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FULL PAPER

Extraction, identification of chemical constituents, and in vitro evaluation against cells and bacterial pathogens of the leaves of Polyalthia rumphii

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Polyalthia rumphii (P. rumphii) is a member of the Annonaceae family with a wide distribution in tropical and subtropical regions. In traditional Chinese medicine, various members of this genus have been employed as medicinal plants to address refractory ailments. The Li ethnic minority, primarily residing in Hainan Island, has traditionally utilised extracts derived from P. rumphii for fever prevention, hypertension management, and the inhibition of cancer cell growth. The current study aimed to extract P. rumphii leaves and identify their chemical components using GS-MS and traditional phytochemical methods. This study evaluated the LPr's biomedical properties using antibacterial and cytotoxicity activities. Three solvents were employed to extract the LPr: hexane, dichloromethane (DCM), and methanol (MeOH). Prepared samples were collected and labelled as HLPr, DLPr, and MLPr, respectively, and their biomedical activities were assessed. The results of phytochemical screening showed the presence of terpenoids and glycosides in all three prepared extracts. At the same time, the alkaloids did not identify as being present in all of them. The results of GC-Mass spectroscopy showed the presence of 22 compounds; the low percentages were Allo-Aromadendrene (0.09%), 2,2-dimethoxyethane (0.27%), and Methyl stearate (0.33%) while Trans-α-bergamotene (11.23%), Benzene, 1-methoxy-4-(4-methyl-4-pentenyl)- (8.97%), 2,5-Pyrrolidinedione, 1-methyl- (6.89%), and 2,6-Dimethylbenzonitrile (5.01%) detected with high percentages. The LPr cytotoxicity was measured against HeLa cells, and the results showed no toxic effect. All of the prepared samples showed a significant impact in inhibiting the bacteria growth. The present study established significant bio-medical properties of LPr and obtained products with nontoxic effect.

KEYWORDS

Phytochemical screening; PLr; GS-Mass spectroscopy; cytotoxicity study; antibacterial study .

Introduction

The *Polyalthia* genus, which contains over 120 species, is one of the more than 130 genera in the Annonaceae family, which is thought to be the most prominent family [1-3]. *Polyalthia rumphii* (Blume ex Hensch.) Merr, it is extensively distributed in the tropics and subtropics [4].

The *Polyalthia genus* is distinguished by its unique effectiveness, which enables it to be employed in the biological and medicinal fields [5]. Previous study has been reported to evaluate their biomedical uses, antioxidant [6], anti-cancer, anti-inflammatory [7], antifungal [8], anti-plasmodial [9,10], anti-DENV2 [11,12] and anti-proliferative [13,14], etc. Previous research was done in several *Polyalthia* genus parts, such as leaves, roots, stem portions, stems, twigs, and barks, to identify the group's compounds [15]. Different parts of the genus of *Polyalthia* are traditionally used in medicine to treat a wide range of illnesses, including skin problems, fever, dysmenorrhea, helminthiasis, hypertension, diabetes, and pharyngeal neurosis [16,17].

Many species of Polyalthia is a traditional Chinese medicine that aromatizes dampness, opens the body, and soothes the mind, and is used medicinally. In recent years, a great deal of studies have shown that the chemical constituents of *Polyalthia* species revealed the presence of several chemical group chemicals, such as terpenoids, tannins, saponins, carbohydrates, flavonoids, alkaloids, etc. Because of their chemical components, and they are considered as a rich in groups of chemical that give them their distinctive efficiency, the *Polyalthia* species exhibit intriguing biological activities. One member of that genus was picked for this investigation, and its physicochemical and biological activity were examined [18,19].

Polyalthia rumphii leaf (LPr) was picked for several reasons, including the fact that no studies have been published to examine their compositions of chemical, cytotoxicity, and antibacterial activity. However, it is possible to obtain comparable phytochemicals from LPr leaves which may have potential antimicrobial activity. In addition, LPr is widely accessible in the area and is inexpensive. Thirdly, because there was little information available on LPr, the current work will serve as a resource for future research. Figure 1 displays the LPs freshness.

In the present study, three solvent systems, i.e. hexane, DCM, and MeOH, were used to extract LPr and its biological activities were evaluated by gauging how well it inhibited six different kinds of bacteria. HeLa cells were also employed for the toxicity study. The results showed that the MeOH extract included carbohydrates, glycosides, terpenoids, and steroids. LPr's biological activity showed that it significantly affects all types (Six) of the bacteria.

Experimental

The Preparation process of LPr

LPr were obtained locally from a proton city, Perak, Malaysia farm. LPr was rinsed in distilled water (D.W) to eliminate all the dust and fungus. Oven dry was used at 40 ^{o}C for three days. To obtain LPr powder, the dried LPr was ground carefully and kept for future work. Figure 2 depicts the scheme of the methodology process of the present work.

