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FULL PAPER

Bioactive chemical constituents of three crude extracts of *Polyalthia Sclerophylla* using GC-mass and phytochemical screening and their antibacterial and cytotoxicity activities

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The aim of the present study was to extract of *Polyalthia sclerophylla* leave (LPS) and identify its chemical constituents using Gas chromatography-Mass (GS-Mass) spectrometry and classical phytochemicals methods. This study included as well the evaluated of the bio-medical properties of LPS using antibacterial (against six bacteria species) and cytotoxicity (M-63 human cell line) activities. Three different solvent system i.e. methanol (MeOH), dichloromethane (DCM) and hexane were used to extract the LPS, three samples were obtained and labelled as MLPS, DLPS, and HLPS, respectively, and evaluated their biomedical activities. Phytochemical screening results showed to present glycosides and terpenoids in MLPS, DLPS, and HLPS, while alkaloids did not detect the presence in all extracts. GC-Mass results were detected to present 21 chemical compounds, the higher percentages were cyclobutanone, 2-methyl-2-oxiranyl-, 2-Undecanol and Pyridine, 2,3,4,5-tetrahydro-3-methyl-, while 1,6-Heptadiene, 1,1,3-Trimethylcyclopentane, and 2(5H)-Furanone, 5-methyl were detected to be presented with low percentages. Alamar blue assay was used to evaluate the LPS cytotoxicity and there was no-toxic effect for all concentrations with higher cell availability average 99.5 %. MLPS, DLPS, and HLPS were showed significant effect to inhibition the bacteria growth, MLPS showed more effect than DLPS and HLPS to be used as a bacterial agent. Current study was establishment for chemical and biomedical properties of LPS and showed good biomedical properties with non-toxic effect.

KEYWORDS

Polyalthia Sclerophylla; antibacterial study; cytotoxicity study; GS-mass; phytochemical screening.

Introduction

Annonaceae famil is considered as the biggest family including more than 130 genus and about 2300-2500 species [2], and one of these is *Polyalthia* genus including about 120 species [3]. *Polyalthia sclerophylla* (PS) is one of the plants belonging to the *Polyalthia* genus. Tree of PS in many south Asian countries, and it is presented at high rates in Malaysia, Thailand, and Indonesia [1], commonly distributed in tropical and subtropical regions [4].

The plants of *Polyalthia* genus are distinguished by their unique efficacy that enables them to be used in the medical and

biological fields. Previous studies were reported for many bio-medical applications for them such as anti-inflammatory, anti-oxidant, anti-cancer [4], anti-plasmodial [5], antifungal [6], antibacterial [7], anti-proliferative [8], and anti-DENV2 [9], etc. Previous studies have been conducted in different parts of *Polyalthia* genus to determine the group's compound isolated stem, stem parts, roots, barks, leaves, and twigs [10]. Various parts of *Polyalthia* genus used in traditional medicine to treat a lot of diseases such as stomach ache, helminthiasis, dysmenorrhea, fever, skin diseases, diabetes, hypertension, and pharynx neurosis [11].

Phytochemical screening of *Polyalthia* species were reported previously and their results were shown to have many chemical group compounds. *Polyalthia longifolia* was evaluated for their phytochemical screening and observed to present alkaloids, flavonoids, tannins, carbohydrates, reducing compounds, phenolic compounds [12], saponins, tannins, terpenoids, [13], etc. According to the previous studies, the *Polyalthia* species were reported to have interesting biological activities due to its chemical compositions which are rich in chemical groups giving these plants their unique effectiveness. In the present study, one of this genus was chosen and studied for its chemical compositions and evaluation of biological activities.

Currently, leaves of *Polyalthia sclerophylla* (LPS) were chosen due to many reasons. Firstly, there was no study reported to study its chemical compositions, antibacterial, and cytotoxicity. However, there is a possibility to get similar or newer phytochemicals from leaves of LPS which may contain potential antimicrobial activity. Secondly, LPS is available locally which is considered as economic (cheap in price). Thirdly, the present study will be considered as database for the researcher because there was no much information about LPS.

In the present study, LPS were extracted using three solvent systems methanol, hexane, and dichloromethane, and also evaluated for their biological activities through determined its antibacterial against six negative and positive bacteria. Moreover, toxicity study was evaluated against MG-63 human cell line. The results were shown to present carbohydrates, glycosides, terpenoides, tannins, and steroids in the methanol extract. The biological activity of the LPS was also show significant effect against bacteria.

Material and methods

Preparation of leaves of Polyalthia sclerophylla

Leaves of *Polyalthia sclerophylla* (LPS) were collected from Perak Malaysia. LPS were washed by distilled water to remove all fungus and dust. 7 days sun-dried was used to dry LPS. The dried LPS were cut into small pieces and keep the LPS powder for the future work.

Extraction of leaves of Polyalthia sclerophylla

The extraction process has been done using soxhlet process using three solvent system.

