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Phytochemical Screening of leaves of *Polyalthia sclerophylla* using Classical Methods and GC-Mass Spectroscopy: Cytotoxicity and Antibacterial Activities

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Abstract

Background: *Polyalthia sclerophylla* (*P. sclerophylla*) 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 current study aims to extract the leaves of *Polyalthia sclerophylla* (TLPS) and determine their chemical contents using GC-MS and standard phytochemical techniques.

Methods: The antibacterial and cytotoxicity activities of TLPS were used to evaluate its bio-medical characteristics. In that sequence, three solvents were used to extract TLPS to produce three samples: MTLPS, DTLPS, and HTLPS.

Results: Phytochemical analysis indicated terpenoids and glycosides in all prepared models, but no alkaloids were observed. The GC-MS data showed twenty-one chemical compounds. Pyridine, 2-Undecanol, 2-methyl-2-oxiranyl- and cyclobutanone were observed with higher percentages, while the 2(5H)-Furanone, 5-methyl, 1,1,3-Trimethylcyclopentane and 1,6-Heptadiene were observed with lower rates. The cytotoxicity study of TLPS was performed using Alamar blue assay using MG-63 cells. However, results show no detrimental influence at any dosage as cell availability increased. MTLPS, DTLPS and HTLPS were treated with six bacteria pathogens, and all of them showed significant effects against these bacteria to inhibit the growth of the bacteria. The extract of methanol (MLPS) was more effective in inhibiting the development of the bacteria compared with the DTLPS and HTLPS.

Conclusion: The current work has shown that the chemical composition of crude TPLS varies, leading to notable chemical, biological, and medicinal features, and their non-toxic impact.

Introduction

The *Polyalthia* genus is part of the *Annonaceae* family, which comprises approximately 130 genera and 2300-2500 species. *Polyalthia*, which has over 120 species, is a tropical and subtropical plant species native to South Asian nations such as Malaysia, Thailand, and Indonesia [1-4]. *Polyalthia* plants are well-known for their excellent medicinal and biological efficiency. They exhibit anti-cancer, anti-inflammatory, anti-oxidant, anti-plasmodial, anti-bacterial, and anti-DENV2 characteristics. *Polyalthia* genus stems, stem sections, roots, barks, leaves, and twigs have all been recognized via research. Traditional medicine uses Polyalthia plants to cure many ailments, including diabetes, dysmenorrhea, fever, helminthiasis, hypertension, stomach aches, pharyngeal neurosis, and skin issues [5-11]. Alkaloids, carbohydrates, terpenoids, tannins, saponins, phenolic compounds, flavonoids, and tannins are among the phytochemicals identified in the *Polyalthia* species. Because of their large chemical groups, these plants have unique biological activities that contribute to their efficiency. This study assesses one of these genera based on its chemical composition and biological activity [12-14]. The leaves of *Polyalthia sclerophylla* (TLPS) have been selected for various reasons. No studies have been reported on its chemical structures, antibacterial activity, or cytotoxicity. Nevertheless, there is a likelihood of getting comparable or unique phytochemicals with antimicrobial action from LPS leaves. Second, LPS is accessible locally and is deemed economical (low-cost). Third, because there was little information regarding LPS, the current study would explore creating a database for the researcher. This study investigated the biological activities of TLPS extracted using methanol, hexane, and dichloromethane, assessing their antibacterial activity against six bacteria. A toxicity investigation was conducted on the MG-63 human cell line. The methanol extract contained carbohydrates, glycosides, terpenoids, tannins, and steroids, indicating its significant influence on bacteria.

Methods

Preparation of TLPS

In Perak, Malaysia, TLPS leaves were collected and cleansed with distilled water to remove any fungal contaminants or dust. TLPS was sun-dried for seven days before being chopped into small pieces and powdered for future use.

Extraction of TLPS

The Soxhlet technique extracted potential chemicals from TLPS via three solvent systems: hexane, dichloromethane (DCM), and methanol (MeOH). 50 g of TLPS were removed to obtain three produced cures were labelled as MTLPS, DTLPS, and HTLPS, which were then kept at four °C for potential research [15].

The Phytochemical analysis of TLPS

MTLPS, DTLPS, and HTLPS were cured using normal chemical procedures to determine their chemical groupings.