FIGURE 1 Fresh leaves of Polyalthia rumphii

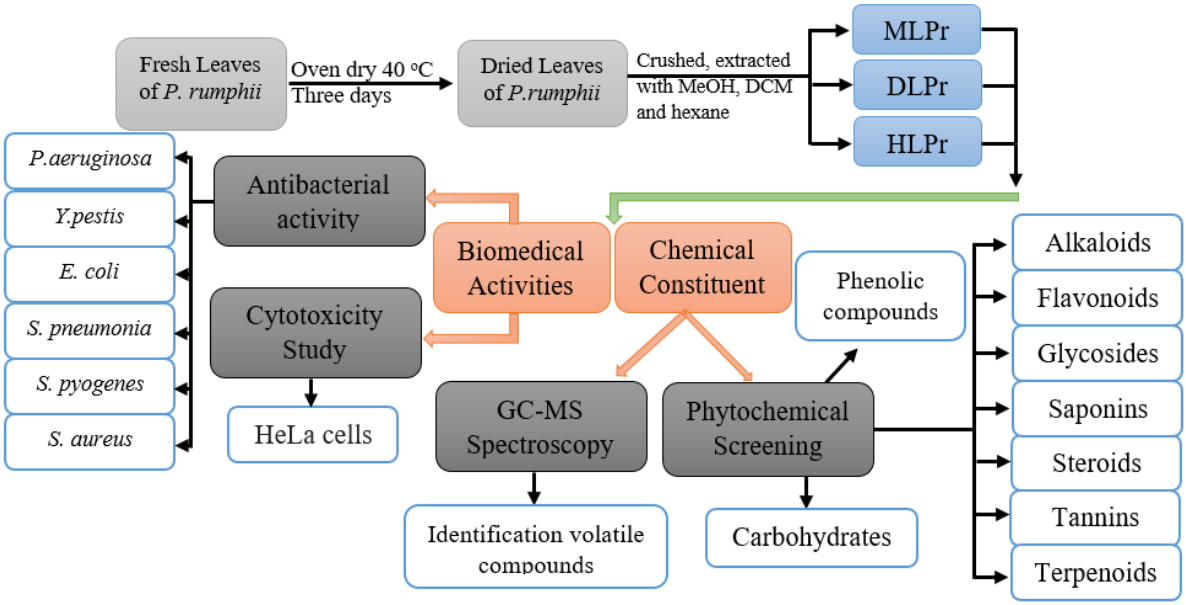


FIGURE 2 The scheme of the methodology process

The extraction method of LPr

Using the hot extraction method, three solvent systems were used to extract LPr, i.e. hexane, DCM, and MeOH.

Briefly, 450 g of LPr were extracted firstly with MeOH to obtain all possible polar compounds, and for the equilibrium polar compound, DCM was used. Finally, hexane was used to remove all potential non-polar compounds, three crude extracts were obtained and labelled as HLPr, DLPr, and MLPr refer to the used solvent hexane, DCM and MeOH, respectively.

The obtained crudes were filtered, dried, and then kept at 4 °C for the next steps.

Chemical characterization of LPr

Prepared samples (HLPr, DLPr, and MLPr) were characterized for their chemical constituent by using Gas chromatography-Mass spectrometry (GC-MS) and classical phytochemical screening technologies.

GC-MS spectrometry analysis of LPr

The chemical constituent of LPr was evaluated using GC-MS (Shimadzu GC-14B) analyser.

The Phytochemical analysis of the LPr

Three extract cured out to evaluate their chemical groups using traditional chemical procedures, as presented in Table 1.

TABLE 1 Traditional chemical procedures of phytochemical of LPr

No	Chemical groups	Method	Ref.
1	Flavonoids	2 g of prepared samples were used to evaluate the presence of flavonoids, and mixed with ethyl acetate as a solvent. The mixture were then heated in the steam bath for 3 min, and then the filtered paper was used to filter the wanted part of the solution. Thereafter, the shaker was used and added dilute of the ammonium. The indicator is observation yellow colour in the solution.	[19]
2	Terpenoids	5 ml of trichloromethane was used to dissolve 20 ml of MLPr, DLPr, and HLPr, and then a few drops of H_2SO_4 added. Presence of reddish-brown is the indicator to presence of Terpenoids	[10]
3	Tannins	DW was used to dissolve 2 g of MLPr, DLPr, and HLPr and were boiled for 20 minutes, and then it was filtered. A few drops of 0.1% ferric chloride was added carefully to the filtered part. The indicator to presence of tannins can be conform by observation of brownish-green and blue-black.	[20]
4	Saponins	20 ml of DW was used to dissolve 2 g of MLPr, DLPr, and HLPr and mixed them for 1 minute. The mixture was shaken and left for 30 minutes. The presence of saponins can be detected by observation of the honeycomb on the surface of the mixture.	[21]
5	Carbohydrates	Two reagents were used to confirm the presence of carbohydrates in LPr. 1 g of the prepared samples dissolved in DW and a few drops of Fehling's reagent were added, a similar procedure was repeated with Molisch's reagent. The observation of brick res gives evidence to test positively.	[22]
6	Steroids	A few drops of acetic anhydride were added to 20 ml of MLPr, DLPr, and HLPr and were used in an ice bath to cool the mixtures. Sulphuric acid was added as a few drops to the mixture. The indicator to conform to a positive test is the colour changing from violet to blue or green.	[23]
7	Alkaloids	Two tests were used to prove the availability of the alkaloids in the LPr. Diluted HCl was used to dissolve 2 g of MLPr, DLPr, and HLPr, and the filtered. The filtered part was treated with two reagents, Dragendroff and Mayer; this is the first test, while the second test was performed by using 40% of $Ca(OH)_2$ to treat with 2 g of the prepared samples. Thereafter, TLC was used to get the spotted. The observation of yellow-orange colours was the indicator of the positive test.	[8]
8	Glycosides	A test of Keller-Killani was used to prove glycosides' presence. DW was used as a solvent to dissolve 2 g of MLPr, DLPr, and HLPr, and then it was torn with a few drops of each of ferric chlorid and sulphuric acid, respectively. The positive result is the observation of two layers of reddish brown.	[24]
9	Phenolic compounds	100 ml of DW was used to dissolve 2 g of MLPr, DLPr, and HLPr, and the solutions were treated with ferric sulfate in a few drops of it. The observation of dark violet can be detected by the positive check.	[25]

Antibacterial activities of PLr

The prepared samples were treated with six bacteria species to evaluate their ability to inhibition of bacteria growth. Three gram positive include *Streptococcus pyogenes* (*S. pyogenes), Streptococcus pneumonia*

(*S.pneumonia)*, and *Staphylococcus aureus* (*S. aureus*), while the gram negative include *Yersinia pestis* (*Y.pestis*), *Yersinia pestis* (*Y.pestis*), and *Escherichia coli* (*E. coli*).