Methanol (MeOH), dichloromethane (DCM), and hexane extract all possible compounds from the LPS. Three crude extracts were obtained, briefly, 25 g of LPS have been extracted using methanol (MLPS), DCM (DLPS), and hexane (HLPS), and these three crudes were stored at 4 ^{o}C for future work [14].

Phytochemical screening of the Leaves of Polyalthia sclerophylla

Three crude extracts were cured out to investigate for their chemical groups of compounds according to the classical chemical methods.

Test for carbohydrates

Molisch's test and Fehling's reagent have been used to detect carbohydrates presence

in the LPS. A pick amount of MLPS, DLPS, and HLPS were dissolved using distilled water (DW), the added dropped from Fehling's reagent, the changed of the colour to brick red proved the presence of reducing sugar in the PS leaves.

Test for alkaloids

To indicate the presence of alkaloids two steps have been used firstly, the preliminary and secondly, the confirmatory testes. The process of preliminary test can be explained briefly by mixing 10 mL of MLPS, DLPS, and HLPS in diluted of HCl, and then the mixture have been filtered. The Mayer's and Dragendroff's reagents were treated with the filtered solution, while the second test were provided by treated 1 g of MLPS, DLPS, and HLPS with 40% of calcium hydroxide solution until alkaline visibly on the paper of litmus, and then the mixture have been extracted twice after treated with chloroform. The thin layer plates have been spotted in the extract of chloroform. To develop the detected and chromatogram solvent system have been used as n-haxaneethyl acetate 4:1 and treated with Dragendorff's reagent during spraying it in the chromatograms solution. The alkaloids presence can be detected with observed the orange colour and yellow in the background [15,16].

Test for glycosides

The glycosides were identified in the LPS via using Keller-Killani test. 1 g of MLPS, DLPS and HLPS were dissolved in DW, then, a few drops of sulphuric acid and ferric chloride were added. The appearance of two layers of reddish brown and reddish brown is evidence of the presence of glycosides [17].

Test for flavonoids

There are many methods to test the presence of the Flavonoids in the medicinal plants. In the percent study three methods have been used to conform the percent of Flavonoids in crude extract of *Polyalthia sclerophylla.*

Test for free flavonoids

10 ml of MLPS, DLPS, and HLPS were mixed with 5 ml ethyl acetate, and then were heated for 3 min in a steam bath. The prepared solution has been filtered with quality filter paper. The filtered solution was shaken after added 1 mL from ammonium dilute. The flavonoids presence can be detected when yellow colour is observed [18].

Reaction with sodium hydroxide

10 ml of MLPS, DLPS, and HLPS were heated in the hotplate. Dilute sodium hydroxide has been then added to the mixture. The flavonoids presence was indicated by formatted yellow precipitate [19].

Lead acetate test

10 ml of MLPS, DLPS, and HLPS were mixed and heated in the hotplate. Thereafter, 1 ml of lead acetate (10%) has been added to the mixture. The flavonoids presence indicated by formatted yellow precipitate [20].

Test for steroids (Lieberman's test)

10 mL of MLPS, DLPS, and HLPS were mixed with acetic anhydride, and then were cooled in bath of ice. A few drops of sulphuric acid were added to the mixtures. The presence of steroids in the mixtures can be detected via changed of the colour from violet to green or blue [8].

Test for terpenoids (Salkowski's test)

10 mL of MLPS, DLPS, and HLPS were mixed firstly with $CHCl_3$ (2 mL), and then careful addition of H_2SO_4 has been done. The terpenoids presence can be detected positively, when the colour of mixture change to reddish brown [21].

Test for tannins (Ferric chloride test)

1 g of MLPS, DLPS, and HLPS were mixed with 50 ml DW and boiled for 20 min in conical flask, and then the samples were filtered. 0.1% ferric chloride was added carefully to the samples as a few drops. To detect the tannins availability in the samples the blue-black and brownish green were observed [22].

Test for saponins (Froth test)

1 g of MLPS, DLPS, and HLPS were dissolved in 10 mL DW in test tube and were mixed for 1 min. The tube have been shaken and stoppered vigorously for 1 min. The tube have been apportioned to attitude in a perpendicular position and realized more than 30 minutes to detect the presence of saponins in the solution froth honey comb observed on the surface [23,24].

Test for Phenolic compounds

1 g of MLPS, DLPS, and HLPS were dissolved in 50 mL DW, and then a few drops of ferric sulfate have added. To indicate the dark violet presence of phenolic group was observed [24].

Gas chromatography-Mass spectrometry CEPS

The crude extract of LPS was cured out to conform the volute compounds using Gas chromatography-Mass spectrometry (GC-MS, Shimadzu GC-14B) analyser.

Cytotoxicity study of LPS

The Alamar Blue assay was used to evaluate the LPS cytotoxicity on MG-63 human cells line. The powder of LPS was immersed in complete media for 24 h with a volume of approximately 200 mg/mL to produce extracts for the cell viability test. The pure extract was filtered using a 0.2 µm syringe for sterilization purposes. The extraction vehicle with no material represented the negative control.