Test for alkaloids

Two stages were employed to detect the presence of alkaloids. The preliminary tests come first, followed by the confirmation testing. Mix 10 ml of diluted TMLPS, TDLPS, and THLPS to summarise the preliminary examination in HCl and filter the liquid. The filtered solution was used to treat Mayer's and Dragendroff's reagents. The second test involved combining 1 g of MTLPS, DTLPS, and HTLPS with 40% Ca(OH)2 solution until alkaline was visible on litmus paper, followed by two chloroform extractions. A Chloroform extract revealed the presence of thin layer plates. The chromatogram was created using ethyl acetate, n-hexane 1:4 solvent system, with a Dragendorff reagent, then sprayed in the chromatogram solution. The existence of alkaloids in the background can be recognised by monitoring the yellow and orange colours [16, 17].

Test for Flavonoids

There are several ways to detect Flavonoids in medicinal plants. Three techniques were utilised in this study to determine the percentage of Flavonoids in TPLS.

Determine the Flavonoids in TLPS

The mixture of MTLPS, DTLPS, and HTLPS with 5 mL of ethyl acetate, then put in the steam bath for heated for 3 minutes and filtered with high-quality filter paper. After adding 1 ml of ammonium dilute, the filtered solution was shaken. The presence of flavonoids may be identified by observing the yellow colour [18].

Test for Carbohydrates

The carbohydrates in TLPS were detected using Molisch's assay and Fehling's reagent. It was discovered that reducing sugar was present in PS leaves by dissolving MTLPS, DTLPS, and HTLPS in distilled water, adding Fehling's reagent, and witnessing the colour shift to brick red.

Detect of Phenolic compound in TLPS

The mixture was prepared by taken 1 g of MTLPS, DTLPS, and HTLPS and solved in 100 ml of distilled water, then added a drop of $Fe_2(SO_4)_3$. The presence of a phenolic group was detected as dark violet [19].

Test of Salkowski to Detect the Terpenoids in TLPS

Initially, 2 ml of $CHCl_3$ was mixed with 10 ml of MTLPS, DTLPS, and HTLPS, followed by adding the H_2SO_4. Terpenoids are present when the mixture's colour turns reddish-brown [20].

Test for Saponins (Froth test)

A test tube was filled with MTLPS, DTLPS, and HTLPS, then combined with 10 ml of distilled water and shaken vigorously for 1 minute. For more than 30 minutes, the tube was connected at an angle, and saponins were discovered by inspecting the surface honeycomb [21, 21].

Detect of Glycosides

The Keller-Killani assay was used to detect glycosides in LPS by dissolving 1 g of MTLPS, DTLPS, and HTLPS in DW, adding sulphuric acid and ferric chloride, and observing the formation of reddish and reddish-brown layers [23].

Test of Ferric chloride to detect Tannins

Before filtering, 1g of mixed MTLPS, DTLPS, and HTLPS was heated in a conical flask with 50 DW for 20 minutes. The samples were carefully soaked in 0.1% $FeCl_3$. Black-Blue and green brownish were seen to determine the availability of tannins in the samples [24].

Test for Steroids (Lieberman's test)

Acetic anhydride was mixed with 10 ml each of MTLPS, DTLPS, and HTLPS before being cooled in an ice bath. Several drops of sulphuric acid have been incorporated into the mixes Combinations of steroids can be identified by a shift in colour to blue or green from violet [25].

The test of Lead acetate

The mixture of MTLPS, DTLPS, and HTLPS (10 ml) was heated using a hot plate. The mixture was then treated with 1 ml of lead acetate (10%). The yellow precipitate format indicates the presence of flavonoids [26].

Reaction with Sodium hydroxide

The hot plate was used to heat 10 ml of MTLPS, DTLPS, and HTLPS. The mixture was then treated with diluted sodium hydroxide. The yellow precipitate format indicates the presence of flavonoids [27].

Detect of volatile compounds using GC-MS

To determine the volatile compounds in the TLPS extracts was analysed using a gas-chromatography-mass spectroscopy (GC-MS) analyser.

The toxicity of TLPS

The cytotoxicity of TLPS on the MG-63 human cell line was assessed using the Alamar Blue assay. For 24 hours, the TLPS powder was incubated in a complete medium containing roughly 250 mg/mL, and then extracts were produced for cell viability test and sterilized using a 0.2 m syringe. The pure extracts were diluted with medium to achieve different weight-to-volume ratios of 50, 100, 150, 200, and 250 mg/mL. The extracts were applied to the healthy MG-63 cell monolayer, and the cells were grown in incubator carbon dioxide for 24 hours at 37°C. The Alamar Blue assay was utilized to assess cell viability, which involves staining and incubating the culture for four hours.

