HAYATI
Journal of Biosciences

Vol. 29 No. 5, September 2022 570-585
DOI:10.4308/hjb.29.5.570-585
ISSN: 1978-3019
EISSN: 2086-4094

Volatile Bioactive Compounds from *Lasiodiplodia pseudotheobromae* IBRL OS-64, an Endophytic Fungus Residing in the Leaf of *Ocimum sanctum*

Mohd Taufiq Mat Jalil[1*], Darah Ibrahim[2]

[1]*School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia*
[2]*Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia*

ARTICLE INFO

Article history:
Received September 23, 2020
Received in revised form August 19, 2021
Accepted October 28, 2021

KEYWORDS:
Lasiodiplodia pseudotheobromae,
Ocimum sanctum,
Disc Diffusion Assay,
Thin layer chromatography (TLC),
Bioautography Assay,
Gas Chromatography-Mass Spectrometer (GCMS)

ABSTRACT

Endophytic fungi are known as potential novel compound producers with promising antimicrobial activities. Hence, the present study aimed to investigate the possible bioactive compounds present in the ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64. The ethyl acetate extract exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria in disc diffusion assay. Thin-layer chromatography (TLC) was performed with chloroform, acetone and ethyl acetate (1:2:1, respectively) used as a solvent system and nine spots with diverse polarities were obtained. The TLC chromatogram with the active spot was localized with bioautography assay and the finding revealed that the dark spot with an Rf value of 0.5882 showed good antibacterial activity against all test bacteria. The fraction F5 exhibited promising antibacterial activity upon partial purification of dark spot via column chromatography and the GC-MS analysis of fraction F5 resulted in the detection of a major compound, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester with 90% matching factor. Thus, this compound may greatly contribute to the antibacterial activity of the fraction and has the potential to be developed as an antibiotic. The findings indirectly indicate that fungal endophytes from the medicinal plant could be a potential candidate for bioactive compounds with pharmaceutical properties.

1. Introduction

Bioactive compounds are substances that possess biological activity or direct effect on a living organism either negative or positive effects, starting with good maintenance of health such as healing effect or be dangerous such as fatal effect (Guaadaoui *et al.* 2014). Medicinal plants are a well-known source for bioactive organic compounds with pharmaceutical potentials. Several bioactive compounds from medicinal plants are reported to demonstrate significant antioxidants, anticancer, antidiabetic, insecticide, and antimicrobial activities. For instance, Ahmad *et al.* (2014) reported the volatile profiling of *Polygonum minus*, an aromatic traditional medicinal plant of Malaysia in different tissues. They found that the volatile compounds isolated from *P. minus* exhibited several favorable biological active activities including antioxidants, anticholinesterase and antimicrobial activities. Furthermore, Siva *et al.* (2016) reported that the medicinal plant, *Ocimum sanctum* possesses various medicinal properties such as antidiabetic, anticancer, antiulcer, antibacterial, and antifungal activities. They also revealed several phytochemical compounds isolated from the plant including alkaloid, phenols, flavonoids, tannins, saponins, and essential oils.

Nowadays, endophytic fungi of medicinal plants also have been reported as one of bioactive compounds sources with promising pharmaceutical potentials. Fungal endophytes are believed to produce secondary metabolites similar to their host as a result of their long-term co-evolution and close relationship (Zhao *et al.* 2011). For example, the production of paclitaxel, a medicinal substance with a high anticancer activity that is widely used in hospitals and clinics form the Pacific yew tree. Mayor (2011) reported that paclitaxel was first discovered in the bark of Taxus brevifolia, a Pacific yew tree by US National Cancer Institute researchers in the 1960s. However, Stierle *et al.* (1995) reported that *Taxomyces andreanae*, an endophytic fungus associated with the Pacific yew tree, *T. brevifolia* was evaluated as a potential source

* Corresponding Author
E-mail Address: taufiqjalil@uitm.edu.my

of the anticancer drug taxol, a secondary metabolite of the host organism. This discovery proved the hypothesis that the fungal endophytes can produce bioactive compounds mimicking their hosts.

The emergence of multi-drug resistant strains has urged the discovery of new drugs the overcome the problem. In bacteria, multi-drug resistance may be generated by one of two mechanisms (Nikaido 2009). Firstly, these bacteria may accumulate multiple genes, each coding the resistance to a single drug within a single cell and the accumulation occurs on resistant plasmids. Second, multi-drug resistance may occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. This resistance strains affected human health worldwide and might lead to death. For instance, multidrug resistance *Pseudomonas aeruginosa* will increase the mortality rate on patients with bacteremia (Morata *et al.* 2012). Wisplinghoff *et al.* (2004) reported that *P. aeruginosa* as one of the leading causes of nosocomial bloodstream infections and the third most frequent cause of Gram-negative infections in the United States. This strain could cause several infections especially in immunocompromised hosts and it is associated with a high mortality rate (Vidal *et al.* 1996).

The antibiotic-resistant strain also occurs in food especially in the ready-to-eat foods (RTE) that lead to several food diseases which are serious widespread health problems in the contemporary world (Vincenti *et al.* 2018). Prior to that, Tewari *et al.* (2012) reported the prevalence of multidrug-resistant *Bacillus cereus* in foods and human stool samples whereby this isolate exhibited a high rate of resistance to ampicillin, carbenicillin, kanamycin, and intermediate resistance to amoxicillin and cephalothin. Due to the emergence of multidrug-resistant strain and the threat to human health, this study was designed to discover a new drug with pharmaceutical potentials from an endophytic fungus, *Lasiodiplodia pseudotheobromae* IBRL OS-64 isolated from the leaf of *Ocimum sanctum* Linn. to overcome the increasing problems.

2. Materials and Methods

2.1. Microorganisms and Cultural Maintenance

The endophytic fungus, *Lasiodiplodia pseudotheobromae* IBRL OS-64 and test bacteria were provided by the Industrial Biotechnology Research Laboratory (IBRL), School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The culture was grown and maintained as described by Taufiq and Darah (2020).

2.2. Preparation of Bacterial Suspension

The bacterial suspension was prepared by picking five single colonies from 24 –h-old culture and transferred them into 5 ml of 0.85% sterile physiological saline (w/v). The turbidity was adjusted using McFarland standards to obtain approximately 1 x 10^8 CFU/ml of the bacterial suspension.

2.3. Fermentation and Extraction

Endophytic fungus was culture in yeast extract sucrose (YES) broth that was prepared according to the method described by Taufiq and Darah (2018). The culture was than ferment and subsequently extracted by following the procedures described by Taufiq and Darah (2019).

2.4. Disc Diffusion Assay

The antibacterial activity of the extract was determined following the method described by NCCLS (2004). Five percent of ethyl acetate was used as a negative control whilst chloramphenicol (30 µg/ml) was set as a positive control. The diameter of inhibition zone formed surrounding the disc was measured and recorded. The experiments were carried out in triplicates on different occasions.

2.5. Thin Layer Chromatography (TLC)

Ethyl acetate partitioned extract was subjected for further separation proses using TLC by the following method described by Atalla *et al.* (2008). The square pieces of 2 × 11 cm aluminum-backed thin layer chromatography (TLC) plates were cut accordingly using a sharp penknife. Following that, the plates were lightly drawn using a pencil to horizontally mark the baseline of the spots at a distance of 1.0 cm of the lower edge of the TLC plate. An aliquot of the ethyl acetate partitioned extract that has been re-dissolved with methanol was dipped using a capillary glass tube and then spotted on the middle point of the horizontal line of the silica plate. The silica plate with the spots was allowed to dry in a moving air stream and the solvents were allowed to run until it reaches 0.5 cm from the top of the TLC plate.

2.6. Agar Diffusion Bioautography Assay

Agar diffusion bioautography assay was carried out according to the method proposed by Choma and Grzelak (2010). The TLC plate that was previously developed was placed on the surface of the brain heart infusion agar (BHIA)/Mueller-Hinton agar (MHA) and left overnight in a fridge at 4°C to allow the diffusion of a developed compound to the agar. The plate was

then inoculated with test bacteria after removing the TLC chromatogram on the agar surface. The final concentration of the bacterial inoculum was set to 5 × 10^5 CFU/ml with the volume of BHIA/MHA was 20 ml per plate. The plates were then incubated in an inverted condition for 24 hours at 37°C. The bacterial growth inhibition appeared as clear zones around the active spot was observed and recorded.

2.7. Staining of Developed TLC Plate and Chemical Profiling of Extract

The TLC plate was developed with chloroform: acetone: ethyl acetate at ratio 1:2:1, respectively. Following that, the plate was dried under an open air-stream to remove the residual organic solvents. The developed TLC plate was then sprayed with five different reagents such as ferric chloride, Wagner's reagent, sodium hydroxide, Iodine vapor, and potassium hydroxide in order to detect the various structural groups of compound viz. flavonoid, lactone, alkaloid, phenol, and anthraquinone. Detection of phenol: The 1% aqueous ferric chloride ($FeCl_3$) was prepared by dissolving 1 g of $FeCl_3$ in distilled water and then sprayed on the TLC plate. The appearance of green, black, blue and purple colors indicated the presence of phenol (Harborne 1973b). Detection of alkaloid: The Wagner's reagent was prepared by dissolving the 2 g potassium iodide and 1.27 g iodine in 5 ml of distilled water and the reagent was top up with distilled water until 100 ml and sprayed on the TLC plate. The presence of alkaloids was indicated by the appearance of reddish-brown on the TLC plate (Raaman 2006). Detection of flavonoid: The TLC plate was sprayed with an increasing amount of 1M sodium hydroxide (NaOH). The presence of flavonoids was indicated by the appearance of yellow color and the spot was discolored after the addition of 1M hydrochloric acid (HCl) (Krvavych *et al.* 2014). Detection of lactone: The developed TLC plate was fumigated with iodine crystals vapor in a closed container. The brown spot indicates the presence of lactone (Harbone 1973a). Detection of anthraquinone: Ten grams of potassium hydroxide (KOH) was dissolved in 100 ml of methanol and the TLC plate was sprayed with 10% methanolic KOH. The presence of anthraquinone was indicated by the change of original color to purple, green, violet or red color (Harborne 1973a).

2.8. Column Chromatography

The slurry packing method was used in the packing of the open column system as described by Salituro and Dufresne (1998). Following that, silica gel 60 with the bead size of 40–63 nm was used as a packing material. A chromatography glass column with 22.0 mm of diameter and 47.0 cm in length was used. Approximately, 35.0 g of silica gel was weighted and added with 100 ml ethyl acetate to acquire a pourable slurry. The slurry was then poured into the glass column system with three-fourths filled. The column was tapped with a rubber pipe to ensure good packing and avoid bubble formation during the packing process. Then, the packed column chromatography was left overnight before loading with the extract. The crude extract was dissolved in methanol and eluted with the same solvent system used during column packing. Due to colorless, the fractions were collected based on the volume viz. 20 ml for each fraction. All collected fractions were concentrated by air-dried under the fume hood chamber. Each fraction was analyzed on a TLC plate and the fractions with similar Rf values were combined.

