



RESEARCH ARTICLE

Phytochemical analysis of *Polyalthia sclerophylla* twigs utilizing classical techniques and GC-MS: Evaluation of cytotoxic and antibacterial properties

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Abstract

The primary objective of the present investigation is to extract compounds from *Polyalthia sclerophylla* twigs (abbreviated as EPST) and subsequently analyze their chemical constituents using GS-MS and conventional phytochemical methods. The bio-medical properties of EPST were assessed by the evaluation of its antibacterial and cytotoxicity activities. In this study, three distinct solvents were used for the purpose of extracting EPST, resulting in the production of three distinct samples, namely Methanol-EPST (M-EPST), Dichloromethane-EPST (D-EPST) and Hexane-EPST (H-EPST). The phytochemical analysis identified glycosides and terpenoids in all examined specimens. Nevertheless, alkaloids were not present in all of them. The gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of a total of twelve organic components. Higher percentages of benzene, 1,3-bis (3-phenoxyphenoxy), furfural and phenol were detected, but lower levels were identified for azetidine and pyridine, 2-fluoro-. The EPST cytotoxicity evaluation was conducted using the Alamar blue test with MG-63 cells. Nevertheless, the findings indicate that no negative impact was observed at any dose level since the availability of cells increased. M-EPST, D-EPST and H-EPST were subjected to experimentation involving six bacterial pathogens. The results of this study demonstrated that all three compounds had substantial efficacy in inhibiting the development of these bacteria. The methanol extract (M-EPST) exhibited greater efficacy in suppressing bacterial growth than the D-EPST and H-EPST extracts. The current investigation has provided evidence that crude PST exhibits a wide range of chemical compositions, resulting in notable chemical, biomedical and biological features and a lack of toxicity.

Keywords: biological activities; EPST; extraction; MG-63 cells; plants

Introduction

Polyalthia is part of the *Annonaceae* family, including over one-hundred twenty species of tropical and subtropical flora Indigenous to South Asian countries, including Indonesia, Thailand and Malaysia (1-4).

The flora of *Polyalthia* has been previously examined and shown to possess considerable medicinal and biological efficacy, including Dengue virus type 2 (anti-DENV2), antibacterial, antiplasmodial, antioxidant, anti-inflammatory and anticancer properties, among others. Extensive studies have identified many components of the genus *Polyalthia*,

including bark, leaves, stems, segments roots, and twigs. *Polyalthia* plants have been historically used in herbal therapy to treat several diseases, including gastrointestinal disorders, metabolic disorders and infectious diseases (5-11). *Polyalthia* species include phytochemicals such as alkaloids, sugars, terpenoids, tannins, saponins, phenolic compounds and flavonoids. Significant functional groups of bioactive compounds in plants provide unique biological effects that enhance their effectiveness. The present study assesses a particular species by analyzing its chemical makeup and biological activity (1, 12, 13).

P. sclerophylla twigs (PST) leaves were chosen for a multitude of causes. There is a lack of known research pertaining to the chemical structures, antibacterial activity and cytotoxicity of the subject in question. However, it is possible to get phytochemicals with antimicrobial properties from leaves that may be either similar or distinct. Furthermore, PST is readily available within the local community and is considered to be cost-effective. Furthermore, because of the limited availability of information pertaining to PST, the present study aims to investigate the development of a comprehensive database to support researchers in this field.

The present work aimed to examine the biological properties of extract of *P. sclerophylla* twigs (EPST) obtained by the utilization of methanol, hexane and dichloromethane solvents. Specifically, the antibacterial efficacy of these extracts was evaluated against a panel of six bacterial strains. An assessment of the toxicity was conducted using MG-63 cells. Methanol extraction was discovered to include sugars, steroids, tannins, terpenoids and glycosides, indicating its potential impact on bacterial activity.

Materials and Methods

Preparation process of PST

The leaves of PST were gathered and subjected to a cleansing process using distilled water in order to remove any fungal contaminants or particulate matter in Perak, Malaysia. The PST sample was subjected to a seven-day sun-drying process, after which it was cut into tiny fragments and pulverized to get a powdered form intended for further use.

Extraction of PST

The Soxhlet method was employed to extract potential groups of the chemical compounds from PST utilizing three distinct solvent systems, namely methanol (MeOH) dichloromethane (DCM) and hexane. A total of 50 g of PST were extracted to create three unique formulations: M-EPST, D-EPST and H-EPST. After that, the formulations were kept at a temperature 4 °C for future research purposes (5).

Phytochemical analysis of PST

The chemical groups of M-EPST, D-EPST and H-EPST were determined by conventional chemical processes, resulting in their successful treatment.