Agar preparation

2000 mL of DW was used to dissolve 40 g nutrient broth agar, and then an autoclave was used to sterilize the solution at 121 °C for 20 minutes. The solution was then cooled, poured into Petri dishes and left to solidify. After that, the plates were stored at 40 °C and kept for future work.

Prepared samples were cured to evaluate their ability to inhibit bacteria growth using the disc diffusion method. The antibacterial determination for the ready samples was performed using measurement of the inhibition zones. Briefly, 100 µL of the bacteria strains were cultured on the plates by spreading them, and then the prepared samples were put on the 6 mm disc and incubated at 37 °C for 24 hours. Finally, the zones were measured using the rule.

Cytotoxicity study of LPr

The LPr cytotoxicity was determined using Alamar blue assay against HeLa cells. Five concentrations of prepared samples were used, i.e. 50, 100, 150, 300, and 500 mg/ml. The models were cultured with HeLa cells in carbon dioxide (CO_2) and incubated for 24 hours at 37 °C.

Results and discussion

The current study was conducted to achieve three objectives. Firstly, extracted of LPr with three different solvents, i.e.

MeOH, DCM, and Hexane, using the hot-extraction method, and three crude extracts were obtained. Secondly, the chemical composition of LPr was determined using screening of the chemical profile and identification of volute compounds using classical screening methods and GC-mass spectroscopy, respectively. The third parameter of the present study aimed to investigate the biomedical properties of the LPr using antibacterial and cytotoxicity studies.

The chemical profile screening evaluated nine groups: Flavonoids, Terpenoids, Tannins, Saponins, Carbohydrates, Steroids, Glycosides, Alkaloids, Phenolic compounds, and Glycosides. The results of the phytochemical profile of MLPr show seven groups out of nine were detected, as demonstrated in Figure 3. Alkaloids. Saponins did not observe to be present.

In the literature, many studies were reported to determine the phytochemical profile of medicinal plants using classical technologies [26,27].

The leaves of the medicinal plants were demonstrated to have various types of chemical groups. The studies of Sadat *et al.* [28] and Kwansa-Bentum *et al.*

[29] were reported to extract the leaves of *Corchorus olitorius* (*C. olitorius*) and *Polyalthia longifolia* (*P. longifolia*) using methanol solvent and evaluated the chemical profile; the results were shown to the presence of saponin, flavonoid, steroid, glycosides, and carbohydrate.

At the same time, tannins were not observed in the leaves of *C. olitorius,* while P. *longifolia* showed the presence of saponin and flavonoid, while alkaloids were not detected to be present. The mentioned results are similar to the present study.

Figure 4 demonstrates the phytochemical analysis of DLPr, and the outcome observed the available of steroids, terpenoids, flavonoids, and glycosides. At the same time, tannins, saponins, carbohydrates, alkaloids, and phenolic compounds were not detected to be available.

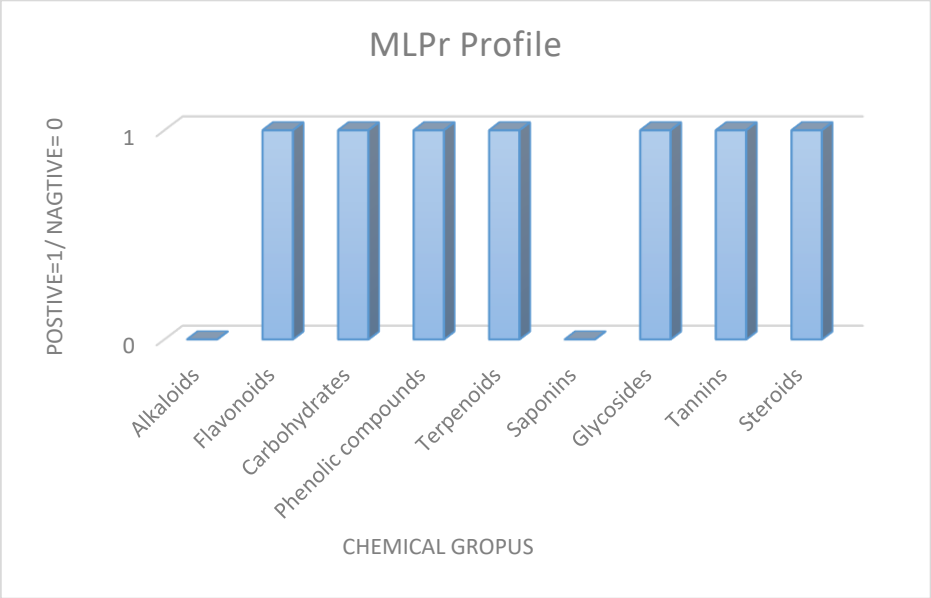


FIGURE 3 Phytochemical profile of MLPr (1 and 0 refer to positive and negative, respectively)