2.9. MIC and MBC Determination

The MIC and MBC values of the extract were determined following the method described by NCCLS (2004). The MIC value of the fungal extract was recorded as the lowest dilution of the extract showing no bacterial growth (no changes in broth color), whereas the MBC value of the extract recognised as the lowest concentration of the MIC well with no visible of bacterial growth.

2.10. Gas Chromatography Mass Spectrometry (GC-MS)

To identify the possible volatile compounds present, the partially purified bioactive fraction that was previously collected from column chromatography was subjected to GC-MS. The faction F5 that exhibited significant antibacterial activity on disc diffusion assay was firstly dissolved with methanol (HPLC grade, Qrec, England) and filtered using sterile Sartorius polytetrafluoroethylene (PTFE) membrane filter (47 mm of diameter and 0.22 µm of pore size) to remove undesired particles, avoid clotting column and prevent contamination. The filtered sample was subsequently injected into the column of the Hewlett-Packed 6890N Network gas chromatography system. The mass spectrometer (Hewlett-Packard 5973 inert mass selective detector) was used in order to detect the presence of possible chemical compounds. The HP-5MS column (Agilent, USA) with an internal diameter of 30.0 m, length of 0.25 mm and fim- 0.25 µm was used in this system. Absolute methanol was used as a blank sample to calibrate the instrument. The GC-MS parameters such as the initial temperature of 280°C, split ratio of 5:1, helium gas carrier column flow of 1.2 ml/minute and injection volume of 1.0 µl were set up. The oven temperature program with an initial temperature of 70°C, hold time of 2 minutes,

Ramp of 30°C, hold time of 20 minutes, the total run time of 32.75 minutes and MSD transfer line heater 285°C were performed consecutively. Finally, the separated bioactive compounds were compared to the NIST02 Mass spectral library database based on chromatograms and their mass peaks.

2.11. Statistical Analysis

All the experiments were performed in triplicates (n = 3) and the experimental data were expressed as mean ± standard deviation (SD). The data were analysed by means of the One Way ANOVA using SPSS 15.0 and Duncan test was used to access the differences between means. The results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Disc Diffusion Assay

Antibacterial activity of ethyl acetate crude extract of *L. pseudotheobromae* IBRL OS-64 isolated from *Ocimum sanctum* leaves are shown in Table 1. The results showed that ethyl acetate extract possesses a significantly broad range of antibacterial activity against test bacteria since it could inhibit both Gram-positive and Gram-negative bacteria. The diameter of the inhibition zone produced by ethyl acetate extract against Gram-positive bacteria ranged between 24 to 30 mm whereas, for Gram-negative bacteria, the inhibition zone was in the range 14 to 20 mm. The finding revealed that the Gram-positive bacteria were more susceptible to the extract compared to Gram-negative bacteria. The present study also showed that bioactive compound that leads to antibacterial activity was secreted extracellularly since the ethyl acetate crude extract was obtained from the fermentative broth.

3.2. Thin Layer Chromatography (TLC)

TLC chromatography was performed to separate the distinct spots in the crude ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64 and the best solvent system was the mixture between chloroform, acetone and ethyl acetate in the ratio of 1:2:1, respectively. Figure 1A shows the TLC chromatogram of fungal isolate, *L. pseudotheobromae* IBRL OS-64 ethyl acetate crude extract viewed under the short UV wavelength whereby Figure 1B shows the graphical illustration of TLC chromatogram viewed under short UV wavelength. Besides that, Figure 1C depicts the TLC plate viewed under long UV light, whilst Figure 1D shows the graphical illustration of the TLC plate viewed under long UV light. Table 2 descripts the color and Rf values of the spot developed on the TLC chromatogram for the ethyl acetate extract of OS-64 viewed under short UV wavelength, long UV wavelength and also visible light. Results revealed that there were 9 separate spots observed on the TLC. There was only one yellow spot observed under visible light with an Rf value of 0.5882. As for observation under short UV wavelength, 9 spots were observed at Rf values ranging between 0.0706 and 0.7056 with all spots were in dark colour except spot number 9 which was in navy blue color (Table 2). Ironically, 9 same spots were also observed under long UV light with the same Rf value but different in color observation. Three spots were observed in turquoise (Rf value of 0.2941, 0.5294, and 0.6471), four in pale turquoise, one in a dark spot (Rf value =0.5882) and another one in light blue color (Rf value = 0.1882).

Table 1. Antibacterial activity of ethyl acetate crude extract against several test bacteria.

Test microorganism	Diameter of inhibition zone (mm)	
	Ethyl acetate crude extract (1,000 µg/ml)	Chloramphenicol (30 µg/ml)
Gram-positive bacteria		
Streptococcus mutans	24.0±0.6	29.4±0.6
Staphylococcus aureus	30.0±1.0	29.3±1.2
MRSA	25.3±1.2	32.3±0.6
Bacillus subtilis	26.0±1.0	28.7±1.2
Gram-negative bacteria		
Yersinia enterocolitica	20.1±0.6	28.3±1.2
Klebsiella pneumoniae	15.3±1.5	29.3±1.2
Shigella boydii	14.3±0.6	30.7±0.6
Escherichia coli	15.7±0.6	31.3±0.6

3.3. Preliminary Detection of Organic Compound

Nine spots detected from TLC were subjected to preliminary chemical analysis. Several tests were performed to detect and investigated the possible bioactive compounds that contributing to antibacterial activity including Wagner's reagent test, Sodium hydroxide test, 10% methanolic KOH test, Iodine vapor test, and 1% aqueous ferric chloride. Table 3 shows the results of the chemical analysis of nine spots detected on TLC. For a 1% aqueous ferric chloride test, only spot S7 gave the positive result which exhibited red-brown color upon the addition of aqueous ferric chloride.

3.4. Agar Diffusion Bio-autography

Agar diffusion bio-autography was performed to detect and investigate the bioactive compounds present in the extract. In this study, the spots were tested against several bacteria including *Streptococcus mutans*, Methicillin-resistant *Staphylococcus aureus, Staphylococcus aureus, Shigella boydii, Yersinia*

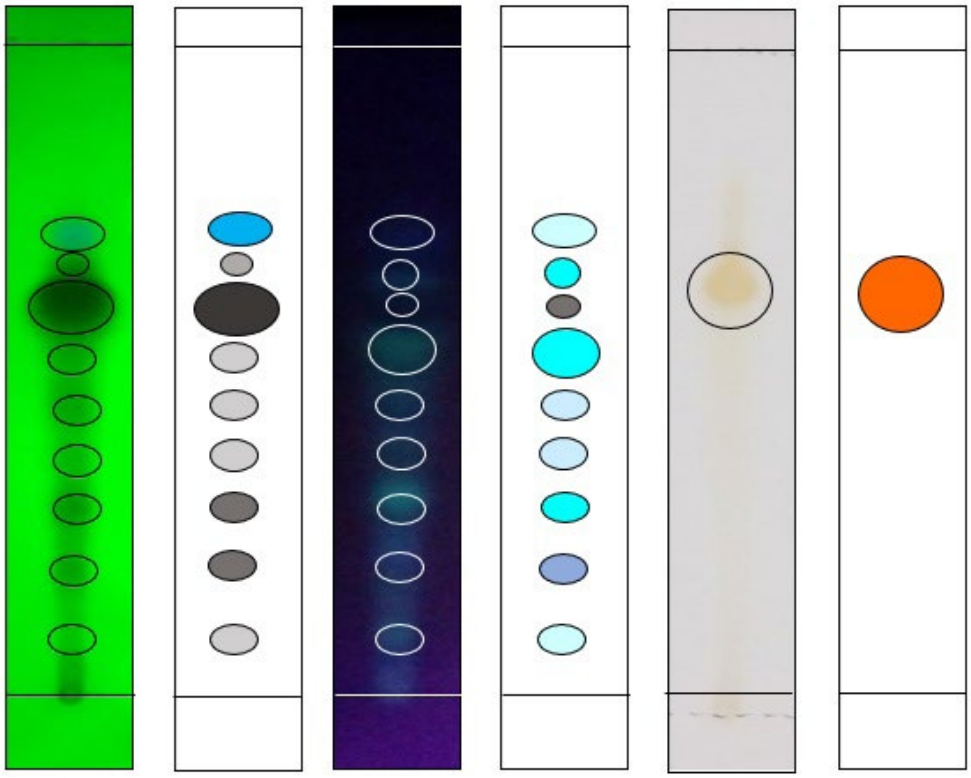

Figure 1. TLC chromatogram of fungal isolate OS-64 crude extract ethyl acetate viewed under different light wavelength with solvent system chloroform: acetone: ethyl acetate at ratio 1:2:1. (A) Short UV wavelength, (B) graphical illustration of short UV wavelength, (C) long UV wavelength, (D) graphical illustration of long UV wavelength, (E) visible light (F) graphical illustration of visible light

Table 2. Colour description and Rf values of the spot developed on TLC chromatogram for the ethyl acetate extract of *L. pseudotheobromae* IBRL OS-64

Spots	Short wavelength	Long wavelength	Visible light	R_f values
1	Dark spot*	Pale turquoise	-	0.0706
2	Dark spot	Light blue*	-	0.1882
3	Dark spot	Turquoise	-	0.2941
4	Dark spot*	Pale turquoise*	-	0.3529
5	Dark spot	Pale turquoise*	-	0.4235
6	Dark spot*	Turquoise	-	0.5294
7	Dark spot	Dark spot	Orange spot	0.5882
8	Dark spot*	Turquoise	-	0.6471
9	Navy blue	Pale turquoise	-	0.7056

enterocolitica, and *Klebsiella pneumoniae*. Table 4 shows the antibacterial activity of extract spots against tested bacteria. Spot S1 was susceptible to Gram-positive bacteria, *S. mutans* whereas spot S2 was susceptible to only Gram-negative bacteria, *Yersinia enterocolitica* and *Klebsiella pneumoniae*. Spot S3, S4, and S5 were observed to exhibit susceptibility towards *Y. enterocolitica* only. Besides that, spot S6 was observed to possess antibacterial activity against Gram-positive and Gram-negative bacteria, MRSA and *Y. enterocolitica*, respectively. Interestingly, spot S7 exhibits antibacterial activity towards five out of six test bacteria excluding *S. boydii*. Spot S7 exhibited a broad range spectrum mode of action since it can inhibit both types of bacteria, Gram-positive and Gram-negative bacteria. Besides that, spot S8 showed a good antibacterial activity towards Gram-positive since only the growth of *S. mutans,* MRSA and *S. aureus* was inhibited. Lastly, Spot S9 was observed to inhibit only the growth of *S. aureus*. Overall, the results revealed that the synergistic effect of bioactive compounds have contributed in the antibacterial activity of the ethyl acetyl crude extract since more than one spot (different compound) showed antibacterial activity against a particular bacteria.