Test of alkaloids

Alkaloid identification was performed using preliminary and confirmatory tests. The initial tests are undertaken before confirming the test. Mix 10 mL of M-EPST, D-EPST and H-EPST to perform a preliminary hydrochloric acid (HCl) analysis after filtering the resultant liquid. The purified liquid has been treated with Dragendorff's and Mayer's reagents. In the next experiment, a combination of 1 g each of M-EPST, D-EPST and H-EPST was mixed with a 40 % solution of calcium hydroxide till alkalinity indicated on the litmus paper. Subsequently, 2 extractions were conducted using CHCl_3 . Thin layer plates were identified in the chloroform extract. The chromatogram was generated with a solvent solution consisting of ethyl acetate and n-hexane in a 1:4 ratio. Subsequently, the chromatographic solution was treated with Dragendorff reagent. Identifying alkaloids in the backdrop may be accomplished by observing the chromatic shifts to

yellow and orange hues (2, 14).

Flavonoid assessment

Various techniques have been developed for the identification of flavonoids in medicinal plants. This study included three approaches to evaluate the concentration of flavonoids in PST.

Identification of flavonoids in PST

M-EPST, D-EPST and H-EPST were prepared by adding 5 mL of $\text{C}_4\text{H}_8\text{O}_2$ (Butyric acid). The resulting mixture was subjected to heating in a steam bath for 3 min. Subsequently, the mixture was filtered using filter paper of superior quality. Following the addition of one ml of diluted NH_4OH , the solution was subjected to agitation. The identification of flavonoids accomplished by the observation of their yellow coloration (16).

The hotplate was used to heat 10 mL of M-EPST, D-EPST and H-EPST. After that, the combination was treated with an appropriately diluted NaOH solution. The formation of a yellow precipitate indicates the presence of flavonoids (23).

Test of carbohydrates of PST

The identification of carbohydrates in PST was achieved via Fehling and Molisch reagents. The presence of reducing sugar in PST was assessed by dissolving M-EPST, D-EPST and H-EPST in distilled water, followed by adding Fehling's solution and observing the resultant colour shift to brick red.

Detection of phenolic compounds in PST

The formulation was achieved by amalgamating 1 g of M-EPST, D-EPST and H-EPST, dissolving them in 100 mL of distilled water, then adding Iron (III) sulfate in drops. A phenolic group was identified as dark violet (15).

Detection of terpenoids in PST

At the outset, a volume of 2 mL of CHCl_3 was combined with 10 mL each M-EPST, D-EPST and H-EPST, after which sulfuric acid H_2SO_4 (sulfuric acid) was introduced. Terpenoids are seen to be present in the combination when a discernible change in coloration occurs, resulting in a reddish-brown hue (17).

Test of saponins of PST

A test tube had been filled with three different solvents: M-EPST, D-EPST and H-EPST. After that, 10 mL of distilled water was introduced into the test tube and the mixture was vigorously agitated for 1 min. Saponins were seen on the surface of the honeycomb by inspecting the tube, which had been positioned at an inclined angle for over 30 min (18, 20).

Glycosides detect of PST

The Keller-Killani test was used to identify glycosides in PST. This included dissolving 1 gram each of M-EPST, D-EPST and H-EPST in distilled water, then adding H_2SO_4 and ferric chloride. The resulting mixture was then observed for the appearance of reddish and reddish-brown layers (19).

Tannins evaluation of PST

Prior to the implementation of any filtration process, a conical flask containing a mixture of M-EPST, D-EPST and H-EPST weighing 1 g was subjected to heating in the presence of 50 mL of distilled water for 20 min. The specimens were meticulously immersed in a solution containing 0.1 % FeCl_3 . The presence of tannins in the samples was assessed by observing the colors black-blue and green brownish (21).

Evaluation of steroids of PST

The experiment included combining acetic anhydride with 10 mL of M-EPST, D-EPST and H-EPST, respectively, and then chilling the mixture in an ice bath. A small quantity of H_2SO_4 has been included in the combinations. The identification of steroid combinations may be achieved by noting a colour shift from violet to blue (7).

Evaluation of lead acetate of PST

The M-EPST, D-EPST and H-EPST (10 mL) were heating on a hotplate. Subsequently, 1 mL of 10 % lead acetate solution was added to the mixture. The emergence of a yellow precipitate indicates the existence of flavonoids (22).

GC-MS spectroscopy of PST

The identification of volatile chemicals in the PST extracts was conducted via the use of a gas chromatography-mass spectroscopy (GC-MS) analyser.

Cytotoxic evaluation of PST

The Alamar Blue test was used to evaluate the cytotoxic effects of PST on the MG-63 human cell line. The PST powder was incubated in a complete medium at a concentration of about 250 mg/mL for 24 hr. Subsequently, extracts were generated from the incubated mixture for the purpose of conducting a cell viability test. The extracts were then sterilized employing a 0.2 m needle.

The concentrated extracts were amalgamated with a medium to attain weight-to-volume ratios of 250, 200, 150, 100 and 50 mg/mL. The extracts were administered to the healthy MG-63 cell monolayer. The cells were cultured in a carbon dioxide incubator for 24 hr at 37 °C. The Alamar Blue test evaluated cell viability by staining after a four-hour incubation period.

