzae pv. oryzae- strainA*

The potential antibacterial effects of EOs were evaluated using the electron microscopy (EM) methods. Scanning electron microscopy (SEM) for visual observation of the pathogen response to the *in-situ* antibacterial activity of the EOs. The test pathogen was treated with the MIC concentration (100 µl/ml), streptomycin (15 µg/ml/disc) and control with DMSO. The method as described by de Oliveira et al., (2011) was used for this analysis. The samples were then fixed with modified Karnovsky's fixative (Karnivsky, 1965) containing 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.05 M sodium cacodylate buffer solution (pH 7.2) and left

at 4 °C overnight. The samples were washed with 0.1 M sodium cacodylate buffer in three changes after 30 mins each, followed by post-fixation in 1% osmium tetroxide in 0.2 M PBS for 2 h, dehydrated through series of graded acetone (35, 50, 75, 95%) for 10 min each and 100% for 15 min. After completing the dehydration process, the samples were transferred into specimen basket and put into critical dryer for 30 min and observed with a scanning electron microscope (SEM: JSM – 5610LV, JOEL, Japan) at the IBS, Universiti Putra Malaysia.

2.10. Data analysis

The data in this present study were analyzed using PROC ANOVA by SAS 9.4 version and significant differences among the means were determined using least significant difference (LSD) at probability level of 0.05. The EPA probit analysis program version 1.5 was used to determine LC values among different concentrations of the essential oil and their overall antimicrobial activity.

3. Results

3.1. The extracted oil and yield (%)

The volume of EOs for the domestic ginger was 8 ml per 3 kg fresh rhizome. The highest yield obtained was 2.46% and the lowest yield obtained was 0.41% per 3 kg whereas for that of wild ginger, the oil yield was poor, the volume of EOs was 0.3 ml per 3 kg. The highest yield obtained was 0.28% and the lowest yield was 0.046% per 3 kg fresh rhizome.

3.2. Chemical constituents of the domestic and wild ginger essential oils by GCMS

The essential oil's chemical constituents of the domestic and wild ginger were identified using GCMS analysis. In total, 82 (42 from domestic and 51 from wild) chemical components were identified, representing 99.0% and 98.9% of the total constituents detected in the EOs from both domestic and wild ginger, respectively. The volatile phytochemical composition of domestic ginger EOs with the most abundant compounds were α-zingiberene (18.56%), geranial (13.88%), neral (10.75%), *Trans*-caryophyllene (9.64%), Eucalyptol (5.05%), β-phellandrene (5.51%), camphene (5.34%), α-pinene (2.05%) and heptan-2-ol (1.05%). The detailed identification and concentrations of the compounds found in both domestic and wild ginger EOs are presented in Table 2. The chemical compounds in the wild ginger EOs were monoterpenes such as isoeugenol, camphene, geranial, geranyl acetate, anethole, fenchyl acetate and neral as well as sesquiterpene hydrocarbons mainly α-humulene, α-urcumene, β-bisabolene, β-sesquiphellandrene, thus the most abundant compounds were isoeugenol (42.17%), caryophyllene (6.72%), β-bisabolene (5.10%), anethole (4.60%), Eucalyptol (1.41%), β-isabolol (1.98%), (-)-globulol (1.90%), α-curcumene (1.54%), α-humulene (1.74%), fenchyl acetate (1.49%), β-pinene (1.85%), alloaromadendrene (1.49%) and geranial (1.15%).

3.3. Chemical constituents of domestic and wild ginger rhizomes by headspace

Using headspace analysis of the domestic and wild ginger rhizomes revealed 27 (24 from domestic and 10 from wild) constituents, representing 99.4% and 99.9%, respectively of the total constituents detected. The dominant constituents of the domestic ginger rhizome were camphene (16.93%), Bisacurone epoxide (16.35%), Eucalyptol (14.90%), β-phellandrene (11.60%), α-zingiberene (7.17%), α-pinene (5.18%), geranial (4.14%), myrcene (4.08%), neral (2.87%), α-farnesene (2.81%), heptan-2-ol (2.31%), β-sesquiphellandrene (2.26%) and β-bisabolene (1.09%). For the wild ginger were β-phellandrene (72.73%), isoeugenol (3.98%), β-pinene (17.21%), and α-pinene (4.21%) (Table 3).

However, monoterpenes were present in the constituents of the two ginger rhizomes. The constituents were monoterpenes alcohols (heptan-2-ol, 4-terpineol); bicyclic monoterpenes (phellandrenes, camphene, borneol, pinenes); acyclic monoterpenoids like myrcene, geranyl acetate, geranial, neral and citronellol. Even though present were several sesquiterpenes such as farnesenes, β-bisabolene, α-zingiberene, curcumenes, caryophyllene, globulol. Some compounds were present in the wild ginger but absent in the domestic ginger rhizomes Tables 2 and 3. Furthermore, the wild ginger oils have isoeugenol (42.17%) as the major component identified, whereas α-zingiberene (18.56%) is the major constituent in the domestic ginger oils.

3.4. Determination of antifungal activity of essential oils (in-vitro)

Findings of this study showed that the assayed EOs had different degrees of growth inhibition against the five fungal species. The antifungal activity of the EOs (Table 4) suggests that the ginger EOs has antifungal effect. Considerable variation in the concentrations of the EOs ranging from 1 to 6 µl/ml was observed to have effect on the fungal pathogens. The fungal pathogens exhibited considerable inhibition even at a lower concentration (1 µl/ml). *Fusarium oxysporum* exhibited the highest inhibition (50.38 ± 0.5) and the lowest was *G. boninense* (27.46 ± 0.5) at a concentration of 1 µl/ml. The order of the sensitivity (descending order) was *F. oxysporum* > *C. falcatum* > *P. oryzae* > *R. microporus* > *G. boninense*. Mycelial inhibition was dependent on the index of concentrations because the diameter of inhibition increased with the increasing concentration of the EOs. Mycelial growth inhibition was observed in *F. oxysporum* and *C. falcatum* to be the same at 2 µl/ml (57.19 ± 0.3).