3.5. Column Chromatography

The ethyl acetate crude extract of *L. pseudotheobromae* IBRL OS-64 was subjected to normal phase column chromatography in order to gain desired bioactive fractions. The mobile phase system used in this chromatography is similar to TLC which was chloroform: acetone: ethyl acetate with a ratio of 1:2:1, respectively. A volume of 1.0 mg/ml of extract was eluted to the column chromatography system and ten fractions were collected based on volume since the extract was almost colorless

Table 3. Chemical groups analysis of the spots appeared on TLC chromatogram

Chemical classes	Method	Reactions								
		S1	S2	S3	S4	S5	S6	S7	S8	S9
Phenol	1% aqueous ferric chloride	-	-	-	-	-	-	+	-	-
Alkaloid	Wagner's reagent test	-	-	-	-	-	-	-	-	-
Flavonoid	Sodium hydroxide test	-	-	-	-	-	-	-	-	-
Anthraquinone	10% methanolic KOH	-	-	-	-	-	-	-	-	-
Lactone	Iodine vapour	-	+	+	-	+	+	+	+	-

Table 4. Spot tested against Gram-positive and Gram-negative test bacteria

Spot (S)	Rf	Reactions							
		SM	MRSA	SA	BS	YE	KP	SB	EC
S1	0.0706	+	-	-	-	-	-	-	-
S2	0.1882	-	-	-	+	+	+	-	-
S3	0.2941	-	-	-	+	+	-	+	-
S4	0.3529	-	-	-	-	+	-	-	-
S5	0.4235	-	-	-	-	+	-	-	-
S6	0.5294	-	+	-	-	+	-	-	-
S7	0.5882	+	+	+	+	+	+	+	+
S8	0.6471	+	+	+	-	-	-	-	-
S9	0.7056	-	-	+	-	-	-	-	-

Key: (+): present of inhibition zone, (–): absent of inhibition zone, (SM): *Streptococcus mutans*, (MRSA): Methicillin resistant *Staphylococcus aureus*, (SA): *Staphylococcus aureus*, (SB): *Shigella boydii*, (YE): *Yersinia enterocolitica*, (KP): *Klebsiella pneumoniae*

when eluted in the silica gel. Ten fractions were re-subjected to the TLC system and re-run using a solvent system of chloroform: acetone: ethyl acetate (ratio 1:2:1 v/v, respectively) to verify targeted spots. The TLC analysis results were tabulated in Table 5. Under short wave view, only 3 fractions showed the presence of active spots that were fractions 4, 5, and 6 with Rf value ranging from 0.55 to 0.69. One spot was detected on fraction 3 with pale turquoise under long wave UV light with Rf value of 0.71. For fraction 4, one sharp turquoise spot can be observed under long-wave UV light with an Rf value of 0.65. The presence of dark spots was observed in fraction 5 at both short and long UV light with an Rf value of 0.57 and 0.58, respectively. Based on the recent finding, the Rf value of fraction F5 was almost similar to spot S7 (Rf value of 0.5882) developed from ethyl acetate crude extract in preliminary detection of bioactive compound using TLC and it can be suggested that those two spots were similar compound. For fraction 6, a dark spot with an Rf value of 0.55 was observed under short wave UV light whereas, under long wave two spots were detected which were dark spot and pale turquoise in colour with Rf value of 0.57 and 0.45, respectively. For fraction 7, only a pale turquoise spot (Rf value: 0.32) was observed under long-wave UV light while no spot was detected under short-wave. Meanwhile, a turquoise spot was detected under long-wave UV light in fraction 8 with an Rf value of 0.26. Two spots, light blue and pale turquoise with an Rf value of 0.15 and 0.10, respectively were detected under a long-wave in Fraction 8 whereas no spot was observed in fraction 9.

3.6. Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) Determination

The MIC and MBC values of fraction F5 against several test bacteria are illustrated in Table 6. In general, the findings showed that fraction F5 inhibited eight test bacteria at a concentration ranged between 125 µg/ml to 500 µg/ml for MIC and 125 µg/ml to 2,000 µg/ml for MBC values. As for Gram-negative bacteria, the MIC values were observed at a concentration of 125 µg/ml to 250 µg/ml while the MBC values were in the ranged between 125 µg/ml to 500 µg/ml. Results revealed that fraction F5 had a bactericidal effect on all Gram-positive bacteria. Meanwhile, MIC values of Gram-negative bacteria were in the range of 250 µg/ml to 500 µg/ml whereas the MBC values were observed at concentration ranged between 500 µg/ml to 2,000 µg/ml. The current findings demonstrated that the fraction F5 of *L. pseudotheobromae* IBRL OS-64 exhibited a bactericidal effect against three Gram-negative bacteria viz. *Yersinia enterocolitica, Shigella boydii,* and *Escherichia coli* while it showed a bacteriostatic effect towards *Klebsiella pneumoniae*.

Table 5. The TLC analysis of each fraction collected from the column chromatography

Fractions	Colour	Colour (R_f)		
		Visible light	Short wave	Long wave
1	colourless	-	-	-
2	colourless	-	-	-
3	colourless	-	-	Pale turquoise (0.71)
4	light yellow	-	Navy blue (0.69)	Turquoise (0.65)
5	light yellow	-	Dark spot (0.57)	Dark spot (0.58)***
6	yellow	-	Dark spot (0.55)	Dark spot (0.57) Pale turquoise (0.45)
7	colourless	-	-	Pale turquoise (0.32)
8	colourless	-	-	Turquoise (0.26)
9	colourless	-	-	Light blue (0.15) Pale turquoise (0.10)
10	colourless	-	-	-

Table 6. MIC and MBC values of fraction F5

Test bacteria	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)
Gram-positive bacteria				
Streptococcus mutans	125	125	1	Bactericidal
Staphylococcus aureus	250	500	2	Bactericidal
MRSA	125	125	1	Bactericidal
Bacillus subtilis	125	125	1	Bactericidal
Gram-negative bacteria				
Yersinia enterocolitica	250	500	2	Bactericidal
Klebsiella pneumoniae	250	2,000	8	Bacteriostatic
Shigella boydii	500	2,000	4	Bactericidal
Escherichia coli	500	2,000	4	Bactericidal

3.7. Gas Chromatography-Mass Spectrometry (GC-MS)

The volatile compounds present in the fungal extract of fraction F5 were screened and identified through GC-MS analysis. Figure 2 shows the gas chromatogram of the bioactive compounds detected using GC-MS instruments. The GC-MS analysis of the fraction F5 of *L. pseudotheobromae* IBRL OS-64 had identified ten possible volatile compounds within the retention time (RT) 9.655 to 26.886 min. The compounds were compared and identified based on the similarity index that matching to the identical registered compounds in NIST 8.0 library. Out of ten bioactive compounds detected, only eight compounds exhibited more than 80% of the similarity index (Table 7). The ten identified compounds were 1-Tridecene (RT 9.655), Hexadecanoic acid, methyl ester (RT 10.363), Cycloeicosane (RT 10.599), Octadecanoic acid, methyl ester (RT 11.286), 1-Nonadecene (RT 11.515), Cyclotetracosane (RT 12.362), Hexanedioic acid, bis [2-ethylhexyl ester] (RT 12.446), 1,2-Benzenedicarboxylic acid, mono [2-ethylhexyl] ester (RT 13.091), 2-Octadecyl-propane-1,3-diol (RT 13.213) and Phenanthro [3,2-b] furan-4-carboxylic acid 1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro 4,7,11btrimethyl,[4R94.alpha.,4a.alpha.,6a.beta.,7.alpha.,11a.alpha., 11b.beta.)]-(RT 26.886).

4. Discussion

Plant endophytic fungi have been recognized as an important and potential novel source of natural bioactive compounds for exploitation in agriculture, medicine, and industry (Joseph and Priya 2011). Endophytes are microorganisms that reside in the part of internal tissues of a plant without causing any disease symptoms to their host and have proven to be rich sources of bioactive natural products and they may provide protection as well as survival conditions to their host by producing substances that may have potential use in medical and agricultural industries (Molina *et al.* 2012). Furthermore, endophytic fungi are believed to produce novel bioactive compounds mirroring their hosts. For instance, the production of paclitaxel (taxol), a bioactive compound found originally from the bark of Pacific yew, *Taxus brevifolia* that has been proved to possess anticancer activity. However, in the year 1993, endophytic fungus, *Taxomyces andreanae* isolated from the pacific yew are reported to have the ability to produce paclitaxel. There are many reports regarding the antimicrobial activity of endophytic fungi that were active against pathogenic microorganisms. Handayani *et al.* (2017) reported the antibacterial activity of endophytic fungi isolated from the mangrove plant, *Sonneratia griffithii* Kurz whereas Desale and Bodhankar (2013) highlighted the antimicrobial activity of fungal endophytes isolated from *Vitex negundo* L. or commonly known as Chinese chaste tree against several bacteria including *E. coli, S. Typhimurium, B. cereus, B. subtilis, K. pneumoniae*, and *S. aureus.*

In this study, the antibacterial activity of the ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64 also demonstrated a significant inhibition effect on Gram-positive and Gram-negative bacteria. However, the findings revealed that the Gram-positive bacteria are more susceptible to the extract compared to Gram-negative bacteria. The different sensitivity of Gram-positive and Gram-negative bacteria towards the