In the meantime, to create the ratios of weight: volume of 25, 50, 75, 100, and 200 mg/mL of the pure extracts were diluted with the complete media, as well as the healthy monolayer of MG-63 cells, the pure, and the diluted extracts. The cells were incubated in a 37 °C CO_2 incubator for 24 h, and then the cell viability was determined using the Alamar Blue assay (Invitrogen, USA), where the Alamar Blue solution was used to stain the culture and placed in the 37 °C CO_2 incubator for 4 h.

Antibacterial activities of Polyalthia sclerophylla

Six species of the bacteria include three gram-negative (*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), and *Yersinia pestis* (*Y.pestis*)) and three gram-positive (*Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumonia*, and *Streptococcus pyogenes*) were used to investigate the ability of MLPS, DLPS, and HLPS samples.

Agar Preparation

The nutrient broth agar were prepared by dissolved 20 g in 1000 mL distilled water (DW), and then sterilized by an autoclave for 20 min at 121 °C. Next, it was cooled to 55 °C and 25 mL of cooled media was added to the plate and left for some time to solidify, and stored at 4 C in the dark for further experimentation.

A method of diffusion was done to identify the antibacterial activity of the crude extracts (MLPS, DLPS, and HLPS). The inhibition zone of the bacteria was evaluated to determine the antibacterial properties. The culture for both bacteria strains was observed using 10 mL of bacterial culture. A 100 mL of bacterial culture spread was taken on the plates of nutrient agar, and then the MLPS, DLPS, and HLPS were put in a plate with a

6 mm filter disc, tailed by the incubation process for 24 h at 37 C. The inhibition zone was then calculated after the incubation process.

Results and discussion

Chemical compositions of LPS

Present study were conducted to the LPS extraction using three solvents MeOH, DCM were named as MLPS, DLPS, and HLPS, and were cured out to evaluate their chemical compositions using phytochemical screening during applied classical procedures and GC-mass spectroscopy.

Phytochemical screening

Phytochemical screening of MLPS showed to present carbohydrates, glycosides, flavonoids, terpenoids, tannins, phenolic compounds, and steroids, while alkaloids and saponins did not indicate to present in LPS, as indicated in Table 1.

Previous studies were reported to evaluate the phytochemical screening using MeOH as a solvent for example study of Kujur *et al.* (25), was reported to evaluate the chemical composition of *Stevia rebaudiana* leaves. Their results were shown to present phenolic compounds, saponins, tannins, and steroids, while alkaloids wasn't appear to present in the leaves. Moreover, study of Kaur *et al.* (26), was reported to used MeOH as a solvent to extract *Caesalpinia sappan* leaves, results have been shown to present of carbohydrates, glycosides, flavonoids, saponins, and tannins, while alkaloids wasn't observed. The results of these studies are consistent with the results obtained in our current study. The available of chemical groups of the compounds in the leaves plants were widely reported and these groups are responsible for their activities, currently the presence of chemical compounds were approved and caused to the activities of MPLS.

TABLE 1 Phytochemical screening of MLPS

Phytochemical test		Indicator	Results
Carbohydrates			**Positive**
1-	Molish test	Observed presence of brick red colour.	
2-	Fehling reagent	Observed presence of brick red colour.	
Alkaloids			**Negative**
1-	Mayer's reagent	Did not observe to present orange colour and yellow.	
2-	Dragendorff's reagent	Did not observe to present orange colour and yellow	
Glycosides			**Positive**
1-	Killer-Killani	Showed the presence of two reddish brown layers.	
Flavonoids			**Positive**
1-	Test of free Flavonoids	Showed the presence of yellow colour.	
2-	Reaction with sodium hydroxide	Formatted yellow precipitate.	
3-	Lead acetate	Formatted yellow	

	test		precipitate.	
	Terpenoids			**Positive**
1-	test	Lieberman's	Noticed the change of colour to reddish brown.	
	Tannins			**Positive**
1-	chloride test	Ferric	Observed blue –black and brownish green.	
	Saponins			**Negative**
1-		Froth test	Didn't observe the honey comb on the surface.	
	Phenolic compounds			**Positive**
1-		Ferric sulfate	Observed dark violet in the colour.	
	Steroids			**Positive**
1-	test	Lieberman's	Change the colour to reddish brown.	

DLPS was cured out to determine its phytochemical screening and results showed to present glycosides, flavonoids, and terpenoids, while carbohydrates, alkaloids, tannins, saponins, and phenolic compounds were not indicated to present, as listed in Table 2.