However, mycelial growth inhibition was also observed in all the sampled fungi. The percentage range of the suppression was from 50% to 100%. The positive control (Azoxystrobin/Difenoconazole) was effective at lower concentration because it demonstrated higher inhibition zones against all the fungal strains. Inhibition zones were not observed with the negative control. The minimum fungicidal concentrations of the ginger EOs on the tested isolates were in the range of 5–6 µl/ml, suggesting the fungicidal effects on the five fungi at > 5 µl/ml (Table 5).

Table 2 Chemical Compounds in Domestic and Wild Ginger Essential oils by GCMS that was identified by comparing their Retention Times (RT), Similarity Index (SI), and Mass Spectral Data with those from FFNSC1.3.lib, NIST11.lib, and WILEY229. Lib Mass Spectral Databases.

S/No	Chemical component	R. Time (mins)	Concentrations (%)		Formula
			DMT	**WLD**	
1	Heptan-2-ol	6.874	1.04812	0.33076	$C_7H_{16}O$
2	α-Pinene	8.084	2.04791	0.75519	$C_{10}H_{16}$
3	Camphene	8.635	5.33798	0.48012	$C_{10}H_{16}$
4	β-Pinene	9.677	0.4184	1.85	$C_{10}H_{16}$
5	6-Methyl-5-hepten-2-one	10.023	0.34674	–	$C_8H_{14}O$
6	Myrcene	10.228	1.81418	–	$C_{10}H_{16}$
7	α-Phellandrene	10.771	0.2967	–	$C_{10}H_{16}$
8	β-Phellandrene	11.861	5.51193	0.54087	$C_{10}H_{16}$
9	Eucalyptol	11.941	5.04681	1.40716	$C_{10}H_{18}O$
10	Terpinolene	14.474	0.27852	–	$C_{10}H_{16}$
11	1,6-Octadien-3-ol,3,7-dimethyl	15.011	1.07906	–	$C_{10}H_{18}O$
12	Citronellal	17.474	0.57812	–	$C_{10}H_{18}O$
13	Isogeranial	18.024	0.40472	–	$C_{10}H_{16}O$
14	Borneol	18.111	0.85195	0.67653	$C_{10}H_{18}O$
15	4-Terpineol	18.651	–	0.70908	$C_{10}H_{18}O$
16	*cis*-Verbenol	18.88	0.65463	–	$C_{10}H_{16}O$
17	α-Terpineol	19.284	–	0.9668	$C_{10}H_{18}O$
18	Linalyl propionate	19.298	1.04619	–	$C_{13}H_{22}O_2$
19	Fenchyl acetate	20.683	–	1.69652	$C_{12}H_{20}O_2$
20	Citronellol	21.128	0.97955	–	$C_{10}H_{20}O$
21	Neral	21.761	10.75466	0.85664	$C_{10}H_{16}O$
22	Geraniol	22.328	0.43753	–	$C_{10}H_{18}O$
23	Geranial	23.183	13.87864	1.14875	$C_{10}H_{16}O$
24	Anethole	23.755	–	4.60316	$C_{10}H_{12}O$
25	2-Undecanone	24.112	0.69251	–	$C_{11}H_{22}O$
26	α-Copaene	27.944	0.32066	–	$C_{15}H_{24}$
27	Geranyl Acetate	28.196	0.21411	–	$C_{12}H_{20}O_2$
28	β-Elemene	28.665	0.79624	0.66772	$C_{15}H_{24}$
29	Cyperene	29.033	–	0.90321	$C_{15}H_{24}$
30	Methyleugenol	29.14	–	1.00219	$C_{11}H_{14}O_2$
31	α-Bergamotene	29.679	–	0.41094	$C_{15}H_{24}$
32	Caryophyllene	29.913	–	6.72124	$C_{15}H_{24}$
33	Elemene	30.477	0.27864	–	$C_{15}H_{24}$
34	α-Humulene	31.39	–	1.73865	$C_{15}H_{24}$
35	*Trans*-β-Farnesene	31.452	0.42654	–	$C_{15}H_{24}$
36	Aromadendrene	31.701	0.29104	–	$C_{15}H_{24}$
37	Alloaromadendrene	31.71	–	1.49034	$C_{15}H_{24}$
38	Selina-4(14),11-diene	32.305	–	0.64802	$C_{15}H_{24}$
39	α-Selinene	32.323	0.21036	–	$C_{15}H_{24}$
40	α-Curcumene	32.613	4.42463	1.54084	$C_{15}H_{22}$
41	β-Chamigrene	32.713	–	0.73115	$C_{15}H_{24}$
42	Eremophilene	32.739	0.20921	–	$C_{15}H_{24}$
43	**β**-Humulene	32.802	–	0.85724	$C_{15}H_{24}$
44	α-Zingiberene	33.264	18.56259	–	$C_{15}H_{24}$
45	Isoeugenol	33.286	–	42.16819	$C_{11}H_{14}O_2$
46	α-Bisabolene	33.456	–	0.63301	$C_{15}H_{24}$
47	β-Bisabolene	33.684	–	5.19785	$C_{15}H_{24}$
48	*Trans*-Caryophyllene	33.728	9.64171	–	$C_{15}H_{24}$
49	γ- Amorphene	33.927	0.36408	–	$C_{15}H_{24}$
50	(Z)-γ Bisabolene	34.014	–	0.59183	$C_{15}H_{24}$
51	3, 7(11)-Eudesmadiene	34.148	–	0.40932	$C_{15}H_{24}$
52	α-Panasinsene	34.183	0.14748	–	$C_{15}H_{24}$
53	β-Sesquiphellandrene	34.42	6.46214	2.46761	$C_{15}H_{24}$
55	(E)-γ -Bisabolene	34.681	–	0.37421	$C_{15}H_{24}$
55	Elemol	35.414	0.5595	–	$C_{15}H_{26}O$
56	Germacrene B	35.788	0.34994	–	$C_{15}H_{24}$
57	(E, E)-Farnesol	35.933	0.57294		$C_{15}H_{26}O$
58	*Trans*-Nerolidol	35.935	–	0.52933	$C_{15}H_{26}O$
59	Spathulenol	36.613	–	0.65573	$C_{15}H_{24}O$