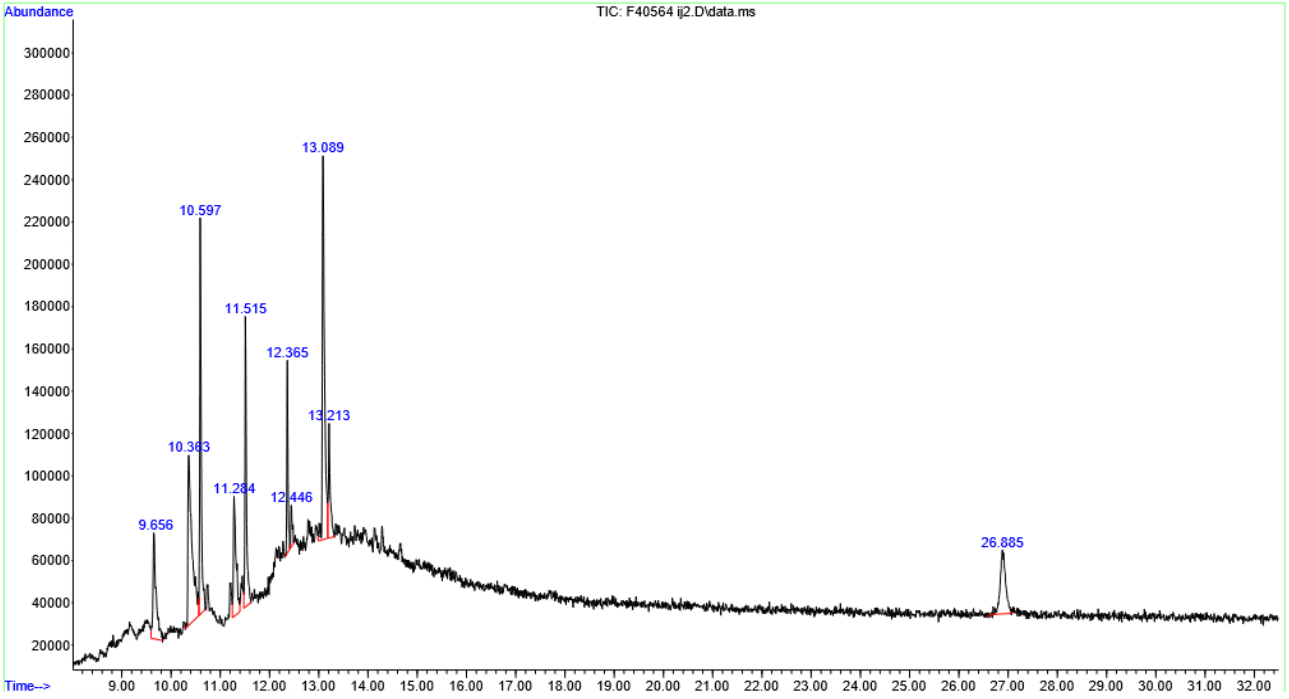

Figure 2. The gas chromatogram of fraction F5 from the ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64

Table 7. Characteristics of the compounds from GCMS analysis of fraction F5 of ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64

Compounds	Retention time (min)	Area	Matching factor (%)	Formula (molecular weight)
1-Tridecene	9.655	8.25	92	$C_{13}H_{26}$ (182.35 g/mol)
Hexadecanoic acid, methyl ester	10.363	17.21	98	$C_{17}H_{34}O_2$ (270.45 g/mol)
Cycloeicosane	10.599	14.00	98	$C_{20}H_{40}$ (280.54 g/mol)
Octadecanoic acid, methyl ester	11.286	7.86	98	$C_{19}H_{38}O_2$ (298.51 g/mol)
1-Nonadecene	11.515	10.83	95	$C_{19}H_{38}$ (266.52 g/ml)
Cyclotetracosane	12.362	5.41	94	$C_{24}H_{48}$ (336.65 g/mol)
Hexanedioic acid, bis (2-ethylhexyl ester)	12.446	1.57	76	$C_{20}H_{40}$ (280.54 g/mol)
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	13.091	21.11	90	$C_{13}H_{26}$ (182.35 g/mol)
2-Octadecyl-propane-1,3-diol	13.216	5.18	58	$C_{21}H_{44}O_2$ (228.58 g/mol)
Phenanthro [3,2-b] furan-4-carboxylic acid 1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,7,11b trimethyl,[4R94.alpha, 4a.alpha.,6a.beta.,7.alpha.,11a. alpha., 11b.beta.)]	26.886	8.58	10	$C_{36}H_{38}O_{10}$ (630.69 g/mol) Pulcherrimin E

extract could be due to the structural and morphological differences between both microorganisms. Generally, Gram-negative bacteria consist of complex structures such as the outer membrane layer, thin peptidoglycan layer, and periplasm compared to Gram-positive bacteria. The outer membrane layer is a structure that differentiates between both bacteria comprised of lipopolysaccharides, proteins, and phospholipids separating the external environment from the periplasm (Beveridge 1999). The outer membrane of Gram-negative bacteria has restricted flow and serves as selective barrier that prevents antibiotic compounds but, allows the transportation of valuable nutrients to the cell. Besides that, the outer membrane also anchored by membrane proteins known as porins which act as a selective channels that allow the transportation of a specific size hydrophilic substances into the periplasm (Miller and Salama 2018). According to Salton and Kim (1996), the high resistance of Gram-negative bacteria towards antibiotic agents might be due to the presence of a complex cell envelops that surrounded the peptidoglycan layer even though it is very thin. Furthermore, the special cell envelops act as a permeability barrier that restricts the flow of outsider molecules into the cell such as hydrophobic, hydrophilic, and charged molecules (Nikaido 2003). In addition to that, it explains why the performance of the extract was poorer against Gram-negative bacteria.

In the process of isolating bioactive compounds and fractioning the ethyl acetate extract of *Lasiodiplodia pseudotheobromae* IBRL OS-64, a bioassay-guided technique was performed starting with thin layer chromatography (TLC). TLC chromatography is a powerful tool and cost-effective method to separate, purify, and identify compounds from natural origin and it is useful for further evaluation (Ebada *et al.* 2008). The mobile phase in TLC travels upwards through the stationary phase and drives the extract mixtures that previously dropped on the lower part of plates upwards with different flow rate until the separation is achieved. By employing the TLC method, the position of substance in the mixture can be presumed by measuring relative mobility and expressed as Rf. The relative mobility value is useful for a qualitative description of the substance in a mixture (Coskun 2016). According to Kumar *et al.* (2016), the compounds that have almost similar physical properties with the mobile phase will stay longer in the mobile phase and it will travel furthest up the TLC plate whereas, the compounds that have high affinity to the silica gel in TLC and less soluble in the mobile phase will travel a shorter distance and stay behind.

The bioautography assay was used to detect the antibacterial activity of a particular spot of the extract against test bacteria. The assay was selected due to cheap, simple and it allows target-directed isolation of bioactive compounds for further test hence preventing isolation of inactive substances (Suleimana *et al.* 2009). In the present study, nine spots were successfully separated from the mixture with Rf values ranging between 0.0706 to 0.7056. The spots were tested on several bacteria and only one spot able to inhibit all test bacteria. It is noteworthy that some bacteria can be inhibited more than one spot. The finding speculated that the antimicrobial activity of crude extract towards certain test bacteria may be due to the synergistic action of several compounds in the mixture. Column chromatography is a useful tool to separate and characterize both organic and inorganic compounds and it also can be used to preliminary isolate of bioactive metabolites from a natural source such as plants (Bajpai *et al.* 2016). Separation of mixture compounds in column chromatography is based on adsorption to the stationary phase and the differences of substance solubility in the mobile phase. The elution process of the more polar compounds was relatively slower as they have a high affinity to the stationary phase whereas the less polar compound was eluted faster from the column since they could travel at a higher speed due to high affinity to the mobile phase (Sapkal *et al.* 2017). The result from column chromatography revealed that one spot named fraction F5 with an Rf value of 0.58 showed significant antibacterial activity towards all test bacteria and it proceeded for further study.

According to Kulip (2003), the appearance of blue, violet, purple, green, or red-brown color indicated a positive result of the fer ric chloride test. Phenol is one of the common phytochemicals that can be found in plants and fungi. According to Baba and Malik (2015), the presence of antioxidant compounds such as phenol in the plant might provide a protection mechanism toward several diseases. For instance, the ingestion process by natural antioxidants has been inversely associated with morbidity and mortality to inhibit degenerative disorders (Gulcin 2012). Yadav *et al.* (2014) reported 36% of endophytic fungal extracts isolated from Black plum, *Eugenia jambolana* Lam possess high total phenolic content. They revealed that endophytic fungi including *Aspergillus* sp., *Aspergillus niger, Aspergillus peyronelii,* and *Chaetomium* sp. strains exhibited phenolic constituent ranging from 58 mg/g to 60 mg/g GAE. In addition, crude extract of endophytic fungus, *Fusarium oxysporum* POS-3 isolated from *Plumeria obtusifolia* L. showed the presence of phenol in its bioactive compound and exhibited a significant antimicrobial activity towards several bacteria including *S. aureus, B. cereus, E. coli, S. thypi,* and yeast, *Candida albicans* (Ramesha and Srinivas 2014). Wagner's reagent test was performed to detect the presence of alkaloids. The current study showed that no alkaloid group was detected in the extract due to the negative result of all tested spots.

The formation of reddish-brown on the TLC indicated the presence of alkaloids (Shibu-Prasanth and Pratap-Chandran 2017). A sodium hydroxide test was carried out to investigate the presence of flavonoid in the fungal extract. However, the test showed a negative result for all tested spots. Generally, the changes colour of intense yellow to colourless with a few drops of diluted acid indicated the presence of flavonoids (Hossain *et al.* 2013). The presence of anthraquinone was tested using 10% methanolic KOH and a negative result was obtained indicated no anthraquinone presence in the extract. For a positive result, the appearance of the yellow and fluorescent yellow spots could be observed indicated the presence of anthrones and anthranols (Wagner *et al.* 1984). All spots on TLC were tested using Iodine vapour to detect the presence of lactone in the fungal extract. Surprisingly, six out of nine spots showed a positive result whereby the brown spot was observed indicating the presence of lactone. The current result was in agreement with Harborne (1973b) that mentioned the appearance of blue, brown, green, yellow, or red spots indicated the presence of lactone. Previous studies revealed that lactone is one of the bioactive compounds that can be produced by endophytic fungi. Chen *et al.* (2017) reported the finding of a novel lactone known as Lasiodiplactone A produced by an endophytic fungus, *Lasiodiplodia theobromae* ZJ-HQ1 isolated from mangrove. They revealed that the unprecedented lactone exhibited a significant anti-inflammatory activity by inhibiting nitric oxide production and potential anti-diabetic activity with an IC_{50} value of 29.4 µM. Besides that, Elfita *et al.* (2014), revealed that two new lactone derivatives were successfully isolated from an endophytic fungus, *Trichoderma* sp. Based on their finding, two lactone derivatives compounds namely 5-hydroxy-4-hydroxymethyl-2H-pyran-2-one and 5-hydroxy-2-oxo-2H pyran-4-yl) methyl acetate obtained from the ethyl acetate extract of endophytic fungus isolated from a small herb, *Tinaspora crispa*. Jimenez-Romero *et al.* (2008) claimed that lactones produced by an endophytic fungus, *Xylaria* sp. isolated from *Siparuna* sp. possess antiplasmodial activity towards *Plasmodium falciparum* and anticancer activity against Vero cells. Their study revealed that one of the lactone compounds, Phomalactone showed a significant antiplasmodial and cytotoxic activity with an IC_{50} value of 13 and 38 µg/ml, respectively.