Antibacterial activities of TPLS

The potential of MTLPS, DTLPS, and HTLPS samples is tested using six species of bacteria. The bacteria consist of six species: three gram-negative and three gram-positive. Include *Yersinia pestis* (*Y. pestis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), along with three Gram-positive including *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumonia* (*S. pneumonia*) *and Streptococcus pyogenes* (*S. pyogenes*).

Agar Preparation

40 g of the nutrient broth agar was dissolved in 2000 ml of DW and sterilized using an autoclave at 121 °C for 25 minutes. Then, it was cooled to 60 °C, and 26ml of cooled media was poured onto the plate and allowed to solidify before being kept in dark conditions at four °C for future experiments. A diffusion approach was used to evaluate the ability of TPLS as an antibacterial agent using MTLPS, DTLPS, and HTLPS. To test the antibacterial capabilities, the inhibition zone of bacteria was studied. 10 mL of bacterial culture was used to observe the culture of both bacterium strains. For 24 hours, the MLPS, DLPS, and HLPS were cultured at 37°C in a 100mL bacterial culture dispersed over nutrient agar plates. The inhibitory zone was then calculated.

Results

Chemical compositions of TLPS

TLPS were extracted using three solvents to produce MTLPS, DTLPS, and HTLPS; then, it was cured to determine the chemical arrangements by employing phytochemical screening using GC-MS spectroscopy and traditional techniques.

Phytochemical screening

Table 1 presents the phytochemical analysis of MLPS, revealing the existence of carbohydrates, flavonoids, glycosides, phenolic compounds, terpenoids, tannins, and steroids, whereas there were no alkaloids or saponins. Table 2 demonstrates that glycosides, flavonoids, and terpenoids were found in the phytochemical screening of DTLPS, while

carbohydrates, alkaloids, tannins, saponins, and phenolic chemicals weren't detected to be present.

The test name	The Observation	Results
Alkaloids		
a- Dragendroff's reagent	The yellow and orange colours didn't observe	Negative
b- Mayer's reagent	The yellow and orange colours didn't observe	
Flavonoids		
a- Test of Lead acetate	Yellow precipitate has been Formatted	Positive
b- Reaction with NaOH	Yellow precipitate has been Formatted	
c- Free Flavonoids test	Yellow colour has been detected to be presence	
Carbohydrates		
a- Fehling reagent	Brick-red colour was detected to be presence	Positive
b- Molish test	Brick-red colour was detected to be presence	
Phenolic compounds		
a- Ferric sulfate test	Colour as dark violet has been observed	Positive
Terpenoids		
a-Lieberman's test	Colour of the solution changed to be reddish-brown	Positive
Saponins		
a- Test of Froth	The honeycomb didn't observe on the surface of the solution	Negative
Glycosides		
a-test of Killer-Killani	Two layers as a reddish-brown in the colour were observed	Positive
Tannins		
a-Test of Ferric chloride	Two colours were observed i.e. brownish-green and blue –black	Positive
Steroids		
a-Test of Lieberman	The colour of the solution was changed to reddish-brown	Positive

Table 1: Phytochemical analysis of MTLPS.

The test name	The Observation	Results
Alkaloids		
c- Dragendroff's reagent	The yellow and orange colours didn't observe	Negative
d- Mayer's reagent	The yellow and orange colours didn't observe	
Flavonoids		
d- Test of Lead acetate	Yellow precipitate has been Formatted	Positive
e- Reaction with NaOH	Yellow precipitate has been Formatted	
f- Free Flavonoids test	Yellow colour has been detected to be presence	
Carbohydrates		
c- Fehling reagent	Brick-red colour wasn't detected to be presence	Negative
d- Molish test	Brick-red colour wasn't detected to be presence	
Phenolic compounds		
b- Ferric sulfate test	Colour as dark violet wasn't observed	Negative
Terpenoids		
a-Lieberman's test	Colour of the solution was changed to be reddish-brown	Positive
Saponins		
a- Test of Froth	The honeycomb didn't observe on the surface of the solution	Negative
Glycosides		
a-test of Killer-Killani	Two layers as a reddish-brown in the colour were observed	Positive
Tannins		
a-Test of Ferric chloride	Two colours weren't observed i.e. brownish-green and blue – black	Negative
Steroids		
a-Test of Lieberman	The colour of the solution was changed to reddish-brown	Positive

Table 2: Phytochemical analysis of DTLPS.