Evaluation of antibacterial properties of PST

The efficacy of M-EPST, D-EPST and H-EPST samples were assessed against the sixth type of bacteria. The bacterial population includes six species: three that are gram-negative and three that are gram-positive. Brought together *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia pestis* and three gram-positive bacteria: *Streptococcus pyogenes*, *S. pneumoniae* and *Staphylococcus aureus*.

Preparation process of the agar

Forty grams of nutritional broth agar were dissolved in 2000 mL of distilled water and sterilised by autoclaving at 121 °C for 25 min. Subsequently, the temperature was lowered to around 60 °C and a volume of 26 mL of refrigerated medium was carefully dispensed onto the plate. The plate was subsequently allowed to solidify before being stored under light-restricted circumstances at a temperature of 4 °C for further experimental use.

The assessment of PST as an antibacterial agent was performed with a diffusion method, utilising M-EPST, D-EPST and H-EPST. In order to assess the antibacterial efficacy, the researchers investigated the bacterial inhibition zone. A volume of 10 mL of bacterial culture was used for the purpose of observing the culture of two distinct strains of bacteria. The M-EPST, D-EPST and H-EPST bacterial strains were subjected to a 24 hr incubation period at a temperature of 37 °C. This incubation was carried out by dispersing a 100 mL bacterial culture onto nutrient agar plates. Subsequently, the calculation of the inhibitory zone was performed.

Results

The PST were obtained by the extraction process using three different solvents, resulting in the production M-EPST, D-EPST and H-EPST. Subsequently, the cured PST samples were subjected to chemical analysis in order to evaluate their molecular compositions. This analysis included the use of phytochemical screening techniques, including GC-MS, as well as standard tests like qualitative chemical tests for alkaloids, tannins, etc.

Phytochemical analysis of M-EPST, D-EPST and H-EPST

Fig. 1 displays the phytochemical examination of M-EPST, indicating the presence of steroids, tannins, terpenoids, phenolic compounds, carbohydrates, glycosides and flavonoids. No alkaloids or saponins were identified.

Fig. 2 presents the results of the phytochemical screening of D-EPST, revealing the presence of glycosides, flavonoids and terpenoids. However, no detection was made for carbohydrates, alkaloids, tannins, saponins and phenolic compounds.

Hexane was used to extract PST in order to identify potential non-polar chemicals inside it. The phytochemical findings of H-EPST are shown in Fig. 3. In the present investigation, it was found that four out of the nine chemical groups examined were discovered, namely glycosides, terpenoids, tannins and saponins.

GC-MS of PST

MeOH was used as the solvent for the extraction of PST. Subsequent photochemical screening showed that M-EPST exhibited a higher number of chemical groups compared to both H-EPST and D-EPST. The identification of chemical compounds was performed using GC-MS of M-EPST. The results presented in this section revealed the presence of 12 chemicals compounds in the PST. Table 1 reveals that benzene, 1,3-bis(3-phenoxyphenoxy) (1P), furfural (6P) and phenol (8P) were seen to occur in more significant proportions, whereas azetidine 2P (2P) and pyridine, 2-fluoro-(7P) were detected in lesser proportions.

Cytotoxicity study of PST

In this part, a study on the cytotoxicity of M-EPST was carried out with the aim of assessing the potential hazards associated with this plant. The toxicity of medical substances is a crucial aspect to evaluate since it directly affects the suitability of the finished product for therapeutic applications. The M-63 cells subjected to varying doses of M-EPST, including five distinct levels. According to the data shown in Fig. 3, it can be seen that all concentrations of M-EPST did not exhibit any detrimental effects. Prior studies conducted in the genus of *Polylathia* have shown that its various species have a benign influence (1). As shown in Fig. 7, the outcomes of the present investigation indicate that PST did not have any detrimental effects.

Antibacterial activities of PST

Table 3 and Fig. 6 shows the antibacterial activity of PST was assessed against a total of six bacterial species, including both gram-negative and gram-positive strains. The efficacy of M-EPST, D-EPST and H-EPST against gram-negative bacteria was shown. Significant inhibition of bacterial proliferation was seen at distances of 8, 9 and 12 mm (a), 6, 7 and 8 mm (b) and 7, 8 and 10

No	The test name	The Observation	Results
1	Alkaloids	The yellow and orange colours didn't observe	Negative
a	Flavonoids	Yellow precipitate has been Formatted	Positive
3	Carbohydrates	Brick-red colour was detected to be presence	Positive
4	Phenolic compounds	Colour as dark violet has been observed	Positive
5	Terpenoids	Ferric sulfate test Colour as dark violet has been observed	Positive
6	Saponins	The honey comb didn't observe on the surface of the solution	Negative
7	Glycosides	Two layers as a reddish-brown in the colour were observed	Positive
8	Tannins	Two colours were observed Le browntsh-green and blue-black	Positive
9	Steroids	The colour of the solution was changed to reddish-brown	Positive
9	Steroids	The colour of the solution was changed to be reddish-brown	Positive

Fig. 1. The evaluation of phytochemical screening of M-EPST.