(*continued on next page*)

Table 2 (*continued*)

S/No	Chemical component	R. Time (mins)	Concentrations (%)		Formula
			DMT	**WLD**	
60	Globulol	36.86	–	1.90228	$C_{15}H_{26}O$
61	Viridiflorol	37.21	–	0.76439	$C_{15}H_{26}O$
62	Guaiol	37.415	–	1.26692	$C_{15}H_{26}O$
63	Ledol	37.652	–	0.46106	$C_{15}H_{26}O$
64	Humulene epoxide	37.897	–	0.26243	$C_{15}H_{24}O$
65	Levomenol	38.005	0.54691	–	$C_{15}H_{26}O$
66	γ-Eudesmol	38.323	0.14552	–	$C_{15}H_{26}O$
67	Zingiberenol	38.669	0.79597	–	$C_{15}H_{26}O$
68	Spathulenol	39.025	–	0.57582	$C_{15}H_{24}O$
69	Isoelemicin	39.444	–	0.39723	$C_{12}H_{16}O_3$
70	Rosifolio	39.514	0.36652	–	$C_{15}H_{24}O$
71	Cadin-4-en-10-ol	39.653	0.30705	–	$C_{15}H_{26}O$
72	Intermedeol	39.683	–	0.90742	$C_{15}H_{26}O$
73	Juniper Camphor	39.856	–	0.77779	$C_{15}H_{26}O$
74	*β*-isabolol	40.198	–	1.97755	$C_{15}H_{24}O$
75	Carotol	40.922	–	–	$C_{15}H_{26}O$
76	1-Chlorooctadecane	41.279	–	0.26508	$C_{18}H_{37}$ cl
77	Farnesal	42.909	–	0.43101	$C_{15}H_{24}O$
78	Squalene	45.164	–	0.49606	$C_{30}H_{50}$
79	Farnesyl Acetate	46.5	–	0.6923	$C_{17}H_{28}O_2$
80	Kauran-18-al	51.637	–	1.0494	$C_{21}H_{34}O$
81	*β*-copaen-4-α-ol	56.145	–	0.37397	$C_{15}H_{24}O$
82	Trispiro [4.2.4.2.4.2.] heneicosane	60.969	–	0.83048	$C_{21}H_{36}$
Total	–	–	99.0	98.9	–

DMT = Domestic ginger EOs, WLD = Wild ginger EOs, - (Absent).

3.5. Determination of in-vitro antibacterial activity of the essential oils

The ginger EOs showed different degrees of growth inhibition of the assayed EOs against the five bacterial species. The growth of the tested pathogens was affected by the concentration EOs which ranged from 100 to 500 µl/ml. In this present study, *X. oryzae* pv. *oryzae*-strain-A was affectively controlled by the EOs at 400 µl/ml and 500 µl/ml. The mean diameters of inhibition zone for 400 µl/ml and 500 µl/ml were 20.66 and 22.66 mm, respectively. The inhibition areas of *X. oryzae* pv. *oryzae*-strain-B at similar concentration were 17.33 and 18.66 mm, respectively (Table 4). *Bacillus* sp. recorded the lowest effect in which the mean diameter of the inhibition zones at 400 µl/ml and 500 µl/ml were 13.00 and 17.66 mm, respectively. These findings suggest significant antibacterial activity against *X. oryzae* pv. *oryzae*-strain-A, *X. oryzae* pv, *oryzae*-strain-B, *R. solanacearum, Klebseilla* sp. and least effective against *Bacillus* sp. The EOs' minimum inhibitory concentration (MIC) was determined and reported in Table 5. The MIC value of ginger EOs was 100 µl/ml. The effectiveness of antibacterial activity varied depending on the species of pathogens.

3.6. Metabolomic analysis of chemical constituents of ginger essential oils

The results obtained from the multivariate analysis of GC-mass spectral data of EOs for the domestic (DMT) and wild (WLD) gingers are shown in the PCA model against *X. oryzae* pv. *oryzae*-strain-A. The validity of PCA model was measured by the relative values of R2X and Q2 respectively, which are the goodness of a model's fit and predictive performance. Model fitness and predictive capacity are considered good when the values Q2 and R2 are > 0.5. R2X (cum) = 0.87 and Q2 = 0.69, indicating how well the model fit and good predictively, each point represented one sample in the score plot.

The PC1 explained 56.5% of the variation between the two different observations whereas PC2 explained 30.5% of the variability. Hence, the model explained a total of 87.0% of the data set variation. Fig. 3 shows the score plot of all the variables from the two EOs group (DMT and WLD) with good separation by PC1. Significant outliers were not observed. The respective loading plot shows a concentrated distribution of metabolites in the middle, suggesting that the two groups might share high similarity in metabolites (Fig. 1).

In the loading column plot (Fig. 2), the elements of the PCA loading vector represent the weights that combine the X-variables (domestic (DMT) and wild (WLD) ginger) to form the score vector. They are also proportional to the correlations between the scores and each X-variable.

The PLS model carried out the detection and comparison of metabolites present in ginger EOs with respect to the bioactivity. The developed PLS model was efficient as shown by parameters R2Y = 0.986 and Q2 = 0.96, respectively. These values indicate the fit and predictability of goodness. The scores plot shows PCs and sample classification. Hence, the model explained 86.2% of the data set variation (Fig. 2A).

Table 3 Phytochemical Compounds in Domestic and Wild Ginger Rhizomes by Headspace that was identified by comparing their Retention times (RT), Similarity Index (SI), and Mass Spectral Data with those from FFNSC1.3.lib, NIST11.lib, and WILEY229. Lib Mass Spectral Databases.