TLPS were extracted using hexane to detect the possible presence of the non-polar compounds in it. Table 3 shows the phytochemical results of HTLPS. Four of nine chemical groups evaluated in the current study were detected to be present, i.e., glycosides, terpenoids, tannins, and saponins.

The test name	The Observation	Results
Alkaloids		
e- Dragendroff's reagent	The yellow and orange colours didn't observe	Negative
f- Mayer's reagent	The yellow and orange colours didn't observe	
Flavonoids		
g- Test of Lead acetate	Yellow precipitate hasn't been Formatted	Negative
h- Reaction with NaOH	Yellow precipitate hasn't been Formatted	
i- Free Flavonoids test	Yellow colour wasn't detected to be presence	
Carbohydrates		
e- Fehling reagent	Brick-red colour wasn't detected to be presence	Negative
f- Molish test	Brick-red colour wasn't detected to be presence	
Phenolic compounds		
c- Ferric sulfate test	Colour as dark violet wasn't observed	Negative
Terpenoids		
a-Lieberman's test	Colour of the solution was changed to be reddish-brown	Positive
Saponins		
a- Test of Froth	The honeycomb didn't observe on the surface of the solution	Negative
Glycosides		
a-test of Killer-Killani	Two layers as a reddish-brown in the colour were observed	Positive
Tannins		
a-Test of Ferric chloride	Two colours weren't observed i.e., brownish-green and blue black	Positive
Steroids		
a-Test of Lieberman	The colour of the solution wasn't changed to reddish-brown	Negative

Table 3: Phytochemical analysis of HTLPS.

Figure 1 shows the presence of phytochemicals groups in the prepared samples, indicating the presence of terpenoids and glycosides in all samples, but no alkaloids were observed.

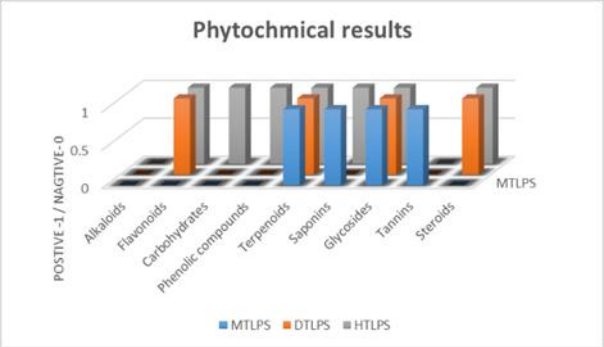

Figure 1: Phytochemical screening of MTLPS, DTLPS, and HTLPS.

GC-MS of TLPS

MeOH was utilized to extract TLPS, and the photochemical screening portion revealed that MTLPS had more chemical groups than DTLPS and MTLPS. GC-MS with MTLPS was used to identify the chemical substances. This section's data identified 21

compounds in the TPLS. As shown in the table4, cyclobutanone,2-methyl-2-oxirany (10), 2-Undecanol(9), and Pyridine,4, 2,3,4,5-tetrahydro-3-methyl (8) were discovered in higher percentages, whereas 2(5H)-Furanone, 5-methyl-(15), 1,1,3-Trimethylcyclopentane(3), and 1,6-Heptadiene(16), were found in lower percentages.

Compound title	Compound formula	Composition %	R. Time
2-Propynenitrile, 3-fluoro-	C_3FN	2.12	8.34
Acetyl cyanide	C_3H_3NO	3.24	9.123
1,2,3,6-Tetrahydropyridine	C_5H_9N	4.51	9.22
1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	6.20	9.888
1-azabicyclo(3.1.0)hexane	C_5H_9N	7.49	10.144
Tans-1-Propenylcyclopropane	C_6H_{10}	2.48	10.333
Ethyl isocyanide	C_3H_5N	4.27	12.378
Pyridine,2,3,4,5-tetrahydro-3-mathyl-	$C_6H_{11}N$	8.58	12.583
2-Undecanol	$C_{11}H_{24}O$	9.25	13.471
Cyclobutanone, 2-methyl-2-oxiranyl-	$C_7H_{10}O_2$	12.74	13.575
2-Hexenal	$C_6H_{10}O$	1.33	13.667
Heptafluorobutyric acid, n-tetradecyl ester	$C_{18}H_{29}F_7O_2$	1.22	13.778
4-Methyl-3-pentenal	$C_6H_{10}O$	1.51	13.789
1,1,3-Trimethylcyclopentane	C_8H_{16}	0.85	13.811
2(5H)-Furanone, 5-methyl-	$C_5H_6O_2$	0.91	13.823
1,6-Heptadiene	C_7H_{12}	0.74	13.823
2-Furanmethanamine	C_5H_7NO	1.23	13.823
2-Pentanone, 3-methylene-	$C_6H_{10}O$	1.23	13841
1-Butene, 2-ethyl-3-methyl-	C_7H_{14}	1.27	13.884
Acetamide, N-2-propynyl-	C_5H_7NO	2.25	13.911
Acetonitrile, 2,2'-iminobis-	$C_4H_5N_3$	2.25	13.911