TABLE 2 Phytochemical screening of DLPS

	Phytochemical test		**Indicator**	**Results**
	Carbohydrates			**Negative**
1-		Molish test	Didn't observe the presence of brick red colour.	
2-	reagent	Fehling	Didn't observe the presence of brick red colour.	
	Alkaloids			**Negative**
1-	reagent	Mayer's	Did not observe the presence of orange colour and yellow.	
2-	reagent	Dragendorff's	Did not observe the presence of orange colour and yellow.	
	Glycosides			**Positive**
1-		Killer-Killani	Showed the presence of two reddish brown layers.	
	Flavonoids			**Positive**
1-	Flavonoids	Test of free	Showed the presence of yellow colour.	
2-	with sodium hydroxide	Reaction	Formatted yellow precipitate.	
3-		Lead acetate	Formatted yellow	

	test		precipitate.	
	Terpenoids			**Positive**
1-	test	Lieberman's	Noticed the change of colour to reddish brown.	
	Tannins			**Negative**
1-	chloride test	Ferric	Didn't observe blue - black and brownish green.	
	Saponins			**Negative**
1-		Froth test	Didn't observe the honey comb on the surface.	
	Phenolic compounds			**Negative**
1-		Ferric sulfate	Didn't observe dark violet in the colour.	
	Steroids			**Positive**
1-	test	Lieberman's	Change the colour to reddish brown.	

DCM as a solvent was used previously in many studies to extract the leaves of medicinal plants and evaluated to their chemical compositions. Many chemical group compounds were identified to present. Study of Maskam *et al.* [27], was reported to extract *Euodia Redleyi* leaves using DCM the results were showed to present flavonoids, saponins, and terpenoids, while phenolic compound and taninns weren't observed. Arzumand *et al.* [28], also mentioned to use DCM to extract *Adenanthera pavonina* L, the results were showed to present alkaloids, tannins, flavonoids, steroids, and terpenoids, while saponins and carbohydrates were not detected. Both of these studies were almost the same as our obtained results.

Hexane was used currently as a solvent to extract LPS to identify non-polar compounds that is possible to be present in LPS. The results showed the presence of glycosides, terpenoids, tannins, and saponins in the LPS, while other chemical groups didn't indicate to be present, as shows in Table 3.

TABLE 3 Phytochemical screening of HLPS

Phytochemical test	Indicator	Results
Carbohydrates		**Negative**
1- Molish test	Didn't observe the presence of brick red colour.	
2- Fehling reagent	Didn't observe the present of brick red colour.	
Alkaloids		Negative
1- Mayer's reagent	Did not observe the presence of orange colour and yellow.	
2- Dragendorff's reagent	Did not observe the presence of orange colour and yellow.	
Glycosides		Positive
1- Killer-Killani	Showed the presence two reddish brown layers.	
Flavonoids		Negative
1- Test of free Flavonoids	Didn't show the presence of yellow colour.	
2- Reaction with sodium	Didn't format yellow precipitate.	

hydroxide		
3- Lead acetate test	Didn't format yellow precipitate.	
Terpenoids		Positive
1- Lieberman's test	Noticed the change of colour to reddish brown.	
Tannins		Positive
1- Ferric chloride test	Observed blue-black and brownish green	
Saponins		Positive
1- Froth test	Observe the honey comb on the surface.	
Phenolic compounds		Negative
1- Ferric sulfate	Didn't observed dark violet in the colour.	
Steroids		Negative
1- Lieberman's test	Didn't change the colour to reddish brown.	

During the previous studies, many reports were available to extract the medicinal plants using hexane as a solvent and showed to present the compounds of chemical group such as saponins, tannins [29], steroids, and flavonoids [30]. Our obtained results are consistent with the results obtained previously.