S/No	Chemical component	R. Time (mins)	Concentrations (%)		Formula
			DMT	WLD	
1	Bisacurone epoxide	1.773	16.35215	–	$C_{15}H_{24}O4$
2	2-Heptanone	5.809	0.36243	–	$C_7H_{14}O$
3	Heptan-2-ol	6.015	2.31573	–	$C_7H_{16}O$
4	α–pinene	6.928	5.47721	4.20737	$C_{10}H_{16}$
5	Camphene	7.321	16.93417	0.08067	$C_{10}H_{16}$
6	β-Pinene	8.055	0.99324	17.20679	$C_{10}H_{16}$
7	Myrcene	8.359	4.07577	0.49867	C_{10} H_{16}
8	Octanal	8.663	0.42944	–	$C_8H_{16}O$
9	α-Phellandrene	8.781	0.79561	–	$C_{10}H_{16}$
10	β-Phellandrene	9.504	11.60409	72.72907	$C_{10}H_{16}$
11	Eucalyptol	9.561	14.99832	0.40929	$C_{10}H_{18}O$
12	Butyl 2-methylvalerate	9.76	0.74342		$C_{10}H_{18}O$
13	Caryophyllene	10.937	–	0.49867	$C_{15}H_{24}$
14	Isoeugenol	11.276	–	3.98642	$C_{11}H_{14}O_2$
15	Linalool	11.503	0.7712	–	$C_{10}H_{18}O$
16	Citronellal	13.084	0.79028	–	$C_{10}H_{18}O$
17	Neral	15.717	2.87096	–	$C_{10}H_{16}O$
18	Geranial	16.564	4.13926	–	$C_{10}H_{16}O$
19	2-Undecanone	17.198	0.3768	–	$C_{11}H_{22}O$
20	Copaene	19.716	0.43628	–	$C_{15}H_{24}$
21	α-Curcumene	22.495	0.50593	–	$C_{15}H_{22}$
22	Germacrene D	22.591	0.59153	–	$C_{15}H_{24}$
23	α-Zingiberene	22.83	7.16701	–	$C_{15}H_{24}$
24	Fenchyl acetate	22.624	–	0.61332	$C_{12}H_{20}O_2$
25	α-Farnesene	23.077	2.81488	–	$C_{15}H_{24}$
26	β-Bisabolene	23.175	2.09026	0.07273	$C_{15}H_{24}$
27	β-Sesquiphellandrene	23.583	2.26332	–	$C_{15}H_{24}$
Total	–	–	99.4	99.9	–

DMT = Domestic ginger rhizome, WLD = Wild ginger rhizome, - (Absent).

Table 4 Inhibitory Effects of Ginger Essential Oils on the Growth of Important phytopathogens. The results were expressed as Mean (n = 3), Mean ± SD for Fungal and Bacterial Inhibition Zones and measured 5 days and 24-48hrs after incubation, respectively.

Phytopathogen	**Inhibition of Radial Growth (%)**						
	1 µl/ml	**2 µl/ml**	**3 µl/ml**	**4 µl/ml**	**5 µl/ml**	**6 µl/ml**	**PC**
Fusarium oxysporum	$50.38^{e} \pm 0.5$	$57.85^{d} \pm 0.1$	$67.43^{c} \pm 0.6$	$78.73^{b} \pm 0.7$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$
Pyricularia oryzae	$42.62^{e} \pm 0.8$	$46.90^{d} \pm 0.2$	$55.42^{c} \pm 0.5$	$71.32^{b} \pm 0.9$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$
Colletotrichum falcatum	$45.02^{e} \pm 0.6$	$57.19^{d} \pm 0.3$	$64.05^{c} \pm 0.4$	$76.82^{b} \pm 0.8$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$
Ganoderma boninense	$27.46^{f} \pm 0.5$	$32.75^{e} \pm 0.2$	$42.41^{d} \pm 0.4$	$59.82^{c} \pm 0.9$	$71.33^{b} \pm 0.7$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$
Rigidoporus microporus	$33.46^{f} \pm 0.8$	$41.65^{e} \pm 0.4$	$49.78^{d} \pm 0.3$	$63.48^{c} \pm 0.8$	$76.67^{b} \pm 0.6$	$100.00^{a} \pm 0.0$	$100.00^{a} \pm 0.0$

	Diameter of Inhibition Zone (mm)							
	50 µl/ml	**100 µl/ml**	**200 µl/ml**	**300 µl/ml**	**400 µl/ml**	**500 µl/ml**	**PC**	**NC**
Xanthomonas oryzae pv. *oryzae*- strain A	0.00^{g}	15.67^{f}	17.33^{e}	19.33^{d}	20.67^{c}	22.67^{b}	25.00^{a}	0.00^{g}
Ralstonia solanacearum	0.00^{g}	13.67^{f}	15.33^{e}	16.67^{d}	17.67^{c}	18.67^{b}	21.33^{a}	0.00^{g}
Xanthomonas oryzae pv. *oryzae*- strain B	0.00^{f}	15.67^{e}	16.67^{d}	16.33^{d}	17.33^{c}	18.67^{b}	20.67^{a}	0.00^{f}
Bacillus sp.	0.00^{e}	10.67^{d}	10.67^{d}	11.67^{c}	12.33^{bc}	13.00^{b}	17.67^{a}	0.00^{e}
Klebsiella sp.	0.00^{e}	14.67^{c}	10.67^{d}	14.33^{c}	14.33^{c}	16.33^{b}	20.33^{a}	0.00^{e}

Means in a row with different superscripts are significantly different ($P < 0.05$). PC-positive control and NC– negative control.