The Test Name	The Observation	Results
Alkaloids a- Dragendorff's reagent a. Mayer's reagent b. Mayer's reagent	The yellow or orange colours didn't observe	Negative
	Yellow precipitate has effected to be precence	Positive
Carbohydrates a. Fehling reagent b. Molish test	Brick-red colour wasn't detected to be	Negative
	Brick-regciour warsn't detected to be presence	Positive
Phenolic compounds a. Ferric sulfate test	Colour as dark violet wasn't observed	Negative
	Colour of the solution was changed to be elnin	Positive
Terpenoids a. Lieberman's test	The honey-comb didn't observe on the surface of the solution	Negative
Saponins a Test of Froth	Two layers as a reddish-brown in the colour were observed	Positive
Glycosides a test of Killer-Killani	Two colours weren't observed i.e brownish-green and blue-black	Negative
Tannins a Test of Lieberman	The colour of the solu-tion was changed to reddish-brown	Positive

Fig. 2. The evaluation of phytochemical screening of D-EPST.

Table 1. The chemical composition of PST

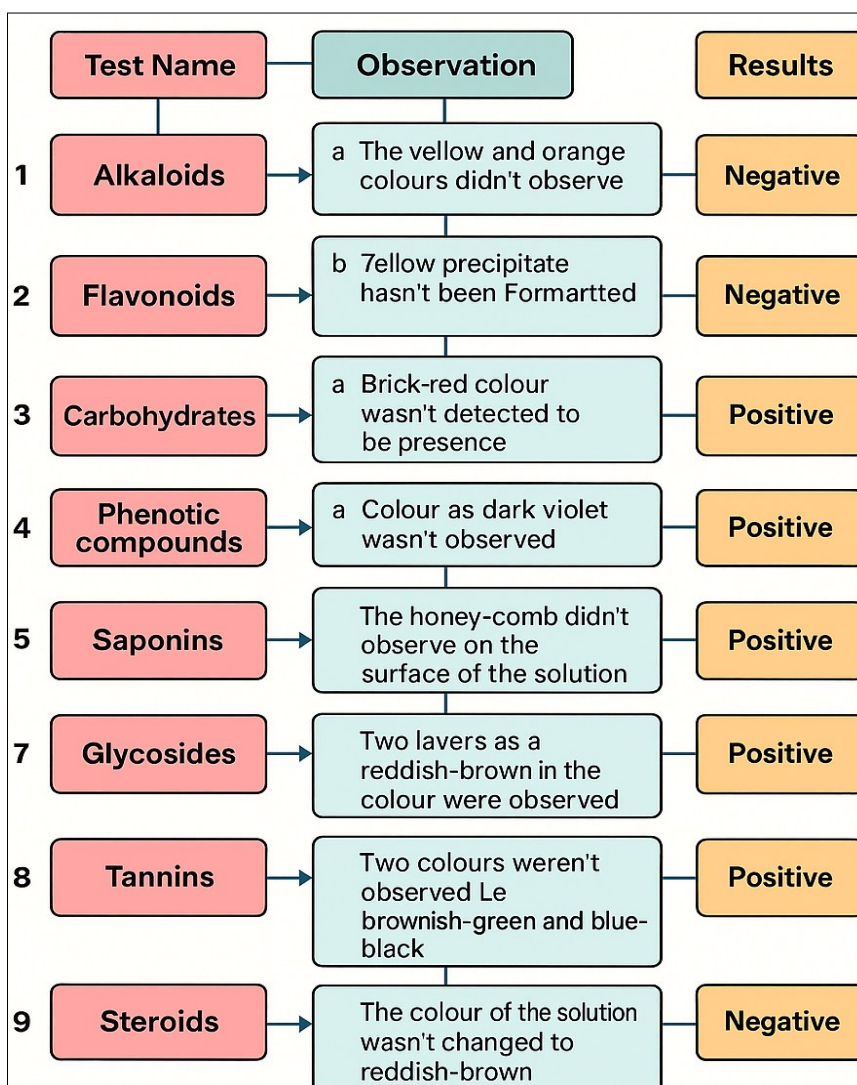
No	R. Time	Compound name	Composition %	Label
1	2.244	Benzene, 1,3-bis(3-phenoxyphenoxy)	16.23	1P
2	2.810	Azetidine	0.43	2P
3	2.817	1-Hydroxy-2-butanone	1.92	3P
4	2.817	Propane, 1-methoxy-2-methyl-	1.92	4P
5	2.818	(S)-(+)-3-Hydroxytetrahydrofuran	1.75	5P
6	3.658	Furfural	17.04	6P
7	4.098	Pyridine, 2-fluoro-	0.01	7P
8	7.703	Phenol	35.80	8P
9	10.178	Phenol, 2-methyl	1.03	9P
10	11.097	Phenol, 2-methoxy	5.22	10P
11	11.097	Mequinol	5.22	11P
12	22.126	Phenol, 2,6-dimethoxy	2.21	12P