Table 4: Chemical formation of TLPS.

Figure 2 shows the chemical structures of the twenty-one chemical compounds obtained from GC-MS spectroscopy of the leaves of *Polyalthia sclerophylla.*

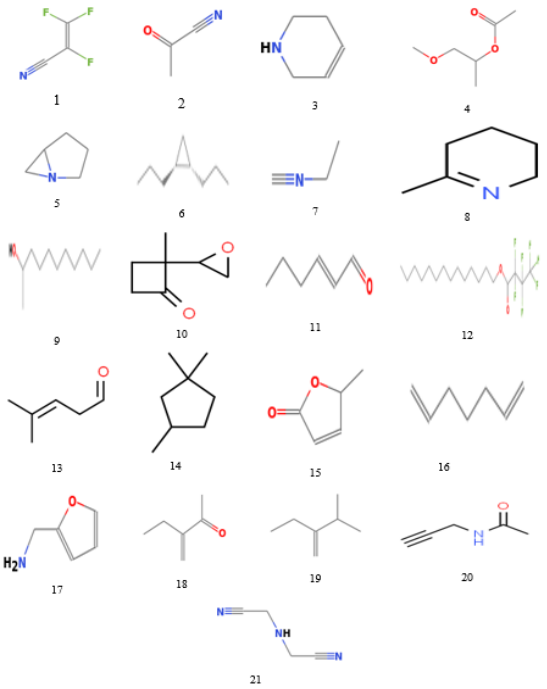

Figure 2: Structures of obtained compounds using GC-Mass.

Cytotoxicity study of TLPS

A cytotoxicity research for MTLPS was conducted in this section to determine whether or not this plant is hazardous. One of the most significant tests for medicinal materials is their poisonous impact, because of the influence on the final product that may be utilised therapeutically. The M-63 human cell line was exposed to five different concentrations of MLPS. Table 5 shows that all concentrations of MTLPS had no harmful impact. Previous research on the *Polyalthia* genus has revealed that its species have a nontoxic impact [35]. The current study's findings revealed that TLPS had no harmful impact.

Concentrations	Availability of the Cells	TLPS	Control
50 mg/ml	98.7755	0.242	0.245
100 mg/ml	98.0469	0.251	0.256
150 mg/ml	100.361	0.278	0.277
200 mg/ml	100.662	0.304	0.302
250 mg/ml	99.6721	0.304	0.305
AVERAGE OF CELLS VIABILITY	99.5668	-	-

Table 5: Alamar Blue assay of TLPS.

Figure 3 shows the availability of M-63 human cell line in the different five concentrations of leaves of Polyalthia *sclerophylla.* The results that obtained were observed there were no toxic effect of the extract on the MG-63 human cell and the availability of the cells were 98%.

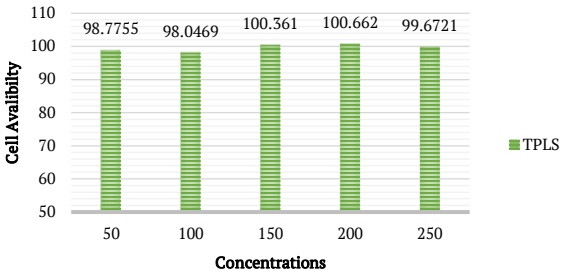

Figure 3: Alamar Blue assay of TLPS

Antibacterial activities of leaves of *Polyalthia sclerophylla*

TLPS was tested for its antibacterial activity against six bacterial species, including gram-negative and gram-positive. As shows in table 6 MTPLS, DTLPS, and HTLPS were found to be effective against gram-negative bacteria. Substantial suppression of bacterial growth was seen at 8, 9, and 12mm (a), 6, 7, and 8mm (b), and 7, 8, and 10mm (c), respectively. The study discovered that crude extracts of MPLS, DLPS, and HLPS successfully suppressed the growth of gram-positive bacteria, S. pneumonia, S. pyogenes, and S. aureus, with growth rates of 10, 9, and 14mm (a), 8, 8, and 10mm (b), and 9, 7 and 12mm (c), respectively. The solvent utilised affects bacterial growth suppression, with methanolic extract having a higher

effect on both bacteria. Grammes showed a smaller effect than dichloromethane extract.