Bioassay of related metabolites are conveniently defined in the loading plot by their correlation with the PCs that distinguish sample groups. Differential metabolites contributing to the correlation were identified using variable importance for the projection (VIP) value. In general, a threshold of VIP > 1 was considered as the relevant metabolites for interpreting

Table 5 Effectiveness of Ginger Essential Oils in Suppressing the Growth of Important Bacterial and Fungal Pathogens, Measured 5 days and 24–48 h after incubation, respectively.

No	Pnytopathogens	LC_{50} (µl/ml)	LC_{90} (µl/ml)	MIC (µl/ml)	MFC (µl/ml)
1.	*Fusarium oxysporum*	1.3	5	1	5
2.	*Pyricularia oryzae*	2.8	5	1	5
3.	*Colletotrichum falcatum*	1.5	5	1	5
4.	*Ganoderma boninense*	2.5	6	1	6
5.	*Rigidoporus microporus*	3.5	6	1	6
6.	*Xanthomonas oryzae* pv. *oryzae*- strain A	300	400	100	
7.	*Ralstonia solanacearum*	400	500	100	
8.	*Xanthomonas oryzae* pv. *oryzae*- strain B	400	500	100	
9.	*Bacillus* sp.	500	> 500	100	
10.	*Klebsiella* sp.	500	> 500	100	

LC_{50} = lethal concentration of the EOs that kills 50% of the cell, LC_{90} = lethal concentration of the EOs that kills 90% of the cell, MIC = minimum inhibitory concentration and MFC/MBC = minimum fungicidal concentration/ minimum bactericidal concentration.

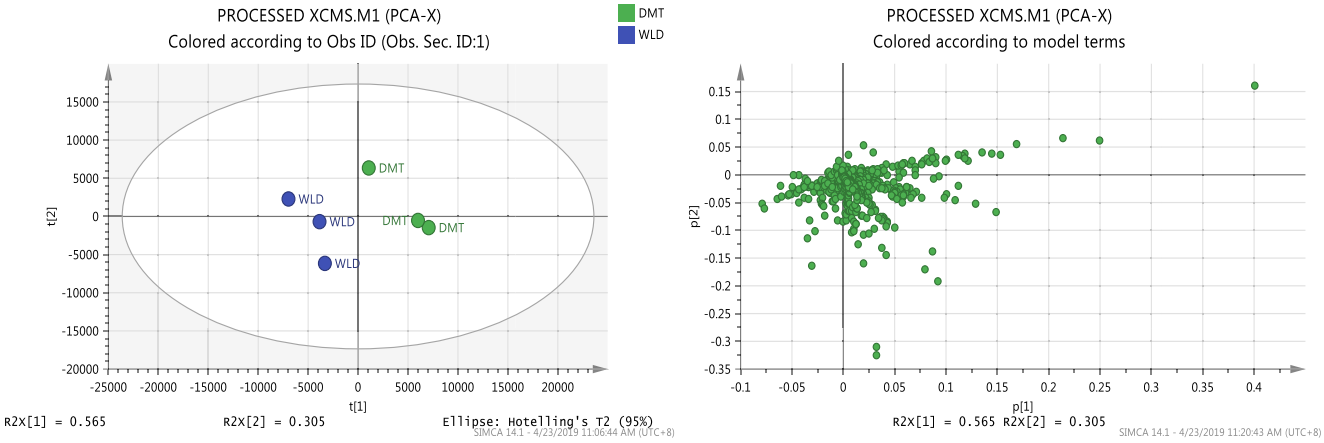

Fig. 1 Score and scattered plot of the observation (PCA) showing all the variables from the two EOs groups (DMT and WLD) have good separation by PC1 suggesting that the two groups might share high similarity in metabolites from the scattered plot.

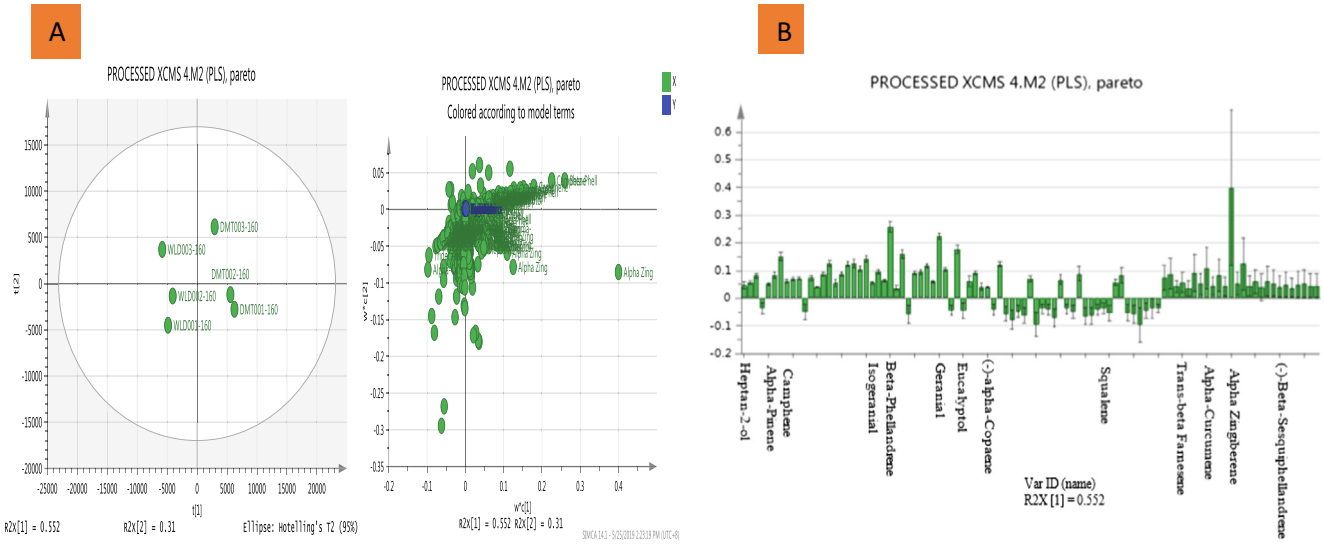

Fig. 2 Metabolomic analysis of domestic and wild EOs. (A) Score and scattered plot of the observation (PLS) showing all the variables from the two EOs group (DMT and WLD) have good separation by PC1. Suggesting that the two groups might share high similarity in metabolites from the scattered plot and (B) Loading column plot (PLS model) of simplified relevant metabolites in a threshold of VIP > 1 representing a total of 13 discriminating metabolites.