mm (c), correspondingly. The research findings revealed that the crude extracts of M-EPST, D-EPST and H-EPST exhibited effective inhibition of gram-positive bacteria, namely *S. pneumonia*, *S. pyogenes* and *S. aureus*. The growth rates observed were 10, 9 and 14 mm (a), 8, 8 and 10 mm (b) and 9, 7 and 12 mm (c) for the corresponding bacteria mentioned. The kind of solvent used has an impact on the inhibition of bacterial growth, with metabolic extract exhibiting a greater efficacy against both bacterial strains. The methanol extract had a comparatively lesser impact in comparison to the dichloromethane extract.

Discussion

Phytochemical screening

In prior research, methanol has been used as a solvent for executing phytochemical screening in *Stevia rebaudiana* leaves (24). Their analysis identified phenolic chemicals, saponins, tannins and steroids, whereas alkaloids were absent. A previous study discovered carbohydrates, glycosides, flavonoids, saponins and tannins in the leaves of *Caesalpinia sappan*, however alkaloids were not found (25). The results correspond with our current research, clarifying the specific chemical groups in plant leaves responsible for their reported effects. The authorisation of these compounds has resulted in the execution of M-EPST measures.

**Fig. 3.** The evaluation of phytochemical screening H-EPST.

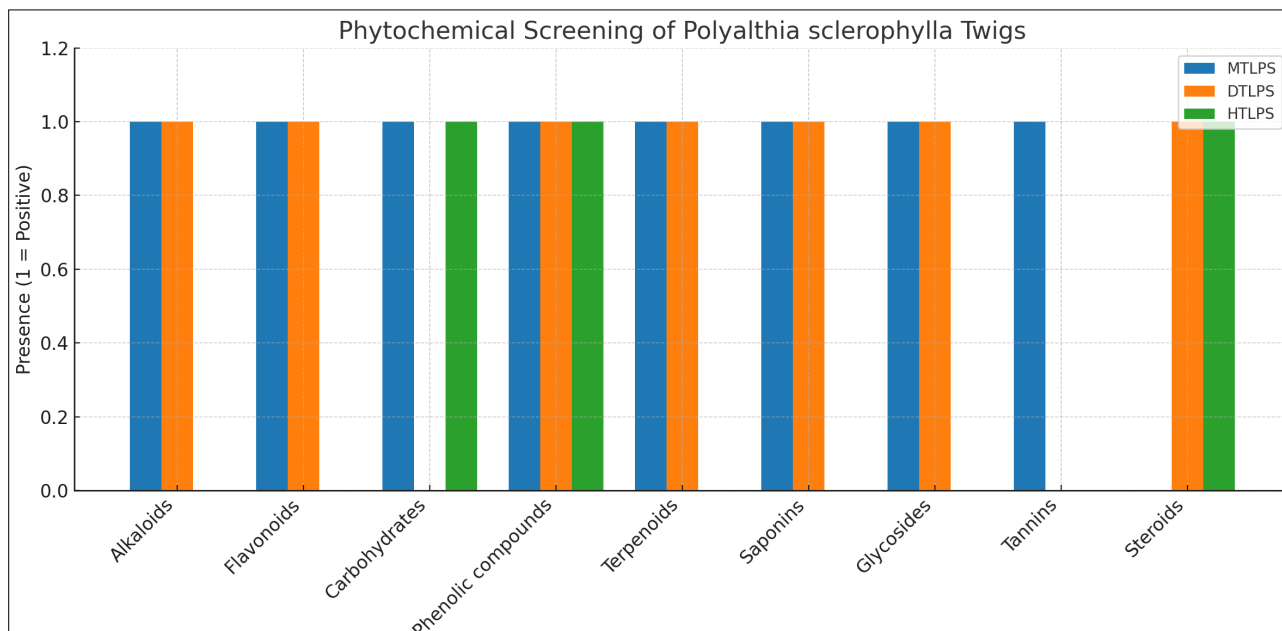


Fig. 4. The investigation of phytochemical screening of M-EPST, D-EPST and H-EPST.