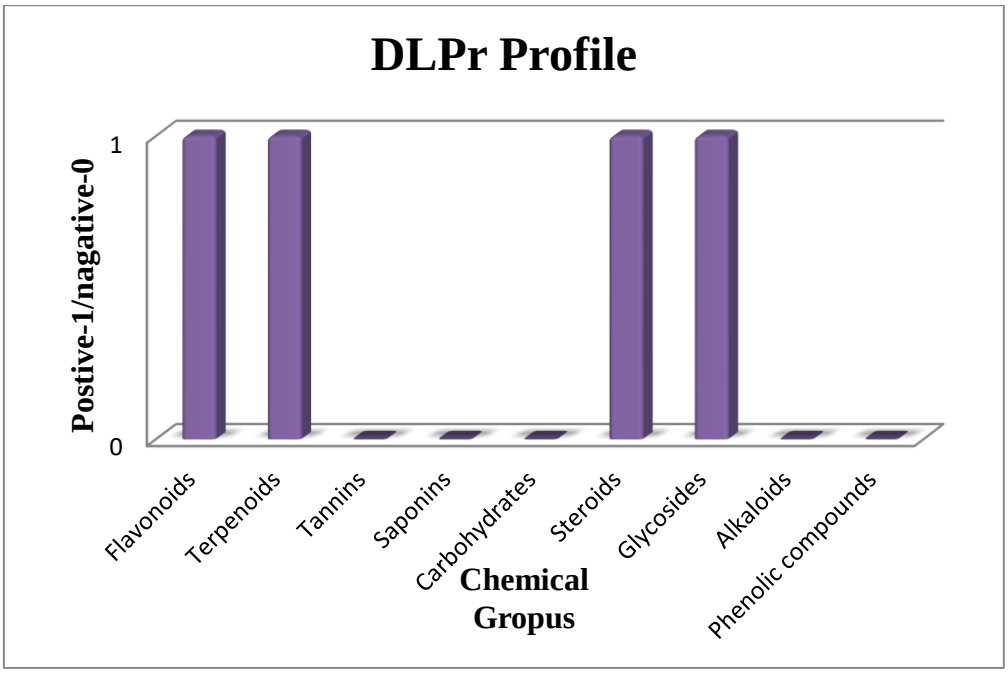


FIGURE 4 Phytochemical profile of DLPr (1 and 0 refer to positive and negative, respectively)

The leaves of the various medicinal plants were reported to be extracted using DCM to investigate their compositions, and the factional chemical groups were demonstrated to be present [30,31].

DCM was used to extract the leaves of *Euodia Redleyi* and *Adenanthera pavonina*. Terpenoids, saponins, and flavonoids were determined to be availably positive, while tannins, phenolic compounds, carbohydrates and saponins were not given positive results [32-34].

The mentioned results were compatible with our obtained results.

Figure 5 illustrates the chemical profile of HLPr which represented to four chemical groups were detected to be presence as a positive test i.e. saponins, tannins, terpenoids, and glycosides while remain five chemical groups were detected to be negative test.

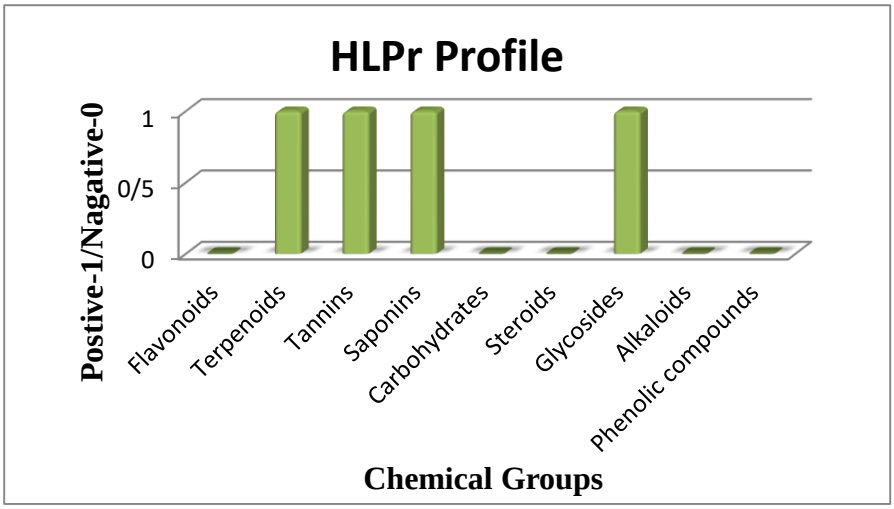


FIGURE 5 Phytochemical profile of HLPr (1 and 0 refer to positive and negative, respectively)

Among the literature, a lot of studies were reported to extract leaves of plants with hexane, and their studies demonstrated various groups to be present, such as flavonoids, steroids, tannins, and saponins [36-38]. The previous results were approximately the same as the current results.

TABLE 2 Phytochemical screening of MLPr, DLPr, and HLPr

No.	Chemical groups	MLPr	DLPr	HLPr
1	Flavonoids	+	+	-
2	Terpenoids	+	+	+
3	Tannins	-	-	+
4	Saponins	+	-	+
5	Carbohydrates	+	-	-
6	Steroids	+	+	-
7	Alkaloids	-	-	-
8	Glycosides	+	+	+
9	Phenolic compounds	+	-	-

(*+ refer to positive results, and – refer to negative results)

Table 2 provides the overall results for the three extracts, i.e. MLPr, DLPr, and HLPr. The results did not indicate the presence of alkaloids in all sections, while terpenoids and glycosides were observed to be present in all extracts. Flavonoids and steroids were observed in two of three quotes, i.e. MLPr and DLPr.

GC-MS spectroscopy of LPr

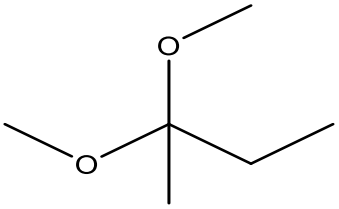
The previous part of the current results was indicated by the phytochemical profile of the three extracts, and the extract of MeOH was shown to have seven of nine evaluated chemical groups, more than other solvents. Based on that, MLPr was utilized to assess its chemical volatile compounds using GC-MS.