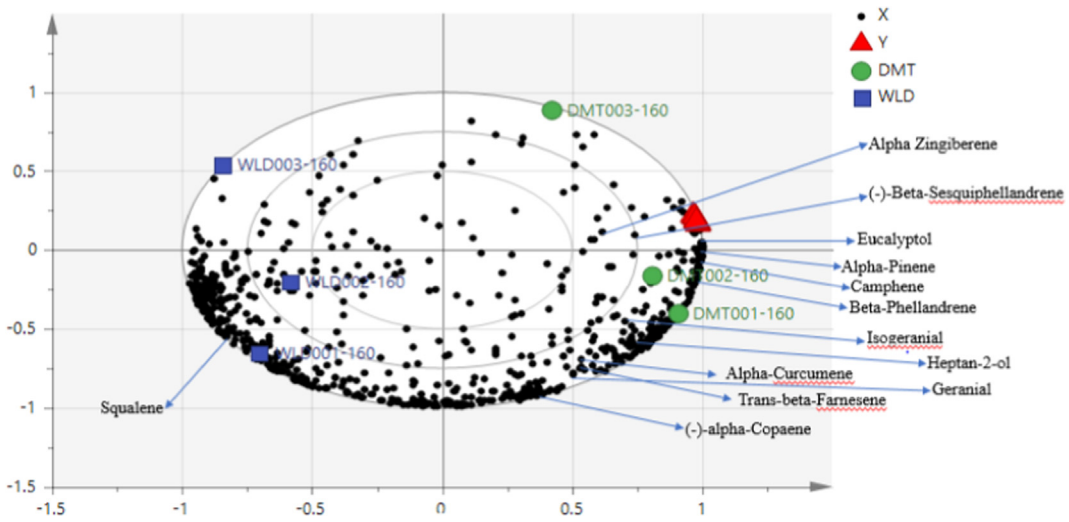

Fig. 3 The bi-plot of the simplified relevant metabolites in a threshold of VIP > 1 showing 13 metabolites with clear contribution from α-Zingiberene, (-)-β-Sesquiphellandrene and Eucalyptol in the bioassay activity.

the discrimination. Subsequently, accurate mass determination, elemental composition analysis, GCMS fragmentation and search of metabolite spectral databases establish the chemical identities of bioactive phytochemicals/reactive metabolites (putative compounds). The robustness of the model was also verified through further testing by a 100-permutation test which signifies a very good prediction. A total of 13 discriminating metabolites (Table 6) resulting from model group were obtained when compared with reported metabolites presented in Table 2.

The cross-validated PLS model showed a strong correlation between ginger EOs and *Xoo*-A bio-efficacy. The results indicate that 13 metabolites in the bioassay (Fig. 2B). This suggest clear activity by α-zingiberene, β-sesquiphellandrene and Eucalyptol (Fig. 3). Their functions in inhibiting *X. oryzae* pv. *oryzae*-strain-A growth were thoroughly ascertained. However, the PLS is a supervised calibration approach which enables the details of ginger components to be compared with the response data from the bioactivity instead of describing the variance of both gingers as PCA does. Conclusively, the results for multivariate analysis of GC-mass spectral data of both ginger EOs were considered with a good fitness and predictability of the constructed PCA and PLS models accordingly. It also showed that the metabolomics approach is an effective way of gaining insights into the metabolite responses to the *X. oryzae* pv. oryzae-strain-A bio-efficacy.

Table 6 The phytochemical Compounds of Ginger EOs in Relation to the Bi-plot Responsible for the Inhibition of the test Phytopathogens in a threshold of VIP > 1 that was considered as the Relevant Metabolites.

S/No	Putative compounds	VIP number	RT	Conc.	Formula	Biological activities
1	Heptan-2-ol	1.38276	6.874	1.04812	$C_7H_{16}O$	Disruption of the mitochondrial membrane - cell viability test thus, antimicrobial
2	α-Pinene	2.60823	8.084	2.04791	$C_{10}H_{16}$	Antifungal and antibacterial -Cellular toxicity
3	Camphene	6.40926	8.635	5.33798	$C_{10}H_{16}$	Insecticidal against cotton leafworm and Cytotoxicity against Homo sapiens (human) HeLa cells.
4	β-Phellandrene	7.3729	11.861	5.51193	$C_{10}H_{16}$	Antifungal against *Colletotrichum gloeosporioides*, *C. fragariae* and *C. acutatum*
5	Eucalyptol	4.29913	11.941	5.04681	$C_{10}H_{18}O$	Insecticidal against cotton leafworm.
6	Isogeranial	1.04368	18.024	0.40472	$C_{10}H_{16}O$	No information found
7	Geranial	2.18994	23.183	13.87864	$C_{10}H_{16}O$	Antimicrobial via cytotoxicity Assay
8	α-Copaene	1.40911	27.944	0.320066	$C_{15}H_{24}$	No information found
9	*Trans*-β-Farnesene	2.03628	31.452	0.42654	$C_{15}H_{24}$	Antimicrobial via cytotoxicity Assay
10	α-Curcumene	2.75977	32.613	4.42463	$C_{15}H_{22}$	Insecticidal, repellents and insect feeding deterrents.
11	α-Zingiberene	11.3492	33.264	18.5629	$C_{15}H_{24}$	Antifungal and Antibacterial against Gram-positive bacteria
12	β-Sesquiphellandrene	1.46082	34.42	6.46214	$C_{15}H_{24}$	Antimicrobial and antioxidant
13	Squalene	1.49348	45.164	0.6923	$C_{30}H_{50}$	Antibacterial through membrane permeation.