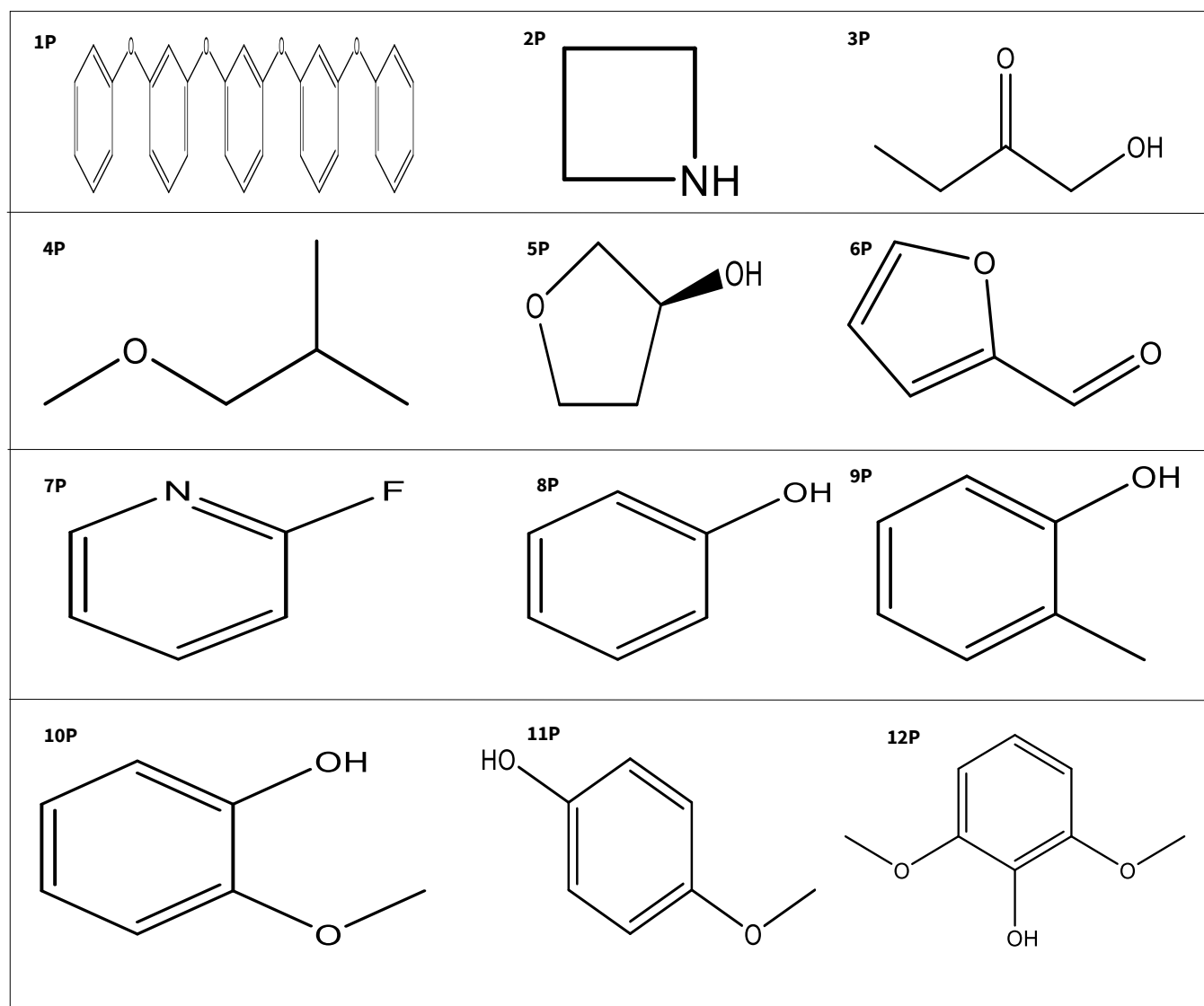
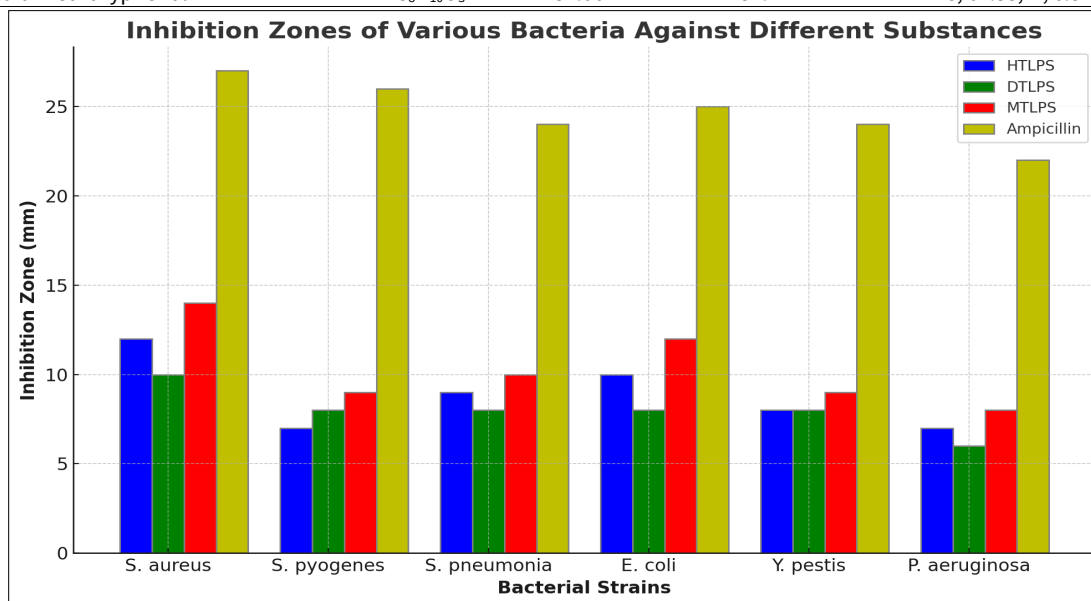


Fig. 5. The compounds obtained from GC-MS.