Bag and Chattopadhyay (2015) evaluated synergistic antimicrobial efficacy by combining essential oils of spices and herbs. They revealed that a combination of cumin and coriander seeds oil was significantly increased antimicrobial activity and this due to synergistic interactions of both compounds. The finding showed that antibacterial activity of the six spots towards *Y. enterocolitica.* The result revealed that the consortium of spots attributing the antibacterial activity. Some spots showed a weak inhibitory effect towards test bacteria and this might be due to the diffusion of bioactive compounds into the agar. According to Dewanjee *et al.* (2015), the low diffusion rate usually occurred to water-insoluble compounds since they are difficult to naturally diffuse from the TLC plate into the agar. The compounds that sharing similar Rf values indicated that they have the same physicochemical properties and also lipophilicity that attributed to the same intermolecular attraction formed between the eluent and the compound (Wang and Zhang 1999; Komsta 2007).

It is noteworthy that fraction F5 showed a significant anti-MRSA activity which is a bacterial strain that resistant to a ß-lactam antibiotic. According to Silhavy *et al.* (2010), MRSA has the capability to express transpeptidase (PBP2A) combined with pentaglycine-branched substrates that enables it to escape from beta-lactams and thus, possess high resistant against ß-lactam antibiotics such as methicillin. Therefore, the compound in fraction F5 might have a different mechanism of action since it could inhibit the growth of MRSA. The present findings also showed that Gram-negative bacteria exhibited relatively low susceptibility towards fraction F5. This phenomenon may be due to the presence of resistance mechanisms such as the outer membrane layer containing lipopolysaccharides (LPS) and the efflux pump system (Fair and Tor 2014). Besides that, results also revealed the susceptibility of some bacteria was increased in fraction as compared to crude extract and vice versa. Soltanian *et al.* (2016) claimed that the crude extract was more efficient than its fraction and they suggested that this phenomenon is due to a synergistic effect by a combination of mycochemical constituents. Furthermore, additive and synergistic effects of several phytochemical substances rather than a single compound influenced the efficacy of natural drugs (Martins *et al.* 2013). According to Ginsburg and Deharo (2011), the additive effect happened when different bioactive compounds are combined and interact in a mixture to exhibit effect similar as a single compound whereas the synergistic effect occurred when the combination of several bioactive compounds could exert greater effect or inhibition greater than a single compound.

The MIC values of the fraction F5 against Gram-positive and Gram-negative bacteria were in the range of 125–250 µg/ml and 250–500 µg/m, respectively. Kuete (2010) postulated that the cut off value for MIC of bioactive compound is as follows: weak (MIC >625 µg/ml), moderate (100≤ MIC ≤625 µg/ml) and significant (MIC ≤100 µg/ml). Therefore, the MIC values of the fraction F5 against a broad range of the test bacteria were in a moderate range of potency. Besides that, the extract demonstrated a wide range of MIC values indicating the different susceptibility level of the

test bacteria towards the extract and the variation in susceptibility could be due to different composition in the extract whereby some compounds in the extract tend to show their selective activity against a particular bacteria (Yenn *et al.* 2014). Besides that, some test bacteria especially Gram-negative bacteria possess high MIC values. According to Wright (2005), this phenomenon may be due to the protective mechanism by the cell component of some microorganisms such as degrading enzyme and extra cell wall layer that contribute to less accessibility of the fungal extract. On the other hand, MBC values of the fraction against Gram-positive and Gram-negative bacteria were 125–500 µg/ml and 500–2,000 µg/ml, respectively. The MBC to MIC ratio of the fraction F5 against all test bacteria were less than 4 except for *Klebsiella pneumoniae* (MBC/MIC ratio = 8). The findings suggested that fraction F5 possesses a bactericidal effect towards the majority of test bacteria, but exhibited a bacteriostatic effect against *K. pneumoniae*. According to Pankey and Sabath (2004), if the MBC/MIC ratio was more than four (MBC/MIC >4), the extract was noted as bacteriostatic whilst if the ratio was less than 4 (MBC/MIC ≤4), it was a bactericidal agent. Furthermore, the same MIC and MBC values of the three test bacteria, *Streptococcus mutans*, MRSA, and *Bacillus subtilis* indicating the sensitivity of the test bacteria towards the extract. Ding *et al.* (2008) postulated that similar MIC and MBC values indicated high sensitivity of test bacteria towards the antibacterial compound in the extract. Moreover, the findings revealed that the fraction F5 exhibited significant antibacterial activity against multi-drug resistant strain such as MRSA due to its low MIC and MBC values. Yenn *et al.* (2012) stated that the low MBC value of the extract towards multidrug-resistant pathogens could validate to some extent the use of endophytic fungi from medicinal plants origin as a source of extremely novel chemotherapeutic agents. Thus, the MIC and MBC values of the fraction F5 could be a useful guideline to choose appropriate and effective doses of therapeutic substances in pharmaceutical and medical practices.

Several compounds were screened using GC-MS analysis with more than 80% of the similarity index that may be contributed to the antibacterial activity of the extract. 1-tridecene is a subclass of compounds known as unsaturated aliphatic hydrocarbons which is an aliphatic hydrocarbon that comprises one or more unsaturated carbons with one or more double or triple bonds. It can be found in nuts, fruits, milk and milk products, fats, and oils and also present in heated oils of nuts, butter, and sunflowers (Anonymous 2018). Sharma and Shah (2015) reported 1-tridecene is a significant constituent in essential oil isolated from *Senecio nudicaulis* Wall. ex DC. growing wild in Himachal Pradesh, India that showed significant antioxidant activity. Besides that, Niu *et al.* (2016) revealed that 1-tridecene was found in volatiles extract of red flour beetles, *Tribolium castaneum* (H.), and this compound was considered as defensive secretions. Mihigo *et al.* (2015) found that 1-tridecene as one of the constituent compounds from leaf extract of the Congolese weedy plant *Emilia coccinea* (SIMS) with moderate antibacterial activity. Naragani *et al.* (2016) revealed the first report on 1-tridecene, a bioactive compound from the genus of Streptomyces, *Streptomyces cheonanensis* VUK-A isolated from mangrove ecosystem that exhibited moderate to significant antimicrobial activity. Besides that, Venkatadri *et al.* (2017), reported 1-tridecene is one of the chemical constituent found in the ethyl acetate extract of *Michelia nilagirica* bark and they postulated that the extract possesses good antibacterial activity. This compound also has been reported to attribute aroma in plant essential oil. According to Jirovetz *et al.* (2006), pleasant warm-herbal-wood-spicy odour-notes of *Acmella radicans* from India might be attributed 1-tridecene, 2-tridecanone, 2-pentadecanone, guaiol, elemol, trans-β-caryophyllene, α-gurjunene, and α-humulene. Best of our knowledge, no previous study reported bioactive compound, 1-tridecene from the fungal extract.

Hexadecanoic acid, methyl ester, or palmitic acid is a common saturated fatty acid found in plants, animals, and microorganisms (Rustan and Drevon 2005). Many previous studies revealed that palmitic acid as phytochemistry compounds with several bioactive properties. Khrisnamoorthy and Subramaniam (2014), reported that hexadecanoic acid, methyl ester was found in methanolic stem extract of *Solena amplexicaulis* (Lam.) Gandhi. Mohamed *et al.* (2014), found that hexadecanoic acid methyl aster in the leaf extract of *Neolamarckia Cadamba* (Rubiaceae) from Malaysia. Besides that, Jegadeeswari *et al.* (2012) revealed that palmitic from Indian native plants, *Aristolochia krysagathra* possessed medicinal properties including antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory. Indrianingsih and Tachibana (2017) reported the ethyl acetate extract of endophytic fungus, *Xylariaceae* sp. QGS01 isolated from the stem of *Quercus gilva* Blume possesses palmitic acid with anti-diabetic activity. Besides that, hexadecanoic acid methyl ester was also considered as an aroma-active compound. Li *et al.* (2017c) reported that hexadecanoic methyl ester is one of the main aroma-active substances identified in traditional fermented Pixian broad bean with 0.19% relative composition and they postulated that esters compound is important indicators in the determination of the quality grade of sauce fragrance.

Octadecanoic acid methyl ester or known as stearic acid is one of the substances from the ester group that

has been reported to possess pharmaceutical potential. According to Othman *et al.* (2015), this compound has successfully isolated from *Jatropha curcas* L root with 22.2% of percentage area with a retention time of 13.559 based on GC-MS analysis and was found to exhibited anti-inflammatory activity. Besides that, Zayed *et al.* (2014) reported that octadecanoic acid methyl ester is one of the major constituents in the leaf extract of *Neolamarckia cadamba* from Malaysia with a percentage area of 11.71%. They revealed that the compound may be responsible for antimicrobial, antioxidant, and anti-diabetic activities of the extract. Furthermore, Rahman *et al.* (2014) also reported the antimicrobial potential of octadecanoic acid methyl ester and their related compounds isolated from leaf extracts of *Jatropha curcas* and *Andrographis paniculata*. Sudharsan *et al.* (2011) also claimed that octadecanoic acid methyl ester isolated from *Trigonella foneum-graecum* L. possess potential antibacterial and antioxidant activities. Their findings revealed that the ethyl acetate extract from the plant root exhibited strong antibacterial activity against *P. aeruginosa* and *B. subtilis* whereas poor activity towards *S. aureus* and *E. coli*.