RT- retension time, VIP -variable importance for the projection.
Source: *https://pubchem.ncbi.nlm.nih.gov*.

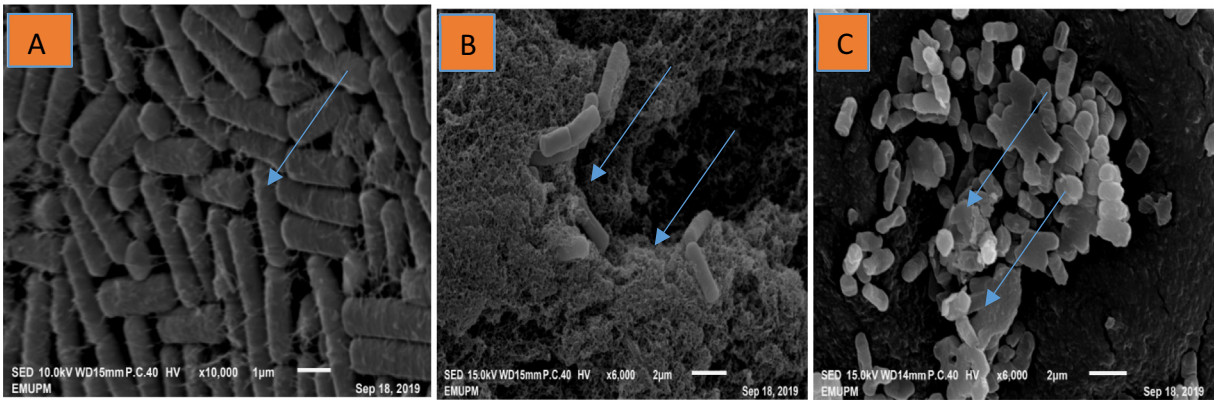

Fig. 4 Scanning electron micrograph of ginger EOs causes ultrastructural modifications in *Xanthomonas oryzae* pv. *oryzae*-strain A cells. (A) The *Xoo* cells treated with DMSO (control) showing *Xoo* cells with a normal rod shape, smooth and bright surface, (B) The *Xoo* cells treated with the ginger EOs showed irregular shape with sunken surfaces, severely disruption of the cells and (C) The *Xoo* cells treated with (Streptomycin (15 μg/ml) display abnormal growth, shrinkage, disruption and aggregation of the cells.

3.7. Scanning electron microscopy (SEM) observation on the mode of action of ginger EOs against Xanthomonas oryzae pv. oryzae-strain A

The examination of the changes on morphology and integrity of *X. oryzae* pv. *oryzae*-strain-A cells in response to ginger EOs treatment at MIC concentration (100 μl/ml), streptomycin (15 μg/ml/disc) and control with DMSO was carried out using scanning electron microscopy. The scanning electron micrograph revealed that normal untreated cells of *X. oryzae* pv. *oryzae*-strain-A was typically rod shaped with a normal, smooth and bright surface without any apparent cellular debris (Fig. 4A). However, the *Xoo* cells with ginger EOs demonstrated irregular shape with sunken surfaces and severely disruption of cells (Fig. 4B). Similarly, effects of the antibiotics on *X. oryzae* pv. *oryzae*-strain-A cells caused abnormal growth, lysis, shrinkage, disruption, and aggregation of cells (Fig. 4C). Thus, by electron microscopy (SEM), it can be inferred that the EOs interrupts the cell's structure with sunken surfaces and produces substantial damage that ultimately leads to *X. oryzae* pv. *oryzae*-strain-A growth inhibition.

4. Discussion

Considerable amount of EOs were extracted the domestic and wild gingers. The EOs extracted from domestic ginger yielded the highest amount (2.46%) than wild ginger (0.28%). The differences in the yields was due to differences of rhizomes texture and odor. Moreover, the amount of the yield is influenced by geographical origin, period of harvest, and condition of the ginger varieties. Similarly, the yield percentage of EOs depends upon on the amount of time spent during extraction, as prolonged extraction time would have a high chance of interaction between the solvent and the sample materials (Hoferl et al., 2015; Lopez et al., 2017). In this present study, the extraction time and geographic region were similar but the oil contents and yield differed because of the difference in the ginger species which might have resulted from variability in genetic composition of the species, plant maturity, and climatic and seasonal conditions in the regions where the crop is cultivated.

As for the findings of GCMS, the wild ginger chemical components of the EOs demonstrated higher number of volatile compounds compared with the domestic ginger. The domestic ginger also showed significantly higher volatile compound content as well as high volume oil and yield. Thus, both ginger EOs concentrations are diverse and present in different proportions. The major contents and compositions of the two EOs were slightly different. The wild ginger EOs had isoeugenol (42.17%) as the major chemical constituent, whereas, the α-zingiberene (18.56%) was the major constituent in the domestic ginger EOs. These results are comparable to those reported in the literature (Hoferl et al., 2015; Sharma et al., 2016.; Khayyat and Roselin, 2018). For some ginger EOs, geranial is the major component while others α--zingiberene and *β*-sesquiterpene were the main components in 10 to 60% range (Sharma et al., 2016.;Sharifi-Rad et al., 2017).

However, different analyses of natural products yield different efficiencies (Sharifi-Rad et al., 2017), the contents of both methods of analysis and the types of ginger which are defined by many compounds in the GCMS analysis of EOs but low compounds in the Headspace approach. This could be due to many factors such as the time taken for the analysis was short and the rhizomes were not efficiently utilized by headspace. In other words, some of the phytoconstituents were hidden in the rhizomes compared to the extracted EOs. The established Headspace analysis adopted in this present study proved to be simple, rapid, and convenient method for fingerprinting the volatile organic compositions characteristic. However, for better qualitative and quantitative scrutiny, extracted EOs should be harness.