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

*The mark – represent to zero inhibition zone

Table 6: Antibacterial activity of TPLS.

Discussion

Previous research has employed MeOH as a solvent for phytochemical screening, i.e., Kujur et al. [28] investigation on *Stevia rebaudiana* leaves, which discovered phenolic compounds, saponins, tannins, and steroids, but no alkaloids were detected. Kaur et al. [29] discovered carbohydrates, glycosides, flavonoids, saponins, and tannins in *Caesalpinia sappan* leaves and no alkaloids. These results are consistent with our present research, identifying the accessible chemical groups in plant leaves responsible for their actions. The presence of these chemicals has been approved, which has resulted in MTPLS actions.

In several investigations, DCM was formerly utilized as a solvent to extract medicinal plant leaves and assess chemical contents. Many chemical group substances have been recognized as being present. DCM extract of *Adenanthera pavonina* L., (DEAP) was observed to be available for terpenoids, steroids, flavonoids, alkaloids, and tannins; in contrast, extract of *Euodia ridleyi* (DERE) was observed to be available for terpenoids, flavonoids, tannins, alkaloids, and steroids, and while phenolic compounds and tannins didn't detect in DERE and saponins and carbohydrates weren't detected in DEAP [30, 31]. Both of these experiments had essentially identical results.

Previous studies on extracting medicinal plants using hexane revealed compounds; flavonoids, steroids, tannins and saponins, [32, 33]. Our findings corresponded with their findings.

To extract TLPS, three solvents are currently used: MeOH, DCM, and Hexane. The data show no alkaloids were detected in any extracts, while terpenoids and glycosides are present in MTLPS, DTLPS, and HTLPS. Figure 1 shows the absence of steroids and flavonoids in HTLPS and present in MTLPS and DTLPS. Hexane is a non-polar solvent. However, chemical groups require polar solvents to dissolve [34].

GC-MS analysis is used in the study to evaluate the chemical structures of twenty-one compounds, as depicted in Figure 2. Three compounds had a more significant proportion of functional groups: compounds 8, 8,9, and 10, highlighting the TLPS's potential in biological sectors. These compounds have a high concentration of productive groups such as NH2, (C(=O)OH), OH, and CH3, indicating their potential for use in various biological applications.

Two critical parameters must be addressed in this section based on the data received. The efficacy of crude extracts in inhibiting bacterial growth is influenced by various factors, including the solvent used in extracting medicinal plants and the compounds present in the plants. Three solvents were examined, each with varying effects on bacterial growth. MTPLS revealed more chemical groups in crude, suggesting more significant potential for bacteria, while DTLPS had fewer chemical groups and less antibacterial activity, indicating a different impact on bacterial growth. The second point concerns TLPS, which has been shown to have a beneficial impact on bacteria. The Polyalthia genus is known for its antibacterial properties against various types of bacteria [36, 37]. Furthermore, TLPS has shown high anti-HIV activity; nevertheless, The TLPS was isolated and evaluated for antibacterial activity against all species of bacteria investigated in the current investigation.

The current study used three solvents to extract TLPS, analyse its chemical constituents, and investigate its biomedical and biological properties. In GC-MS spectroscopy, the methanolic extract of TLPS revealed 21 components. Three of the 21 compounds were found in high percentages, compound number, 8,9, and 10. While three were found in low percentages, compounds 3, 3,15, and 16, The researchers discovered that three crude extracts had substantial antibacterial efficacy against all bacterium species, indicating they can be used as antibacterial agents. MTLPS had a more significant impact than the other extracts. These crudes are allowed for biomedical safety as the cytotoxicity testing showed no hazardous effects on MG-63 cells. Our research will serve as a valuable resource for future TLPS research.

Author Contributions

All authors contributed equally in conducting and reporting this research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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