Another presumed bioactive compound that has been identified in the fraction F5 was 1-nonadecene. Smaoui *et al.* (2012) reported the finding of 1-nonadecene isolated from a broth culture of a new actinomycetes strain, *Streptomyces* sp. TN256. Their study revealed that the pure bioactive compound possesses antifungal and antibacterial activity. Besides that, wild type cyanobacterium strain, *Synechococcus* sp. has been reported to have the capability to biosynthesis hydrocarbon such as 1-nonadecene (Yoshino *et al.* 2015). Other reports revealed the finding of 1-nonadecene in the Brazilian medicinal herb, *Erythrina velutina* Willd (Correia *et al.* 2011). In their study, the bioactive compound presented in chromatographic peak in the time range of 10.49 to 10.51 min. Devi and Singh (2013) claimed that endophytic fungus, *Colletotrichum gloeosporioides* isolated from the medicinal plant, *Phlogacanthus thyrsiflorus* Nees produced potential bioactive compounds including 1-nonadecene. Bio-control fungus, *Acremonium* sp. (MPHSS-2.1) was reported to possess significant antifungal activity against phytopathogens with IC_{50} value in the range of 0.21 to 0.31 mg/ml (Chowdhary and Kaushik 2018). Their further study through GC-MS analysis confirmed the presence of 1-nanodecene in the hexane extract of endophytic fungus isolated from peppermint plants. Besides that, cyclotetracosane is one of the constituent compounds that has been extracted from exotic weed (*Croton bonplandianus* Bail) was found to possess a significant α-amylase inhibitory, antioxidant, and antidiabetic activities (Karuppiah Vijayamuthuramalingam *et al.* 2017). Besides that, cyclotetracosane also present in the *n*-hexane extract of *Myristica fatua* leaves (Fajriah *et al.* 2017). In their study, the bioactive compound with a retention time of 23.172 min was contributed in cytotoxic activity towards breast cancer MCF-7 cell line with an IC_{50} value of 2.19 µg/ml. Bughio *et al.* (2018) reported that the chemical constituents of cyclotetracosane in the essential oil of *Tamarix dioica* are similar in leaves and flowers and a higher composition of hydrocarbons and phenolic compounds were observed. They also claimed that essential oils of flowers and leaves exhibited a good antimicrobial activity towards Gram-negative bacteria, *E. coli*, and Gram-positive bacteria, *S. aureus*.

Peng *et al.* (2017) reported the antibacterial molecular activities of poplar wood extractives. Their study revealed that 24 compounds were identified from wood extracts of *Populus tomentosa* (LD-021) including hexanedioic acid, bis (2-ethylhexyl) with 1.10% of P.A. Meanwhile, 27 components successfully identified from wood extracts of *Populus lasiocarpa* (LD-174) with the ester compound comprise 0.90% of the total amount. They also reported that hexanedioic acid, bis (2-ethylhexyl) are one of the rare components in the wood extract of poplar woods since it was an industrial chemical that was commonly used in Canada for several industrial purposes such as in cosmetic products. On the other hand, the current study revealed that the fungal extract might contain 2-octadecyl-propane-as one of the compound constituents with a matching percentage of 58. Ara *et al.* (2012) reported that local medicinal plants from Saudi Arabia exhibited antimicrobial activity towards *Staphylococcus aureus, Bacillus subtilis, Candida albicans, Escherichia coli*, and *Shigella sonnei*. Their GC-MS analysis showed that one of the medicinal plants, *Datura stramonium* possesses 2-octadecyl-propane-1,3-diol in its extract and the compound might contribute to antimicrobial properties. Besides that, 2-octadecyl-propane-1,3-diol represents one of the major constituents in the bioactive compounds extracted from *P. harmala* oil with a percentage area of 6.18% (Selim *et al.* 2013b). They also claimed that the volatile oil from *P. harmala* exhibited significantly higher antibacterial activity towards *B. cereus* and *B. subtilis*.

The present study revealed that 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester is a major compound in the fraction F5 with 21.11 retention area and 90% of matching factor. According to Rizwan *et al.* (2012), 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester is a common plasticizer that was found to exert antimicrobial activity. Their GC-MS analysis showed that the methanolic extract fraction of *Agave attenuate* Salm-Dyck comprises of 31 components with 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester

was observed as one of the major constituents with a percentage area of 6.33%. Besides that, Verma *et al.* (2014a) claimed that the secondary metabolite from an endophytic fungus, *Aspergillus flavipes* KF671231 composes of 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester as the main constituent of the bioactive compound with antifungal activity. They revealed the partially purified fraction that previously subjected to bioassay-guided fractionation exhibited one sharp peak at retention time (RT) 14.648 which covers 73.40% of the total area. They conclude that the antifungal property might be from 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester that 98% matched with the standard library (NIST) database. Besides that, Jenecius *et al.* (2012) also reported that the compound possesses antifouling and antimicrobial activity. Besides that, the results through GC-MS analysis showed that major compounds in the fungal extract were belonging to fatty acid methyl ester, the hydrocarbon derived from fatty acids such as alkenes and alkanes. According to Agoramoorthy *et al.* (2007), several fatty acids are known to possess antibacterial and antifungal activities such as myristic and palmitic acids. Besides that, Ramanathan *et al.* (2016) revealed that alkenes and alkanes are two out of eight bioactive compounds present in the methanolic extract of *Rhizophora mucronata* that demonstrated significant antibacterial and antifungal activities towards test microorganisms. The presence of stearic and palmitic acids as one of the functional group constituents in the fixed oil extract from the seed of *Ocimum sanctum* has been previously reported (Singh *et al.* 2012). Furthermore, Theantana *et al.* (2012), claimed that hexadecanoic acid and octadecanoic acid methyl ester were mainly found in endophytic fungi of Thai medicinal plants including *Eupenicillium tropicum, Eupenicillium shearii, Phomopsis conorum, Penicillium steckii*, isolated from *Stemona tuberosa* Lour. and *Fusarium proliferatum* isolated from *Houttuynia cordata* Thunb with antimicrobial and antioxidant potentials. Moreover, Henry *et al.* (2002) reported that fatty acids could exhibit good antioxidant activity.

The potential pharmacological bioactive compounds from the endophytic fungus, *Lasiodiplodia pseudotheobromae* IBRL OS-64 extract demonstrated promising antibacterial activity against a broad-range of test pathogenic bacteria. The yellow bioactive fraction procured exhibited that the 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester was the major compound with a 21.11 retention area and 90% of matching factor and together with other several compounds may contribute to the antibacterial property of the extract.

Conflict of Interest

Authors have declared that no competing interests exist.

Acknowledgements

The authors are grateful to Universiti Sains Malaysia for awarding the RUI research grant scheme (ac: 1001/PBIOLOGI/811326) to support this study and Ministry of Education, Malaysia for granting Taufiq, MMJ MyBrain scholarship.

References

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., Hsu, M.J., 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove. *Braz. J. Microbiol,* 38, 739-742. https://doi.org/10.1590/S1517-83822007000400028

Anonymous., 2018. The human metabolome database. The Metabolomics Innovation Centre. Available at: http://www.hmdb.ca/metabolites/HMDB30930. [Date accessed: 24 April 2018]

Ahmad, R., Baharum, S.N., Bunawan, H., Lee, M., Mohd Noor, N., Rohani, E.R., Ilias, N., Zin, N.M., 2014. Volatile profiling of aromatic traditional medicinal plant, *Polygonum minus in* different tissues and its biological activities. *Molecules.* 19, 19220-19242. https://doi.org/10.3390/molecules191119220

Ara, I., Bukhari, N.A., Solaiman, D., Bakir, M.A., 2012. Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis. *J. Med. Plants. Res.* 6, 5688-5694.

Atalla, M., Zeinab, H.K., Eman, R.H., Amani, A.Y., Abeer, A.A., 2008. Production of some biologically active secondary metabolites from marine-derived fungus *Varicosporina ramulosa. Malays. J. Microbiol.* 4, 14-24.

Baba, S.A., Malik, S.A., 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J. Taibah. Univ. Sci.* 9, 449-454. https://doi.org/10.1016/j.jtusci.2014.11.001

Bag, A., Chattopadhyay, R.R., 2015. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PLoS ONE* 10, e0131321. https://doi.org/10.1371/journal.pone.0131321

Bajpai, V.K., Majumder, R., Park, J.G., 2016. Isolation and purification of plant secondary metabolites using column-chromatographic technique. *Bangladesh. J. Pharmacol.* 11, 844-848. https://doi.org/10.3329/bjp.v11i4.28185

Beveridge, T.J., 1999. Structures of Gram-negative cell wall and their derived membrane vesicles. *J. Bacteriol.* 181, 4725-4733. https://doi.org/10.1128/JB.181.16.4725-4733.1999

Bughio, S.H., Samejo, M.Q., Memon, S., Bano, S., Mughal, M.A., Memon, A.A., 2018. Chemical composition of the essential oils from *Tamarix dioica* and determination of its antibacterial activity. *Int. J. Food. Prop.* 20, 2660-2667. https://doi.org/10.1080/10942912.2017.1387138

Chen, S., Liu, Z., Liu, H., Long, Y., Chen, D., Lu, Y., She, Z., 2017. Lasiodiplactone A, a novel lactone from the mangrove endophytic fungus *Lasiodiplodia theobromae* ZJ-HQ1. *Org. Biomol. Chem.* 15, 6338-6341. https://doi.org/10.1039/C7OB01657C

Choma, I.M., Grzelak, E.M., 2010. Bioautography detection in thin-layer chromatography. *J. Chromatogr. A.* 1218, 2684-2691. https://doi.org/10.1016/j.chroma.2010.12.069

Chowdhary, K., Kaushik, N., 2018. Biodiversity study and potential of fungal endophytes of peppermint and effect of their extract on chickpea rot pathogens. *Arch. Phytopathol. Plant. Prot.* 51, 139-155. https://doi.org/10.1080/03235408.2018.1440707

Correia, L.P., Procopio, J.V.V., Santana, C.P., Santos, A.F.O., Cavalcante, H.M.M., Macedo, R.O., 2011. Characterization of herbal medicine with different particle sizes using pyrolysis GC/MS, SEM, and thermal techniques. *J. Ther. Anal. Cal.* 111, 1691-1698. https://doi.org/10.1007/s10973-011-2129-x

Coskun, O., 2016. Separation techniques: chromatography. *North. Clin. Istanbul.* 3, 156-160. https://doi.org/10.14744/nci.2016.32757

Desale, M.G., Bodhankar, M.G., 2013. Antimicrobial activity of endophytic fungi isolated from *Vitex negundo* Linn. *Int. J. Curr. Microbiol. App. Sci.* 2, 389-395.

Devi, N.N., Singh, M.S., 2013. GC-MS analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* Nees. *International. J. Pharm. Sci. Rev. Res.* 23, 392-395.

Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R., Dua, T.K., 2015. Bioautography and its scope in the field of natural product chemistry. *J. Pharm. Anal.* 5, 75-84. https://doi.org/10.1016/j.jpha.2014.06.002

Ding, G., Liu, S., Guo, L., Zhou, Y., Che, Y., 2008. Antifungal metabolites from the plant endopytic fungus *Pestalotiopsis foedan. J. Nat. Prod.* 71, 615-618. https://doi.org/10.1021/np070590f

Ebada, S.S., Edrada, R.A., Lin, W., Proksh, P., 2008. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates. *Nat. Prot.* 3, 1820-1831. https://doi.org/10.1038/nprot.2008.182

Elfita, Munawar., Muharni., Sudrajat, M.A., 2014. Identification of new lactone derivatives isolated from *Trichoderma* sp., an endophytic fungus of Brotowali (*Tinaspora crispa*). *HAYATI. J. Biosci.* 21, 15-20. https://doi.org/10.4308/hjb.21.1.15

Fair, R.J., Tor, Y., 2014. Antibiotics and bacterial resistance in the 21st century. *Perspect. Med. Chem.* 6, 25-64. https://doi.org/10.4137/PMC.S14459

Fajriah, S., Megawati, Hudiyono, S., Kosela, S., Hanafi, M. 2017. Chemical constituents and potential cytotoxic activity of n-hexane fraction from *Myristica fatua* Houtt leaves. *AIP. Conf. Proc.* 1862, 030087. https://doi.org/10.1063/1.4991191

Ginsburg, H., Deharo, E., 2011. A call for using natural compounds in the development of new antimalarial treatments - an introduction. *Malaria. J.* 10, 1. https://doi.org/10.1186/1475-2875-10-S1-S1

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., Hamal, A., 2014. What is a bioactive compound? A combined definition for a preliminary consensus. *Int. J. Nutr. Food. Sci.* 3, 174-179. https://doi.org/10.11648/j.ijnfs.20140303.16

Gulcin, I., 2012. Antioxidant activity of food constituents: an overview. *Arch. Toxicol.* 86, 345-391. https://doi.org/10.1007/s00204-011-0774-2

Handayani, D., Rivai, H., Hutabarat, M., Rasyid, R., 2017. Antibacterial activity of endophytic fungi isolated from Mangrove plant *Sonneratia griffithii* Kurz. *J. App. Pharm. Sci.* 7, 209-212.

Harborne, J.B., 1973a. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, UK, Chapman and Hall Ltd.

Harborne, J.B., 1973b. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, UK, Chapman and Hall Ltd.

Henry, G.E., Momin, R.A., Nair, M.G., Dewitt, D.L., 2002. Antioxidant and cyclooxygenase activities of fatty acids found in food. *J. Agric. Food. Chem.* 50, 2231-2234. https://doi.org/10.1021/jf0114381

Hossain, M.A., Al-Raqmi, K.A.S., Al-Mijizy, Z.H., Weli, A.M., Al-Riyami, Q., 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris. Asian. Pacific. J. Trop. Biomed.* 3, 705-710. https://doi.org/10.1016/S2221-1691(13)60142-2

Indrianingsih, A.W., Tachibana, S., 2017. A-glucosidase inhibitor produced by an endophytic fungus, *Xylariaceae* sp. QGS 01 from *Quercus gilva* Blume. *Food. Sci. Human. Well.* 6, 88-95. https://doi.org/10.1016/j.fshw.2017.05.001

Jegadeeswari, P., Nishanthini, A., Muthukumarasamy, S., Mohan, V.R., 2012. GC-MS analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). *J. Curr. Chem. Pharm. Sci.* 2, 226-232.

Jenecius, A.A., Uthayakumari, F., Mohan, V.R., 2012. GC-MS determination of bioactive components of *Sauropus bacciformis* BLUME (Euphorbiaceae). *J. Curr. Chem. Pharm. Sci.* 2, 347-358.

Jimenez-Romero, C., Ortega-Barria, E., Arnold, A.E., Cubilla-Rios, L., 2008. Activity against *Plasmodium falciparum* of lactones isolated from the endophytic fungus, *Xylaria* sp. *Pharm. Biol.* 46, 1-4. https://doi.org/10.1080/13880200802215859

Jirovetz, L., Buchbauer, G., Abraham, G.T., Shafi, M.P., 2006. Chemical composition and olfactoric characterization of *Acmella radicans* (Jacq.) R.K. Jansen var. radicans from southern Indian. *Flav. Fragr. J.* 21, 88-91. https://doi.org/10.1002/ffj.1524

Joseph, B., Priya, R.M., 2011. Bioactive compounds from endophytes and their potential in pharmaceutical Effect: a Review. *Am. J. Biochem. Mol. Biol.* 1, 291-309. https://doi.org/10.3923/ajbmb.2011.291.309

Karuppiah Vijayamuthuramalingam, U.D., Rajaram, R., Madhavaram Kuppusamy, K., Jonnalagadda, B., Arokiasamy, S., 2017. Anti-hyperglycemic and antioxidant potential of *Croton bonplandianus*. Bail fractions in correlation with polyphenol content. *Iran. J. Basic. Med. Sci.* 20, 1390-1397.

Khrisnamoorthy, K., Subramaniam, P., 2014. Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *Int. Scholar. Res. Not.* 2014, 13. https://doi.org/10.1155/2014/567409

Komsta, L., 2007. Prediction of the retention in thin layer chromatography screening systems by atomic contributions. *Anal. Chim. Acta.* 593, 224-237. https://doi.org/10.1016/j.aca.2007.04.059

Krvavych, A.S., Konechna, R.T., Petrina, R.O., Kyrka, M.S., Zayarnuk, N.L., Gulko, R.M., Stadnytska, N.E., Novikov, V.P., 2014. Phytochemical research of plant extracts and use *in vitro* culture in order to preserve rare wild species *Gladiolus imbricatus. Res. J. Pharm. Biol. Chem. Sci.* 5, 240-246.

Kuete, V., 2010. Potential of cameroonian plants and derived products against microbial infections: a review. *Plant. Med.* 76, 1479-1491. https://doi.org/10.1055/s-0030-1250027

Kulip, J., 2003. An ethnobotanical survey of medicinal and other useful plants of Muruts in Sabah, Malaysia. *Telopea*. 10, 81-98. https://doi.org/10.7751/telopea20035608

Kumar, S., Jyotirmayee, K., Sarangi, M., 2016. Thin layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. *International Journal of Pharmaceutical Sciences Review and Research*. 18, 126–132.

Li, X., Zhao, C., Zheng, C., Liu, J., Vu, V.H., Wang, X., Sun, Q., 2017c. Characteristics of microbial community and aroma compounds in traditional fermentation of *Pixian broad* bean paste as compared to industrial fermentation. *Int. J. Food. Prop.* 20, 2520-2531. https://doi.org/10.1080/10942912.2017.1373358

Martins, S., Amorim, E.I.C., Sobrinho, T.J.S.P., Saraiva, A.M., Pisciottano, M.N.C., Aguilar, C.N., Texeira, J.A., Mussatto, S.I., 2013. Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentate* leaves. *Ind. Crops. Prod.* 41, 306-311. https://doi.org/10.1016/j.indcrop.2012.04.037

Mayor, S., 2011. Tree that provides paclitaxel is put on list of endangered species. *BMJ.* 343, d7411. https://doi.org/10.1136/bmj.d7411

Miller, S.I., Salama, N.R., 2018. The gram-negative bacterial periplasm: size matters. *PLoS Biol.* 16, e2004935. https://doi.org/10.1371/journal.pbio.2004935

Mihigo, S.O., Tekayela, M., Masesane, I.B., Sichilongo, K., 2015. Preliminary GC profiling and anti-bacterial activity investigation of *Emilia coccinea* (SIMS) G. DON (Asteraceae). *Int. J. Chem. Aqua. Sci.* 1, 31-36.

Mohamed, Z.Z., Fasihuddin, B.A., Wei-Seng, H., Shek-Ling, P., 2014. GC–MS analysis of phytochemical constituents in leaf extracts of *Neolamarckia cadamba* (Rubiaceae) from Malaysia. *Int. J. Pharm. Pharm. Sci.* 6, 123-127.

Molina, G., Pimentel, M.R., Bertucci, T.C.P., Pastore, G.M., 2012. Application of fungal endophytes in biotechnological processes. *Chem. Eng. Trans.* 27, 289-294.

Morata, L., Cobos-Trigueros, N., Martínez, J.A., Soriano, A., Almela, M., Marco, F., Sterzik, H., Núñez, R., Hernández, C., Mensa, J., 2012. Influence of multidrug resistance and appropriate empirical therapy on the 30-day mortality rate of *Pseudomonas aeruginosa* bacteremia. *Antimicrob Agents Chemother.* 56, 4833-4837. https://doi.org/10.1128/AAC.00750-12

Naragani, K., Mangamuri, U., Muvva, V., Poda, S., Muganti, R.K., 2016. Antimicrobial properties of *Streptomyces cheonanensis* VUK-A from mangrove origin. *Int. J. Pharm. Pharm. Sci.* 8, 53-57.

[NCCLS] National Committee for Clinical Laboratory Standards., 2004. Performance standards for antimicrobial disk and dilution susceptibility testing. Fourteenth Informational Supplement NCCLS documents M100-S14. Wayne, Pennsylvania, USA; 2004.

Nikaido, H., 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* 67, 593-656. https://doi.org/10.1128/MMBR.67.4.593-656.2003

Nikaido, H., 2009. Multidrug resistance in bacteria. *Ann. Rev. Biochem.* 78, 119-146. https://doi.org/10.1146/annurev.biochem.78.082907.145923

Niu, Y., Hardy, G., Agarwal, M., Hua, L., Ren, Y., 2016. Characterization of volatiles *Tribolium castaneum* (H.) in flour using solid phase microextraction–gas chromatography mass spectrometry (SPME–GCMS). *Food. Sci. Human. Well.* 5, 24-29. https://doi.org/10.1016/j.fshw.2015.11.002

Othman, A.R., Abdullah, N., Ahmad, S., Ismail, I.S., Zakaria, M.P. 2015. Elucidation of *in vitro* anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC. Comp. Alter. Med.* 15, 11. https://doi.org/10.1186/s12906-015-0528-4

Pankey, G.A., Sabath, L.D., 2004. Clinica lrelevance of bacteriostatic versus bactericidal mechanism of action in the treatment of gram negative bacterial infections. *Clin. Infect. Dis.* 38, 864-870. https://doi.org/10.1086/381972

Peng, W., Li, D., Zhang, M., Ge, S., Mo, B., Li, S., Ohkoshi, M., 2017. Characteristics of antibacterial molecular activities in poplar wood extractives. *Saudi. J. Biol. Sci.* 24, 399-404. https://doi.org/10.1016/j.sjbs.2015.10.026

Raaman, N., 2006. Phytochemical Techniques. New Delhi, New India Publishing Agency.

Rahman, M.M., Ahmad, S.H., Mohamed, M.T.M., Ab Rahman, M.Z., 2014. Antimicrobial compounds from leaf extracts of *Jatropha curcas, Psidium guajava*, and *Andrographis paniculata. Sci. World. J.* 2014, 635240. https://doi.org/10.1155/2014/635240

Ramanathan, T., Arunprabu, S., Dinesh, P. 2016. Antimicrobial activity of crude methanolic extracts of *Rhizophora mucronata. Int. Sci. Inv. Today*. 5, 520-527.