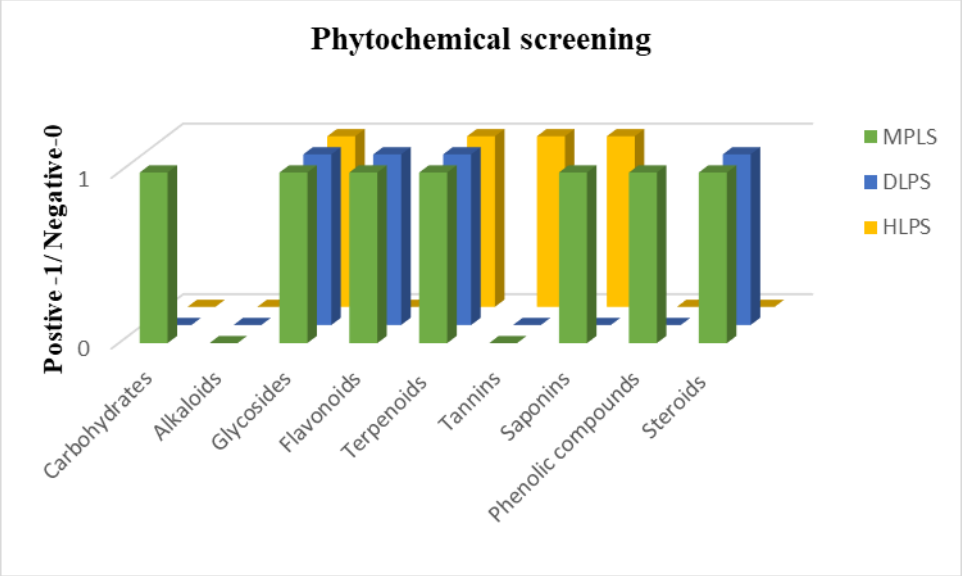


FIGURE 1 Phytochemical screening of MLPS, DLPS, and HLPS

Three solvents were used currently to extract LPS including MeOH, DCM, and Hexane. Results show to present glycosides and terpenoids in MLPS, DLPS, and HLPS, while alkaloids did not detect to present in all extracts. Steroids and flavonoids were detected to present in MLPS and DLPS, while they were not detected in HLPS, as displayed in Figure 1. As mentioned in the previous studies, these chemical groups need polar solvent to dissolve in and hexane is non-polar solvent [30].

Gas chromatography-mass spectrometry (GC-MS) of CEPS

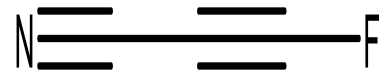
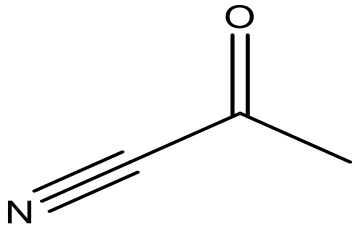
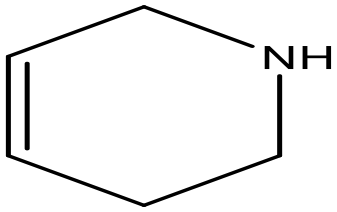
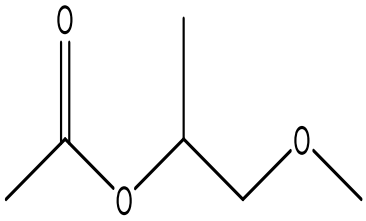
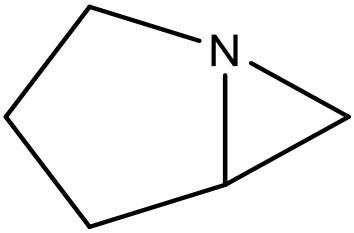
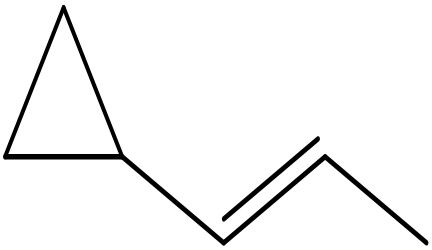
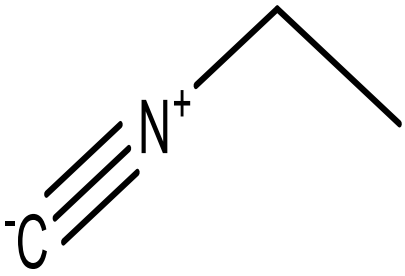
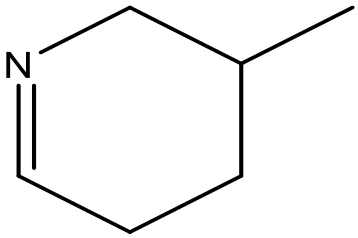
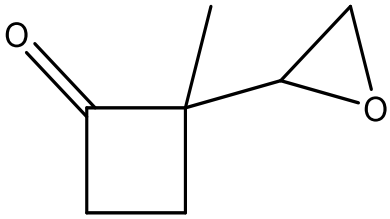
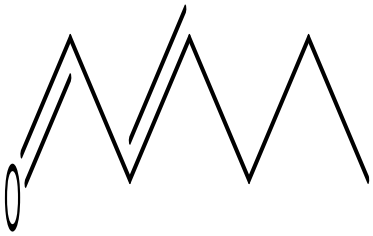
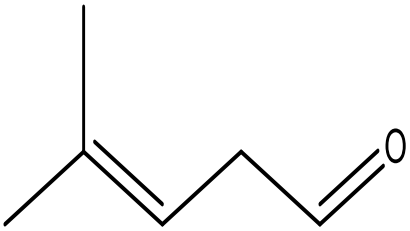
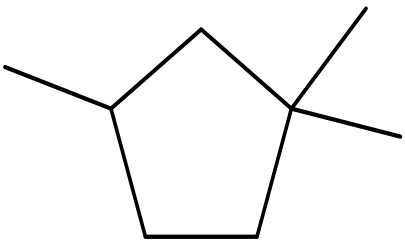
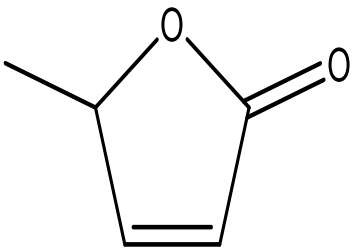
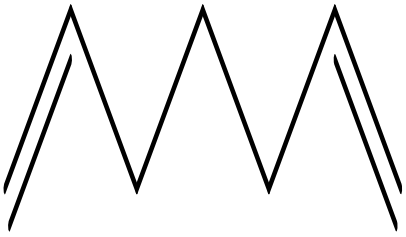
MeOH was used as a solvent to extract LPS, according to the results mentioned in the photochemical screening part, MLPS indicated to have more chemical groups of the compounds compare with DLPS and MLPS. MLPS were chosen to identify the chemical compounds using GC-MS. The results of this part were showed 21 compounds in the CEPS. The higher