The results showed the presence of twenty-two compounds. Four of them were indicated with higher percentages, including 2,5-Pyrrolidinedione, 1-methyl- (8), Benzene, 1-methoxy-4-(4-methyl-4-pentenyl)- (9) Trans-α-bergamotene (17), and 2,6-Dimethylbenzonitrile (21) while three compounds were showed lower percentages including 2,2-Dimethoxybutane (1), Methyl stearate (6), and Allo-Aromadendrene (22), as summarized in Table 3.

TABLE 3 Composition of chemical profile of LPr

No.	Name of compounds	Chemical groups	Chemical formula	R. Time	Composition %
1	2,2-Dimethoxybutane	Alkane	$C_6H_{14}O_2$	7.122	0.27
2	11,13-Dimethyl-12- tetradecen-1-ol acetate	Acetate	$C_{18}H_{34}O_2$	7.77	2.57
3	α-Copaene	Alkene	$C_{15}H_{24}$	8.333	4.54
4	Isoledene	Alkene	$C_{15}H_{24}$	8.512	4.32
5	Methyl 14-methyl Pentadecanoate	Fatty acid	$C_{17}H_{34}O_2$	8.591	2.22
6	Methyl stearate	Ester	$C_{19}H_{38}O_2$	9.267	0.33
7	2,5-Pyrrolidinedione, 1-methyl-	Amide	$C_5H_7NO_2$	9.812	6.89
8	Benzene, 1-methoxy-4-(4-methyl-4-pentenyl)-	Aromatic Ether	$C_{13}H_{18}O$	9.835	8.97
9	3-ethyl-3-hydroxyandrostan-17-one	Ketone	$C_{21}H_{34}O_2$	9.982	3.44
10	3',5′-dimethoxyacetophenone	Ketone	$C_{10}H_{12}O_3$	10.189	2.15
11	Tetradecanoic acid	Carboxylic acid	$C_{14}H_{28}O_2$	10.399	3.89
12	9-Octadecenoic acid, (E)-	Carboxylic acid	$C_{18}H_{34}O_2$	10.901	4.31
13	1,2-Benzenedicarboxylic acid	Carboxylic acid	$C_8H_6O_4$	11.222	2.73
14	1,2-Epoxy-4-Methylpentane	Alkane	$C_6H_{12}O$	11.308	3.79
15	12-Oleanen-13,11-dione	Alcohol	$C_{30}H_{46}O_2$	11.59	2.09
16	Humul-1,6-dien-3-ol	Alcohol	$C_{15}H_{26}O$	11.607	3.88
17	Trans-α-bergamotene	Alkene	$C_{15}H_{24}$	11.681	11.23
18	Thymol	Alcohol	$C_{10}H_{14}O$	12.671	2.7
19	Cedrane-8,13-diol	Alcohol	$C_{15}H_{26}O_2$	12.975	1.81
20	Hexadecanoic acid	Carboxylic acid	$C_{16}H_{32}O_2$	13.873	2.37
21	2,6-Dimethylbenzonitrile	Amide	C_9H_9N	14.569	5.01
22	Allo-Aromadendrene	Alkene	$C_{15}H_{24}$	15.023	0.09

The twenty-two compounds were drawn using ChemDraw software, as shown in Figure 6. Based on the structures, the obtained compounds were observed with interesting factional groups, such as methyl, hydroxyl, amide, and carboxyl, groups that can give evidence to the activities of the LPr, which make it able to have significant results in biomedical application.



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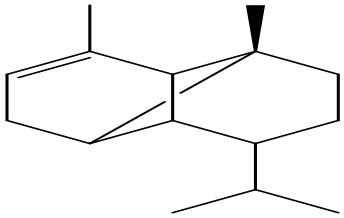

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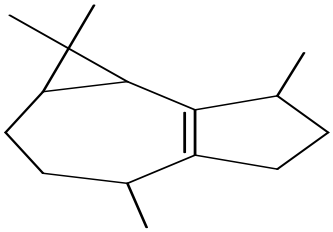

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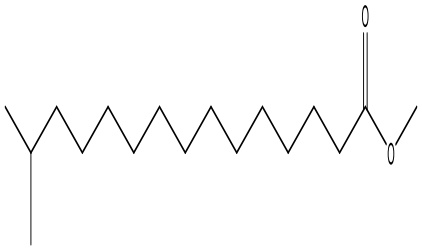

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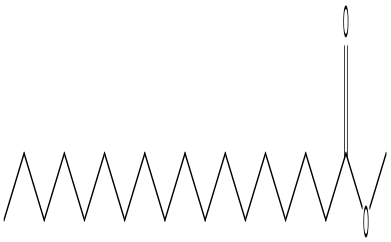

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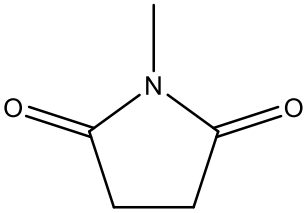

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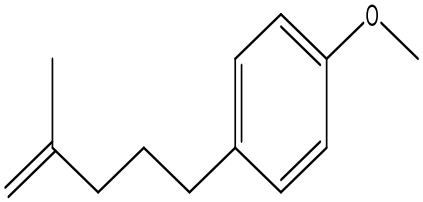

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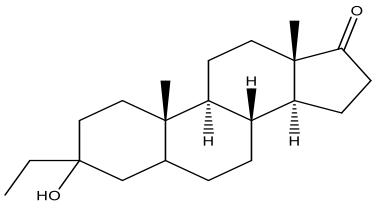