Table 2. The physicochemical properties of the obtained compounds

No.	IUPAC	Chemical Formula	Exact Mass	Molecular Weight	Elemental Analysis
1P	1,3-bis(3-phenoxyphenoxy)benzene	C ₃₀ H ₂₂ O ₄	446.15	446.5	C, 80.70; H, 4.97; O, 14.33
2P	Azetidine	C ₃ H ₇ N	7.06	57.10	C, 63.11; H, 12.36; N, 24.53
3P	1-hydroxybutan-2-one	C ₄ H ₈ O ₂	88.05	88.11	C, 54.53; H, 9.15; O, 36.32
4P	1-methoxy-2-methylpropane	C ₅ H ₁₂ O	88.09	88.15	C, 68.13; H, 13.72; O, 18.15
5P	(S)-tetrahydrofuran-3-ol	C ₄ H ₈ O ₂	88.05	88.11	C, 54.53; H, 9.15; O, 36.32
6P	Furan-2-carbaldehyde	C ₅ H ₄ O ₂	96.02	96.09	C, 62.50; H, 4.20; O, 33.30
7P	2-fluoropyridine	C ₅ H ₄ FN	97.03	97.09	C, 61.85; H, 4.15; F, 19.57; N, 14.43
8P	Phenol	C ₆ H ₆ O	94.04	94.11	C, 76.57; H, 6.43; O, 17.00
9P	o-cresol	C ₇ H ₈ O	94.04	108.14	C, 77.75; H, 7.46; O, 14.79
10P	2-methoxyphenol	C ₇ H ₈ O ₂	124.05	124.14	C, 67.73; H, 6.50; O, 25.78
11P	4-methoxyphenol	C ₇ H ₈ O ₂	124.05	124.14	C, 67.73; H, 6.50; O, 25.78
12P	2,6-dimethoxyphenol	C ₈ H ₁₀ O ₃	154.06	154.17	C, 62.33; H, 6.54; O, 31.13

**Fig. 6.** The evaluation of the antibacterial activity of M-EPST, D-EPST and H-EPST against bacterial species.

DCM was once used as a solvent in many investigations to extract medicinal plant leaves and assess their chemical contents. A multitude of chemical substances has been recognised as present. The *Adenanthera pavonina* L. (DEAP) DCM extract was identified to include tannins, alkaloids, flavonoids, steroids and terpenoids. The extract of *Euodia redleyi* (DERE) was determined to contain terpenoids, flavonoids, tannins, alkaloids, and steroids. Phenolic chemicals and tannins were absent in DERE, whereas saponins and carbohydrates were absent in DEAP (26, 27). Both trials had almost identical results.

Previous investigations on the extraction of medicinal plants using hexane have shown the existence of several compounds, including saponins, tannins, steroids and flavonoids (28, 29). Our study outcomes corresponded with their findings.

Currently, three solvents, including MeOH, DCM, and hexane are used for the extraction of TLPS. Based on the data

analysis, it can be seen that there was an absence of alkaloids in all of the extracts examined. However, terpenoids and glycosides were found to be present in the D-EPST and H-EPST extracts. Fig. 4 illustrates the lack of flavonoids and steroids in H-EPST, whereas their presence is seen in M-EPST and D-EPST. Hexane is classified as a non-polar solvent. Chemical groups need the use of polar solvents for dissolution (30).

GC-MS of PST

GC-MS analysis was used in the investigation to assess the chemical compositions of a total of twenty-one chemicals, as seen in Fig. 5 and Tables 1 & 2, compounds exhibited a higher percentage of functional groups, namely compounds 1P, 6P and 8P, hence emphasizing the promise of the PST in biological applications. The compounds above exhibit a notable abundance of functional groups, including CH₃, OH and (C=O) OH) which suggests their promising suitability for diverse biological applications.