percentages were cyclobutanone, 2-methyl-2-oxiranyl-, 2-Undecanol, and Pyridine, 2,3,4,5-tetrahydro-3-methyl-, while 1,6-Heptadiene, 1,1,3-Trimethylcyclopentane, and 2(5H)-Furanone, 5-methyl were detected to present with low percentages, as presented in Table 4.

Figure 2 demonstrated the chemical structures of compounds observed in the GC-mass analysis. As mentioned above, 21 compounds were obtained and identified using their R. time in the GC-MS and the current study analysed their structures and chemical formula. According to the results, three compounds were observed as higher percentage (cyclobutanone, 2-methyl-2-oxiranyl-, 2-Undecanol, and Pyridine, 2,3,4,5-tetrahydro-3-methyl-), and based on the structures of these compounds are very rich in functional groups such as carboxyl, amide, hydroxyl, and methyl, these functional groups can explain clearly the LPS ability to use in biomedical applications.

TABLE 4 Chemical compositions of LPS

No.	Molecular formula	R. Time	Name of compound	Composition %
1	8.34	C_3FN	2-Propynenitrile, 3-fluoro-	2.12
2	9.123	C_3H_3NO	Acetyl cyanide	3.24
3	9.22	C_5H_9N	1,2,3,6-Tetrahydropyridine	4.51
4	9.888	$C_6H_{12}O_3$	1-Methoxy-2-propyl acetate	6.20
5	10.144	C_5H_9N	1-azabicyclo(3.1.0)hexane	7.49
6	10.333	C_6H_{10}	Tans-1-Propenylcyclopropane	2.48
7	12.378	C_3H_5N	Ethyl isocyanide	4.27
8	12.583	$C_6H_{11}N$	Pyridine, 2,3,4,5-tetrahydro-3-methyl-	8.58
9	13.471	$C_{11}H_{24}O$	2-Undecanol	9.25
10	13.575	$C_7H_{10}O_2$	Cyclobutanone, 2-methyl-2-oxiranyl-	12.74
11	13.667	$C_6H_{10}O$	2-Hexenal	1.33
12	13.778	$C_{18}H_{29}F_7O_2$	Heptafluorobutyric acid, n-tetradecyl ester	1.22
13	13.789	$C_6H_{10}O$	4-Methyl-3-pentenal	1.51
14	13.811	C_8H_{16}	1,1,3-Trimethylcyclopentane	0.85
15	13.823	$C_5H_6O_2$	2(5H)-Furanone, 5-methyl-	0.91
16	13.823	C_7H_{12}	1,6-Heptadiene	0.74
17	13.841	C_5H_7NO	2-Furanmethanamine	1.23
18	13841	$C_6H_{10}O$	2-Pentanone, 3-methylene-	1.23
19	13.884	C_7H_{14}	1-Butene, 2-ethyl-3-methyl-	1.27
20	13.911	C_5H_7NO	Acetamide, N-2-propynyl-	2.25
21	13.911	$C_4H_5N_3$	Acetonitrile, 2,2'-iminobis-	2.25

 2-Propynenitrile, 3-fluoro-	 Acetyl cyanide	 1,2,3,6-Tetrahydropyridine
 1-Methoxy-2-propyl acetate	 1-azabicyclo(3.1.0)hexane	 Tans-1-Propenylcyclopropane
 Ethyl isocyanide	 Pyridine, 2,3,4,5-tetrahydro-3-methyl-	 2-Undecanol
 Cyclobutanone, 2-methyl-2-oxiranyl-	 2-Hexenal	 4-Methyl-3-pentenal
 1,1,3-Trimethylcyclopentane	 2(5H)-Furanone, 5-methyl-	 1,6-Heptadiene