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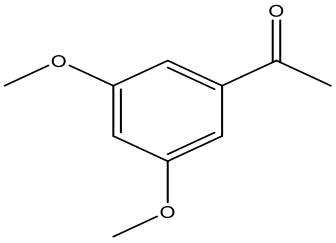

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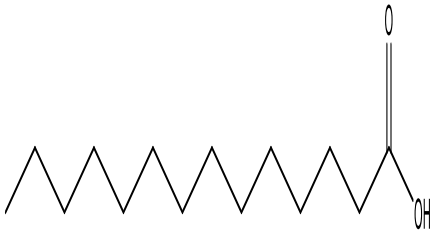

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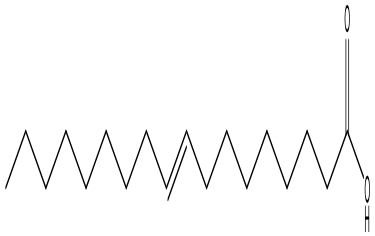

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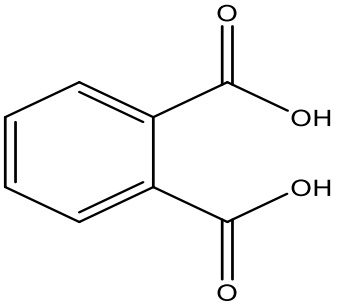

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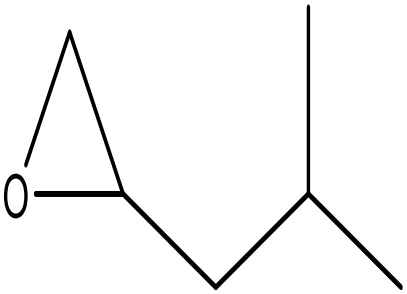

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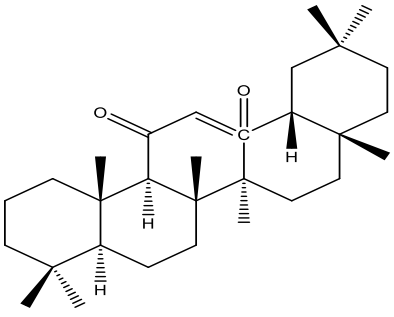

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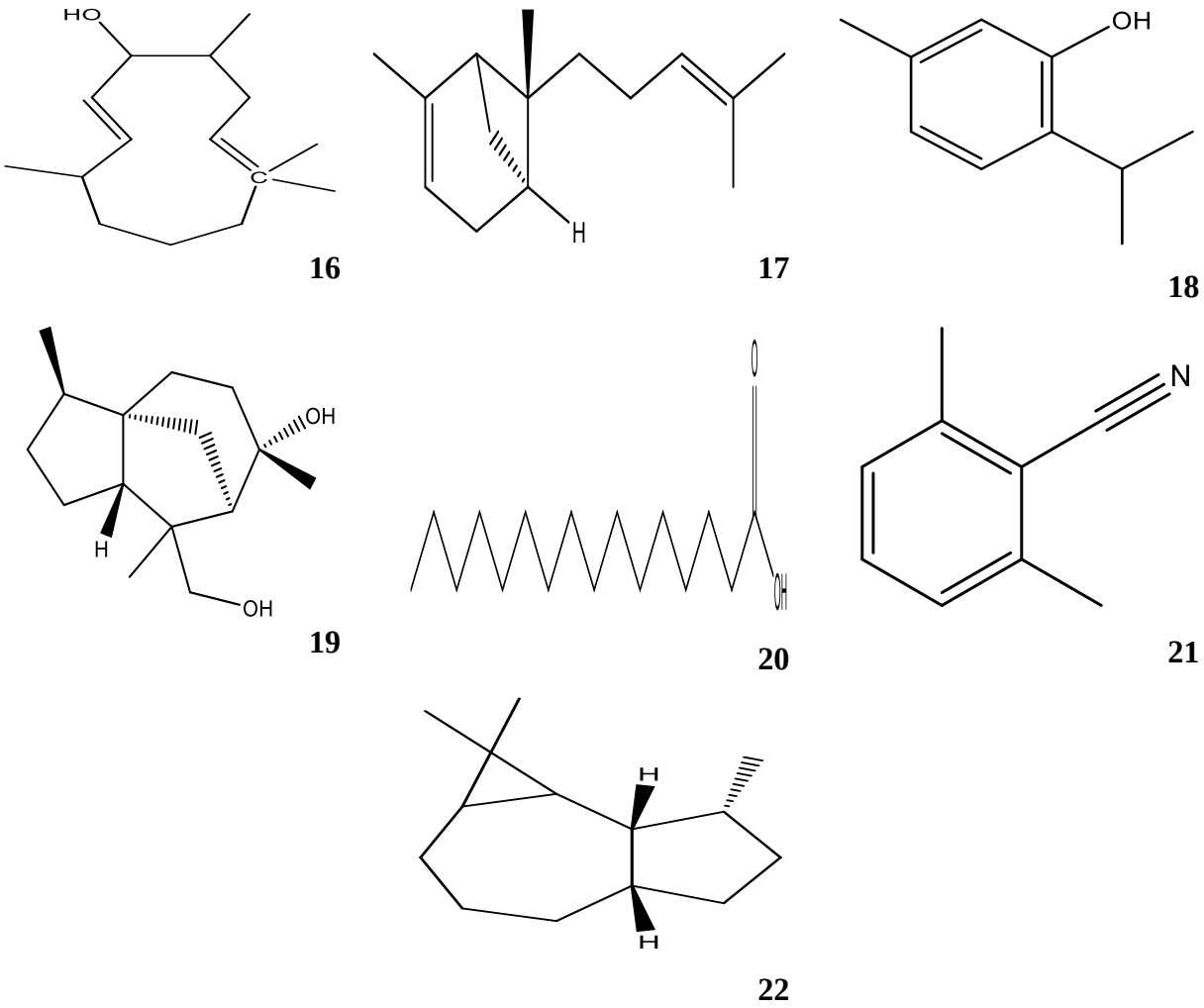

FIGURE 6 Chemical structures of twenty two obtained compounds

As indicated in Table 4, the chemical-physical properties of all obtained compounds were analyzed using ChemDraw software, including exact mass, molecular weight, mass to charge (m/z), and elemental analysis. Compound number 15 had a higher molecular weight (440.71 g/mol), while compound number 14 had a lower molecular weight (100.16 g/mol). Based on the element analysis, all obtained compounds showed Carbone and hydrogen, which is expected because the extract is considered organic. The other elements obtained are oxygen and nitrogen due to function groups.