The results of the *in-vitro* antifungal screening showed that ginger EOs are highly antifungal and potent against all the tested fungal pathogens. The LC_{50} value resulting from exposure to the EOs varied among fungal pathogens because of the ability of the pathogens to resist against the active volatile organic components. As the EOs concentration increased, the activity against the fungi increased. The findings of the present study are consistent with the reported in the literature. The *in-vitro* tests indicated that the ginger EOs exhibited effective antimicrobial activity against major phytopathogenic fungi. A study by El-Baroty et al. (2010) revealed that ginger EOs inhibited the growth of the common spoilage fungus *Aspergillus niger* at dilutions of 75 μg/ml and 100 μg/ml. Moreover, the EOs showed similar inhibitory effect against *P. notatum, M. heimalis* and *F. oxysporum*. Similarly, the

antimicrobial activity of EOs of *Z. officinale* showed that all of the fungal strains were sensitive (Lopez et al., 2017). Furthermore, Kumar et al. (2016) reported that EOs exhibited significant antimicrobial activity against *B. subtilis*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *A. niger* compared with standards. The EOs are made up of many sesquiterpenes and monoterpenes and exhibited significant antimicrobial activity against pathogenic microorganisms. The MIC and MFC of the ginger EOs on the tested isolates showed fungicidal effect on *F. oxysporum, P. oryzae, C. falcatum, G. boninense* and *R. microporus* at 5–6 µl/ml. Gakuubi et al. (2017) studied EOs of *Eucalyptus camaldulensis* Dehnh on the antifungal activity against *Fusarium* spp and found that MIC and MFC values of the EOs on the test pathogens were at 7–8 µl/ml and 8–10 µl/ml, respectively.

However, the results obtained for antibacterial activity against bacterial pathogens including *X. oryzae* pv. *oryzae*-strain A, *R. solanacearum, Klebseilla* sp., *X. oryzae* pv. *oryzae*-strain B and *Bacillus* sp. showed different degrees in growth inhibition of the assayed EOs. The bacterial growth of the tested pathogens was affected by the EOs concentration (100–500 µl/ml). In this present, the inhibition increased with the increasing concentration of the EOs used. The results obtained is in conformity Wonni et al., (2016) who also reported the antibacterial effects of EOs. Rajip et al., (2016) revealed that EOs of palmarosa (*Cymbopogon martinii*) at 5% and 1% concentrations demosntrated the highest pathogen inhibition followed by lemongrass oil (*Cymbopogon flexuous*), cinnamon oil (*Cinnamomum zeylanicum*) and vetiver oil (*Chrysopogon zizanioides*).

In the present study, the Intensity of the antibacterial activity varied depending on the species of bacteria. These findings are consistent with those reported in the literature. The study by Debbarma et al., (2013) on the antibacterial activity of ginger EOs (*Z. officinale*), eucalyptus (*E. camaldulensis*), and sweet orange (*Citrus sinensis*) were assessed against fish spoilage caused by pathogenic bacteria. Among the three EOs analyzed, ginger EOs demonstrated the best antibacterial activity against all the examined bacteria. In some studies, ginger EOs and extracts demonstrated strong antimicrobial activity and inhibitory to selected food-spoilage microorganisms (Nikolic et al., 2014; Bohme et al., 2014).

The metabolomics of GC-mass spectral data of the wild and domestic ginger EOs provides a systematic solution in addition to addressing the challenges of recognizing novel phytoconstituents in a complex interaction among phytochemicals and the microbial organisms using analytical software called SIMCA. Metabolomics ability to distinguish antimicrobials from different sources and expose the correlation with the bioassay arises from the advanced scientific base of data acquisition of GCMS. Data collection and preparation methods adopted in metabolomics-based research are used to promote and recognize novel phytoconstituents variability in the complex interaction of metabolites. The identification of the principal components (PC1 and PC2) in a complex dataset are meant to get clear sample clustering in the scores plot as well as the contribution of individual ions to PCs and group separation in the loading plot, which portrays the relationships between ions and PCs using the unsupervised method. The supervised multivariate data analysis (MDA) is used for model construction purposes by PLS whereby data properties and intent of the MDA analysis are usually determined by their correlation with antimicrobial activity against the tested microbes.

The action of EOs on the cell structure was fully identified by observing the changes on morphology and integrity of the *Xoo* cells with the MIC concentration of ginger EOs (100 µl/ml) and streptomycin (15 µg/ml). The integrity of the cell is very important for the survival of organisms as it is the key factor for critical biological activities taking place in the cells (Chouhan et al., 2017). The cell or cell membrane establishes an effective barrier between internal and external structures; important substances and chemicals are exchanged through the cell membrane. Thus, the effects of antimicrobial activity is achieved when cell morphology is disturbed (Wu et al., 2019). The work of de Oliveira et al. (2011) on assessment of the antibiotic efficacy of extracellular compounds generated by *Pseudomonas* strain against *X. citri* pv, *citri* 306 strain revealed that the cell integrity was completely disrupted by the action of the said compounds. Similarly, Sahu et al., (2018) found that niclosamide inhibited *Xoo* growth by impeding the formation of biofilms and disrupting *Xoo* cells. Based on the findings of this present study, ginger EOs have the potential application for controlling plant diseases caused by various phytopathogens. However, there are some limitations in our understanding on the regulating synergism and/or antagonism of the individual bioactive compounds in the EOs. Consequently, research in the future should explore the mechanism of action of the individual EO components, along with an initiation in systematically investigating on the mechanisms of synergistic interaction between components. New techniques for synergistic studies could provide an important platform for this field of research.

5. Conclusion

The chemical constituents in the two ginger EOs are diverse and present in different proportions. The overall finding of this study suggested that ginger EOs could be used as a new antimicrobial agent in suppressing the growth of phytopathogens and as a potential alternative for synthetic fungicides and bactericides for sustainable production agriculture.

6. Compliance with ethical principles

This research conducted by the authors includes no experiments with human and animal participant, rather than phytopathogens.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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