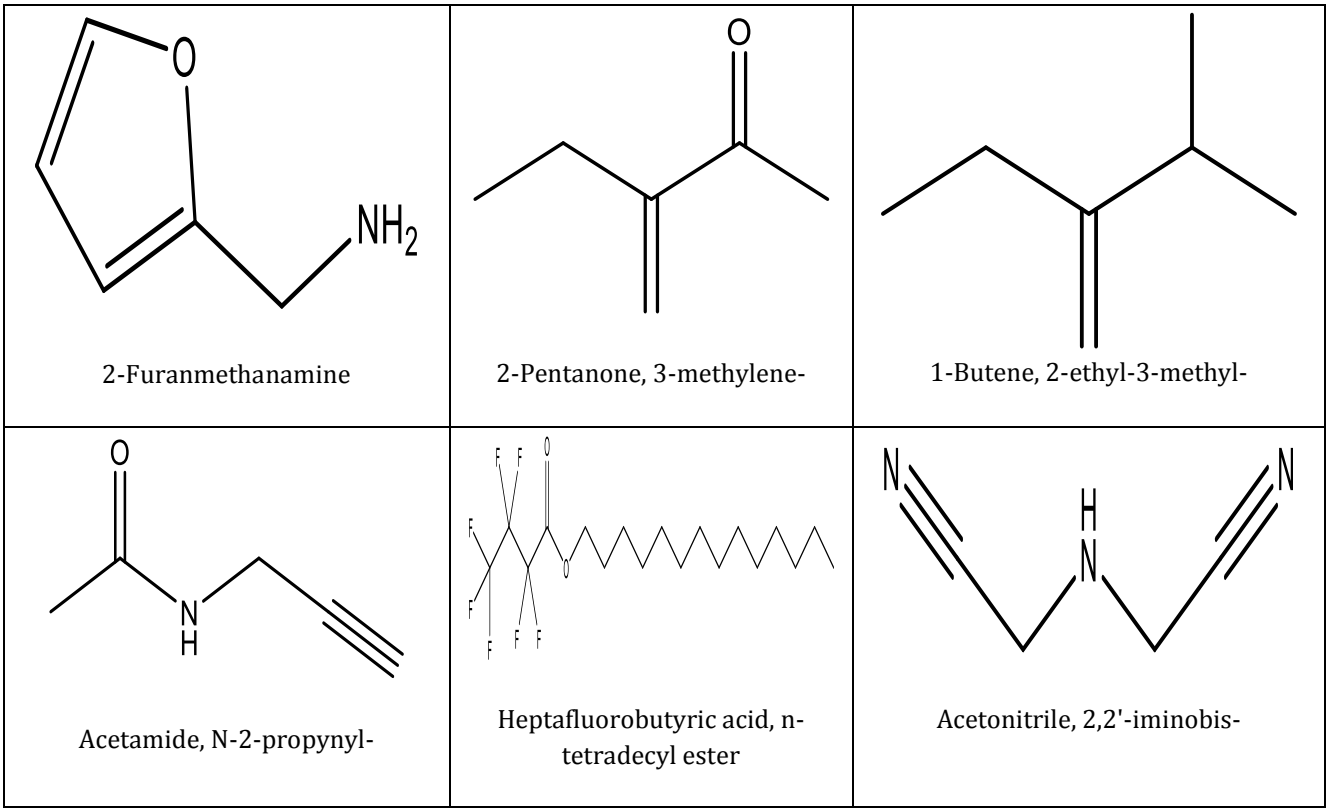


FIGURE 2 Chemical structures of the GC-Mass spectroscopy obtained compounds

Cytotoxicity study of LPS

In this part, cytotoxicity study was investigated for MLPS to screen wither this plant has toxic or not. One of the important tests for the materials that deal with medical fields is toxic effect of them, due to its effect for the final product that is possible to be clinically used. Five concentrations of MLPS were used against M-63 human cell line. All concentrations showed there is no toxic effect in MLPS, as presented in Table 5. According to the previous studies, that were reported about *Polyalthia* genus and they show there is nontoxic effect for its species [31]. The results of the present study showed that there is no toxic effect in the LPS as shows in figure 3.

TABLE 5 Cytotoxicity study of LPS

No.	Concentrations	Control	LPS	Cell availability
1	25 mg/mL	0.245	0.242	98.7755
2	50 mg/mL	0.256	0.251	98.0469
3	75 mg/mL	0.277	0.278	100.361
4	100 mg/mL	0.302	0.304	100.662
5	200 mg/mL	0.305	0.304	99.6721
	AVERAGE	0.277	0.2758	
	CELL VIABILITY AVERAGE	100	99.5668	