TABLE 4 Chemical-physical properties of LPr

Com.	Exact Mass	Molecular Weight	m/z	Elemental Analysis
1	118.10	118.18	118.10 (100.0%), 119.10 (6.5%)	C, 60.98; H, 11.94; O, 27.08
2	282.26	282.47	(100.0%), 283.26 (19.5%), 284.26 (1.8%)	C, 76.54; H, 12.13; O, 11.33
3	204.19	204.36	204.19 (100.0%), 205.19 (16.2%), 206.19 (1.2%)	C, 88.16; H, 11.84
4	204.19	204.36	204.19 (100.0%), 205.19 (16.2%), 206.19 (1.2%)	C, 88.16; H, 11.84
5	270.26	270.46	270.26 (100.0%), 271.26 (18.4%), 272.26 (1.6%)	C, 75.50; H, 12.67; O, 11.83
6	298.29	298.51	298.29 (100.0%), 299.29 (20.5%), 300.29 (2.0%)	C, 76.45; H, 12.83; O, 10.72
7	113.05	113.12	113.05 (100.0%), 114.05 (5.4%)	C, 53.09; H, 6.24; N, 12.38; O, 28.29
8	190.14	190.29	190.14 (100.0%), 191.14 (14.1%)	C, 82.06; H, 9.54; O, 8.41
9	318.26	318.50	318.26 (100.0%), 319.26 (22.7%), 320.26 (2.5%)	C, 79.19; H, 10.76; O, 10.05
10	180.08	180.20	180.08 (100.0%), 181.08 (10.8%	C, 66.65; H, 6.71; O, 26.63
11	228.21	228.38	228.21 (100.0%), 229.21 (15.1%), 230.22 (1.1%)	C, 73.63; H, 12.36; O, 14.01
12	282.26	282.47	282.26 (100.0%), 283.26 (19.5%), 284.26 (1.8%)	C, 76.54; H, 12.13; O, 11.33
13	166.03	166.13	166.03 (100.0%), 167.03 (8.7%)	C, 57.84; H, 3.64; O, 38.52
14	100.09	100.16	100.09 (100.0%), 101.09 (6.5%)	C, 71.95; H, 12.08; O, 15.97
15	440.37	440.71	440.37 (100.0%), 441.37 (32.4%), 442.37 (2.7%), 442.37 (2.4%)	C, 81.76; H, 10.98; O, 7.26
16	223.21	223.38	223.21 (100.0%), 224.21 (16.2%), 225.21 (1.2%)	C, 80.65; H, 12.18; O, 7.16
17	204.19	204.36	204.19 (100.0%), 205.19 (16.2%), 206.19 (1.2%)	C, 88.16; H, 11.84
18	150.10	150.22	150.10 (100.0%), 151.11 (10.8%)	C, 79.96; H, 9.39; O, 10.65
19	238.19	238.37	238.19 (100.0%), 239.20 (16.2%), 240.20 (1.2%)	C, 75.58; H, 10.99; O, 13.42
20	256.24	256.43	256.24 (100.0%), 257.24 (17.3%), 258.25 (1.4%)	C, 74.94; H, 12.58; O, 12.48
21	131.07	131.18	131.07 (100.0%), 132.08 (9.7%)	C, 82.41; H, 6.92; N, 10.68
22	204.19	204.36	204.19 (100.0%), 205.19 (16.2%), 206.19 (1.2%)	C, 88.16; H, 11.84

Test of cytotoxicity of LPr

The crude extract of LPr was cured to evaluate its cytotoxicity against HeLa cells, as mentioned in the methodology part. Five different concentrations of LPr were used to prepare five samples and treated with the cells. All models showed a no-toxic effect, and the average cell availability was 97.7%, as presentd in Table 5 and Figure 7. Based on the literature studies, the crude extracts of the genus *Polyalthia* were reported as having a non-toxic effect on the sections of the cells [14]. The current study is constituent with the previous ones.

TABLE 5 HeLa cells availability cultured with five various concentrations of LPr

Concentrations of LPr	LPr	Control	HeLa cells Availability
50 mg/mL	0.159	0.166	95.78313253
100 mg/mL	0.24	0.241	99.58506224
150 mg/mL	0.286	0.296	96.62162162
200 mg/mL	0.31	0.313	99.04153355
250 mg/mL	0.301	0.316	95.25316456
HeLa cells Average	0.24875	0.254	97.75783748