Table 3. The evaluation of antibacterial activity of PST

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

*The mark - represent to zero inhibition zone

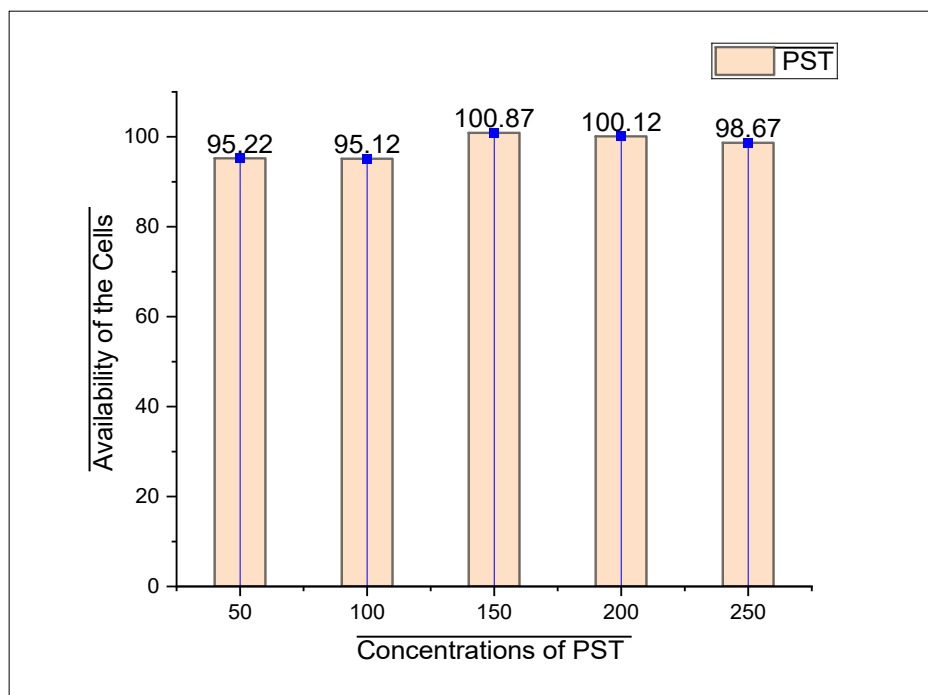


Fig. 7. The cytotoxicity evaluation of PST.

Cytotoxicity study of PST

In this part, a study on the cytotoxicity of M-EPST was undertaken to ascertain its potentially harmful nature. The evaluation of the toxicological effects of medicinal substances is a crucial aspect in determining their suitability for therapeutic application. This is due to the potential impact these substances may have on the end product's therapeutic efficacy. The M-63 was subjected to 5 various quantities of M-EPST. According to the data shown in Fig. 7, it can be seen that all concentrations of M-EPST did not exhibit any detrimental effect. Prior studies conducted on the genus of *Polyalthia* have shown that its various species have a benign influence (1). The outcomes of the present investigation indicate that the use of PST did not have any detrimental effects.

Antibacterial activities of PST

This part necessitates the examination of two crucial parameters in light of the data obtained. The effectiveness of crude extracts in the inhibition of bacterial growth is subject to several variables, such as the choice of solvent used for the extraction of medicinal plants and the specific chemicals found within these plants. The study investigated the impact of three different solvents on bacterial growth, observing the various impacts of each solvent. The results of the M-EPST analysis demonstrated a more significant number of chemical groups present in the crude sample, therefore implying a higher degree of potential for bacterial activity. Conversely, the D-EPST analysis indicated a reduced quantity of chemical groups and a diminished antibacterial effect, so suggesting a distinct influence on bacterial development. The second aspect is PST, which has been shown to have a favourable influence on bacterial populations. The antibacterial characteristics of the *Polyalthia* genus have been shown in previous studies, demonstrating efficacy against a range of bacterial strains (31, 32). In addition, it has been shown that PST has significant anti-HIV properties. However, in the present study, the PST compound was isolated and assessed for its antibacterial efficacy against all bacterial species examined.

Conclusion

In this particular investigation, three distinct solvents were used to extract PST, therefore facilitating the analysis of its chemical ingredients and the exploration of its biomedical and biological features. In GC-MS, the metabolic extract of PST revealed 12 components. Three out of the total of 12 compounds were seen to occur in significant proportions; namely compounds numbered 1P, 6P and 8P. 1P, 6P and 8P were detected in petite proportions, the researchers observed these 3 extracts exhibited significant antibacterial activity against all bacterial species. This finding suggests that these extracts have the potential to be used as antibacterial agents. The influence of M-EPST was shown to be more substantial compared to the other extracts. The biological safety of these crudes has been established since cytotoxicity tests revealed no adverse effects on MG-63 cells. The study conducted will provide a significant contribution to the field of PST research, serving as a valuable resource for future studies in this area. This locating helps the capability of using PSTs in biomedical applications, especially in tissue engineering, wound recovery or as additives in biocompatible scaffolds. Moreover, this study provides a valuable foundation for future research exploring the therapeutic properties, molecular mechanisms and capability modifications of PSTs to enhance their bioactivity and specificity.

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Authors' contributions

MM designed the study and performed the experiments, while HAA and IZ prepared the manuscript and helped in data analysis. Finally, RMH, MZ and QRL interpreted the data, wrote the paper, supervised the experiments, corrected the manuscript and gave significant suggestions to upgrade the assessment.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Ethical issues: None

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