Ramesha, A., Srinivas, C., 2014. Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata* L. and *Plumeria obtusifolia* L. *Eur. J. Exp. Biol.* 4, 35-43.

Rizwan, K., Zubair, M., Rasool, N., Riaz, M., Zia-Ul-Haq, M., Feo, V., 2012. Phytochemical and biological studies of *Agave attenuate. Int. J. Mol. Sci.* 13, 6440-6451. https://doi.org/10.3390/ijms13056440

Rustan, A.C., Drevon, C.A., 2005. Fatty Acids: Structures and Properties, In Encyclopaedia of Life Sciences. New York, John Wiley and Sons, Ltd. https://doi.org/10.1038/npg.els.0003894

Salituro, G.M., Dufresne, C., 1998. Isolation by low-pressure column chromatography, in: Salituro, G.M. (Eds.), Natural Products Isolation. Methods in Biotechnology, vol 4. Humana Press. https://doi.org/10.1007/978-1-59259-256-2_4

Salton, M.R.J., Kim, K.S., 1996. Structure. In: Baron S (Eds.). *Medical Microbiology, 4th Edition*. Galveston, University of Texas Medical. pp. 1-19.

Sapkal, P.M., Madane, P.D., Palshikar, M.N., 2017. An overview on different chromatographic techniques. *Int. J. Pharm. Pharm. Res.* 8, 149-161.

Selim, S.A., Abdel Aziz, M.H., Mashait, M.S., Warrad, M.F., 2013b. Antibacterial activities, chemical constitutes and acute toxicity of Egyptian *Origanum majorana* L., *Peganum harmala* L. and *Salvia officinalis* L. essential oils. *Afr. J. Pharm. Pharmacol.* 7, 725-735.

Silhavy, T.J., Kahne, D., Walker, S., 2010. The bacterial cell envelope. *Cold. Spring. Harb. Perspect. Biol.* 2, a000414. https://doi.org/10.1101/cshperspect.a000414

Singh, N., Verma, P., Pandey, B.R., Bhalla, M., 2012. Therapeutic potential of *Ocimum sanctum* in prevention and treatment of cancer and exposure to radiation: an overview. *Int. J. Pharm. Sci. Drug. Res.* 4, 97-104.

Siva, M., Shanmugam, K.R., Shanmugam, B., Venkata, S.G., Ravi, S., Sathyavelu, R.K., Mallikarjuna, K., 2016. *Ocimum sanctum*: a review on the pharmacological properties. *Int. J. Basic. Clin. Pharmacol.* 5, 558-565. https://doi.org/10.18203/2319-2003.ijbcp20161491

Sharma, P., Shah, G.C., 2015. Composition and antioxidant activity of *Senecio nudicaulis* Wall. ex DC. (Asteraceae): a medicinal plant growing wild in Himachal Pradesh, India. *Nat. Prod. Res.* 29, 883-886. https://doi.org/10.1080/14786419.2014.990904

Shibu-Prasanth, C.R., Pratap-Chandran, R., 2017. Phytochemical and antimicrobial analysis of leaf samples of different Rubus species. *Int. J. Chem. Tech. Res.* 10, 359-368.

Smaoui, S., Mathieu, F., Elleuch, L., Coppel, Y., Merlina, G., Karray-Rebai, I., Mellouli, L., 2012. Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp. TN256 strain. *World. J. Microbiol. Biotechnol.* 28, 793-804. https://doi.org/10.1007/s11274-011-0872-6

Soltanian, H., Rezaeian, S., Shakeri, A. Janpoor, J., Pourianfar, H.R. 2016. Antibacterial activity of crude extracts and fractions from Iranian wild-grown and cultivated *Agaricus* spp. *Biomed. Res.* 27, 56-59.

Stierle, A., Strobel, G., Grothaus, P., Bignami, G., 1995. The search for a taxol-producing microorganism among the endophytic fungi of the Pacific yew, *Taxus brevifolia. J. Nat. Prod.* 58, 1315-1324. https://doi.org/10.1021/np50123a002

Sudharsan, S., Saravanan, R., Shanmugam, A., Vairamani, S., Mohan kumar, R. Menaga, S., Ramesh, N., 2011. Isolation and characterization of octadecanoic acid from the ethyl acetate root extract of *Trigonella foneum-graecum* L. by using hydroponics method. *J. Bioterr. Biodefense.* 2, 105. https://doi.org/10.4172/2157-2526.1000105

Suleimana, M.M., McGaw, L.J., Naidoo, V., Eloff, J.N., 2009. Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *Afr. J. Trad. Comp. Alter. Med.* 7, 64-78. https://doi.org/10.4314/ajtcam.v7i1.57269

Taufiq, M.M.J., Darah, I., 2018. Anti-MRSA of the ethyl acetate crude extract from *Lasiodiplodia pseudotheobromae* IBRL OS-64, an endophytic fungus isolated from the leaf of *Ocimum sanctum* Linn. *Int. J. Pharm. Pharm. Sci,* 10, 50-55. https://doi.org/10.22159/ijpps.2018v10i8.26527

Taufiq, M.M.J., Darah, I., 2019. Antibacterial activity of an endophytic fungus *Lasiodiplodia pseudotheobromae* IBRL OS-64 residing in leaves of a medicinal herb, *Ocimum sanctum* Linn. *J. App. Biol. Biotech.* 7, 35 41. https://doi.org/10.7324/JABB.2019.70207

Taufiq, M.M.J., Darah, I., 2020. Biological activity of *Lasiodiplodia pseudotheobromae* IBRL OS-64 extracts, an endophytic fungus isolated from medicinal herb, *Ocimum sanctum* against foodborne diarrhea-caused bacteria. *Pharmacogn. J.* 12, 897-904. https://doi.org/10.5530/pj.2020.12.127

Tewari, A., Singh, S.P., Singh, R., 2012. Prevalence of multidrug resistant *Bacillus cereus* in foods and human stool samples in and around Pantnagar, Uttrakhand. *J. Adv. Vet. Res.* 2, 252-255.

Theantana, T., Kanjanapothi, D., Lumyong, S., 2012. *In vitro* inhibition of lipid peroxidation and the antioxidant system of endophytic fungi from Thai medicinal plants. *Chiang. Mai. J. Sci.* 39, 429-444.

Tong, W.Y., Darah, I., Latiffah, Z., 2011. Antimicrobial activities of endophytic fungal isolates from medicinal herb *Orthosiphon stamineus* Benth. *J. Med. Plants. Res.* 5, 831-836.

Venkatadri, B., Khusro, A., Aarti, C., Rameshkumar, M.R., Agastian, P., 2017. *In vitro* assessment on medicinal properties and chemical composition of *Michelia nilagirica* bark. *Asian. Pac. J. Trop. Biomed.* 7, 782-790. https://doi.org/10.1016/j.apjtb.2017.08.003

Verma, A., Johri, B.N., Prakash, A. 2014a. Antagonistic evaluation of bioactive metabolite from endophytic fungus, *Aspergillus flavipes* KF671231. *J. Mycol.* 2014, 5. https://doi.org/10.1155/2014/371218

Vidal, F., Mensa J, Almela M, Martínez, J., Marco, F., Casals, C., Gatell, J., Soriano, E., de Anta, M.J., 1996. Epidemiology and outcome of *Pseudomonas aeruginosa* bacteremia, with special emphasis on the influence of antibiotic treatment: analysis of 189 episodes. *Arch. Intern. Med*. 156, 2121-2126. https://doi.org/10.1001/archinte.1996.00440170139015

Vincenti, S., Raponi, M., Sezzatini, R., Giubbini, G., Laurenti, P., 2018. Enterobacteriaceae antibiotic resistance in ready-to-eat Foods collected from hospital and community canteens: analysis of prevalence. *J. Food. Prot.* 81, 424-429. https://doi.org/10.4315/0362-028X.JFP-17-317

Wagner, H., Bladt, S., Zgainski, E.M., 1984. Drug Containing Anthracene Derivatives. In Plant Drug Analysis. Springer-Verlag Berlin Heidelberg GmbH, Berlin. https://doi.org/10.1007/978-3-662-02398-3_4

Wang, Q.S., Zhang, L., 1999. Review of research on quantitative structure-retention relationship in thin-layer chromatography. *J. Liq. Chromatogr. Rel. Technol.* 22, 1-4. https://doi.org/10.1081/JLC-100101639

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P., Edmond, M.B., 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective surveillance study. *Clin. Infect. Dis.* 39, 309-317. https://doi.org/10.1086/421946

Wright, G.D., 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv. Drug. Deliv. Rev.* 57, 1451-1470. https://doi.org/10.1016/j.addr.2005.04.002

Yadav, M., Yadav, A., Yadav, J.P., 2014. *In vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. *Asian. Pac. J. Trop. Med.* 7, 256-261. https://doi.org/10.1016/S1995-7645(14)60242-X

Yenn, T.W., Ibrahim, D., Latiffah Zakaria, L., 2012. Antimicrobial activity of *Phomopsis* sp. ED2, an endophytic fungus isolated from *Orthosiphon stamineus* Benth. *In: Proceedings of The 2nd Annual International Conference Syiah Kuala University 2012 and The 8th IMT-GT Uninet Biosciences Conference.* Banda Aceh: Syiah Kuala University. pp. 22-24.

Yenn, T.W., Ngim, A.S., Ibrahim, D., Zakaria, L., 2014. Antimicrobial activity of *Penicillium minioluteum* ED24, an endophytic fungus residing in *Orthosiphon stamineus* Benth. *World. J. Pharm. Pharm. Sci.* 3, 121-132.

Yoshino, T., Liang, Y., Arai, D., Maeda, Y., Honda, T., Muto, M., Kakunaka, N., Tanaka, T., 2015. Alkane production by the marine cyanobacterium *Synechococcus* sp. NKBG15041c possessing the α-olefin biosynthesis pathway. *Appl. Microbiol. Biotechnol.* 99, 1521-1529. https://doi.org/10.1007/s00253-014-6286-2

Zayed, M.Z., Ahmad, F.B., Ho, W.S., Pang, S.L., 2014. GC-MS analysis of phytochemical constituents in leaf extracts of Neolamarckia cadamba (Rubiacieae) from Malaysia. *Int. J. Pharm. Pharm. Sci.* 6, 123-127.

Zhao, J., Shan, T., Mou, Y., Zhou, L., 2011. Plant-derived bioactive compounds produced by endophytic fungi. *Mini. Rev. Med. Chem.* 11, 159-168. https://doi.org/10.2174/138955711794519492