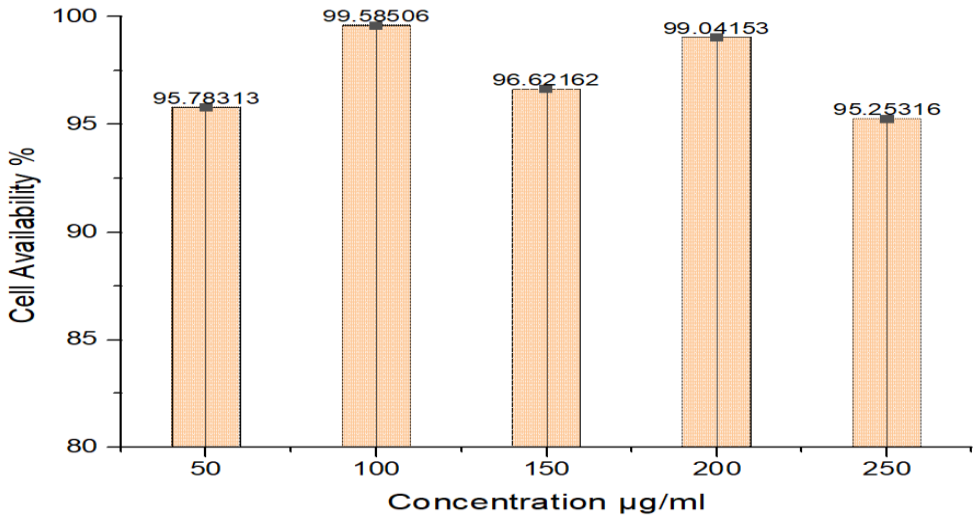

FIGURE 7 Evaluation of cytotoxicity of LPr

The evaluation of antibacterial activity of LPr

As mentioned in the methodology part, six species of bacteria were used to estimate the antibacterial activity of LPr. Prepared samples, including MPLS (i), DLPS (ii), and HLPS (iii), were treated with all bacteria species. The ampicillin was used as a positive control, while the solvents (MeOH, DCM, and Hexane) were a negative control; all solvents did not show any inhibition for the growth of the bacteria. The obtained samples showed practical activities against these six species of bacteria. MLPr was indicated to have a higher effect than DLPr and DLPr, where it showed effectiveness, i.e. 11 mm, 8 mm, and 13 mm against *S. pyogenes, S.pneumonia* and *S. aureus*, and 8.8 mm, 9.2 mm, and 11 mm against *P.aeruginosa, Y.pestis*, and *E. coli*. Figure 8 demonstrates the effect of prepared samples against these bacteria.

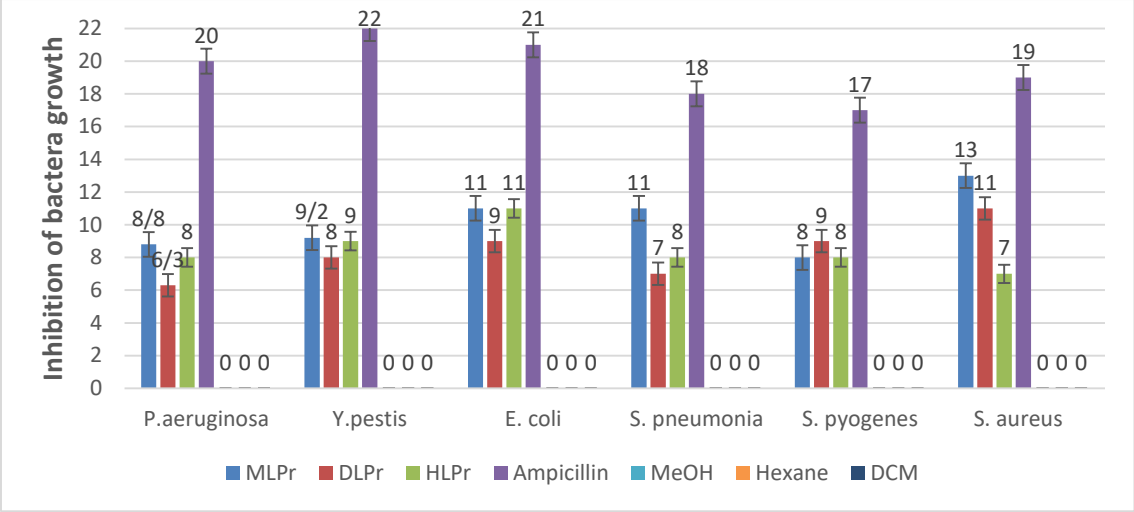

FIGURE 8 The evaluation of antibacterial effect of LPr

According to the results that were obtained from the antibacterial study, the previous study demonstrated the reason that the ability of the medicinal plants to have a significant effect on the bacteria was the functional groups present in the plant; this is due to the diversity in composition of these plants [40-43], based on the chemical analysis of the LPr that mentioned in the previous part, various chemical groups were detected to be present. These groups were responsible for the effectiveness of it against these bacteria.

MLPr was observed to have a higher effect on the bacteria. The reason was observed due to the presence of seven of nine investigated groups, while DPLr showed less impact because only four of nine chemical groups were detected. The current study demonstrated the ability of LPr to be used as an antibacterial agent against both grams of the bacteria (gram-negative and gram-positive).

Conclusion

The current study reported hot extraction of LPr using MeOH, DCM, and hexane. Secondly, its chemical composition was estimated using chemical profile analysis and chemical identification of its compounds. Thirdly, evaluated its biomedical and biological properties. Twenty-two compounds were identified, four of them were detected with higher concentrations, i.e., compound number 8, 9, 17, and 21, while and three compounds showed **1**, **6**, and **22**. As shown in the cytotoxicity analysis, the LPr could be used as an antibacterial agent against six bacteria species, with a non-toxic effect. A current study demonstrated that LPr has various chemical groups with chemical compounds with multiple functional groups. The present study also showed the unique biological properties of the LPr and gave an excellent product without any toxic effects.

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Authors' Contributions

All authors contributed to the article and approved the submitted version.

Conflict of Interest

This study is the contribution of the authors who do not have any conflict of interest with any other individual or institution.

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