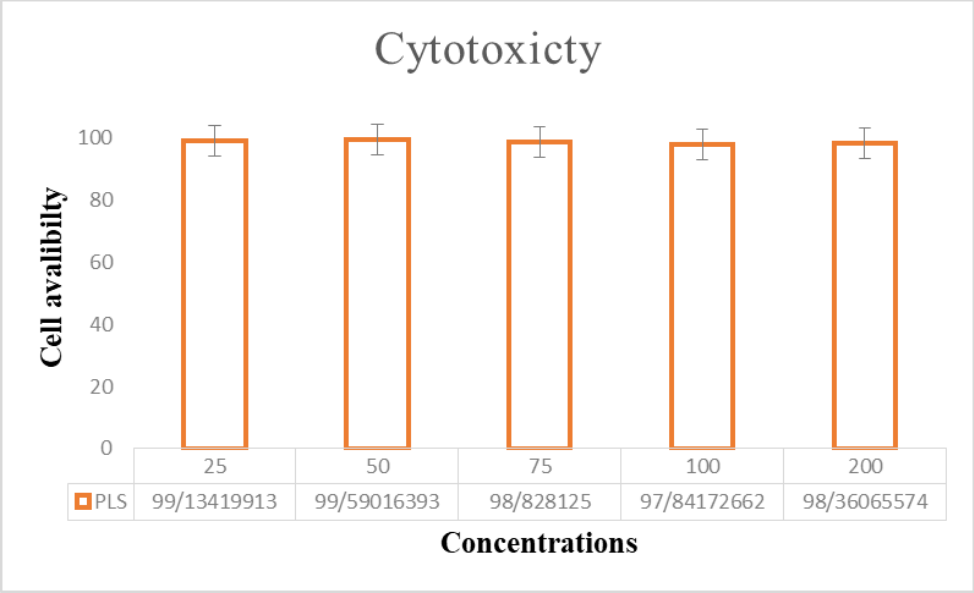


FIGURE 3 Cytotoxicity study of LPS

Antibacterial activities of leaves of Polyalthia sclerophylla

The antibacterial properties of LPS was evaluated against six bacterial species included three gram-negative i.e. *P.aeruginosa, Y.pestis, E. coli*, and three gram-positive i.e. *S. pneumonia*, *S. pyogenes*, and *S. aureus*. MPLS (a), DLPS(b), and HLPS(c) were cured out against gram-negative *P.aeruginosa*, *Y.pestis*, and *E. coli*, and also shows significant inhibition of bacteria growth were 8, 9, and 12 mm (a), 6, 7, and 8 mm (b), 7, 8, and 10 mm (c), respectively, while against gram-positive include *S. pneumonia, S. pyogenes, and S. aureus* were 10, 9, and 14 mm (a), 8, 8, and 10 mm (b), 9, 7, and 12 mm (c), respectively. Results show that all crude extracts indicates significant effects against all species of the bacteria. As listed in Table 6, it was clearly observed that the inhibition of bacteria growth depending on the types of solvent, metabolic extract was shows more effect on both of bacteria grams compare with dichloromethane extract was showed as a less effect compare with others.

TABLE 6 Antibacterial effect of *Polyalthia sclerophylla*

	MLPS	DLPS	HLPS	Ampicillin	MeOH	DCM	Hexane
Gram-negative	**Inhibition zone**						
P.aeruginosa	8 mm	6mm	7mm	22mm	0	0	0
Y.pestis	9 mm	7mm	8mm	23mm	0	0	0
E. coli	12mm	8mm	10mm	25mm	0	0	0
Gram-positive	**Inhibition zone**						
S. pneumonia	10 mm	8mm	9mm	24mm	0	0	0
S. pyogenes	9 mm	8mm	7mm	26mm	0	0	0
S. aureus	14mm	10mm	12mm	27mm	0	0	0

In this part, two important parameters should be addressed according to the obtained results. Firstly, the ability of crude extracts to inhibit the growth of bacteria depending on many reason such as solvent that use to extract the medicinal plants [32], the types of chemicals that available in the plants [33], etc. In the current study, three different solvents were used which showed three different effects on the bacteria growth. Solvents are working depending on the polarity of chemical groups and as mentioned above MPLS showed more chemical groups compounds to be available in the crude that means more chance to compounds to effect on the bacteria. While the DLPS was shows to get less chemical groups compare with other and its ability against bacteria was less that other. The second point should be address about the LPS has been successfully showed to have good effect on the bacteria. As mentioned in the previous studies, the *Polyalthia* genus showed good biological properties and one of them was antibacterial activates against wide range of bacteria species [7, 10]. Moreover, LPS was showed good activity to be used as an anti-HIV (1). However, LPS was chosen currently to extracted and evaluated for its antibacterial study, and also revealed good activity against all these six bacteria species included in the present study.

Conclusion

The present study was conducted to extract LPS using three solvents system and characterization its chemical constituents as well as biomedical and biological properties. The metabolic extract of LPS showed 21 compounds in GC-mass spectroscope of which 3 compounds were observed in height percentage i.e. 2-methyl-2-oxiranyl-, 2-Undecanol, and Pyridine, 2,3,4,5-tetrahydro-3-methyl-, while three compounds of 21were appear in low percent including 1,6-Heptadiene, 1,1,3-Trimethylcyclopentane, 2(5H)-Furanone, and 5-methyl-. Antibacterial activates of three extract crude were showed significant results against all bacteria species, which give evidence to use them as antibacterial agent. MLPS showed more effect than other extract. The results of the cytotoxicity study revealed that there was no toxic effect of the cells of MG-63 which make these crudes can be used in biomedical safety. Our study will be good database for the future work on the LPS.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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