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Phytochemical Analysis and Biological Activity of the Leaf Extracts of *Polyalthia longifolia* (Masquerade Tree)

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Abstract: The serious global health concern of antimicrobial resistance has prompted the search for new antimicrobials. Plants are considered a rich source of potent anti-infective agents. This study aimed to assess the phytochemical constituents of the Nigerian *Polyalthia longifolia and* its antimicrobial potency, based on its ethno-medicinal use. The crude extract was obtained by extracting the powdered air-dried leaf of *Polyalthia longifolia* with methanol. Standard chemical procedures were used to screen the extract for phytochemicals. Solvent-solvent extraction was used to separate the crude extract into hexane, neutral, acid, and base fractions. The crude extract and fractions were tested for activity against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Candida albicans,* and *Aspergillus niger.* FT-IR and GC-MS analyses were employed to identify functional groups and specific bioactive compounds in the crude extract. Phytochemical analysis revealed that terpenoids, alkaloids, saponins, tannins, flavonoids, steroids, and volatile oils were present. The neutral and base fractions exhibited notable antibacterial efficacy, particularly against *Salmonella typhi and Staphylococcus aureus*, with inhibition zones comparable to standard antibiotics. The fungi *Aspergillus niger* and *Candida albicans* were not inhibited by the crude extract or its fractions. Major compounds such as diisooctyl phthalate, oleic acid, palmitic acid, farnesol formate, n-hexadecanoic acid, octadecanoic acid, and methyl kolavenate were identified by comparing the crude extract's GC-MS analysis with reference library computer mass spectrometry data. These compounds may be responsible for the observed bioactivity. The findings support the traditional use of *P. longifolia* which is a potential source of antibacterial agents for novel drugs.

Keywords: *Polyalthia longifolia , Leaf Extracts, Phytochemicals, Antimicrobial Activity.*

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I. INTRODUCTION

Antimicrobial resistance (AMR) caused by antibiotic misuse and overuse [1] poses a threat to human life, marked by microorganisms developing resistance to drugs designed to combat them [2]. The abuse of antibiotics makes resistance genes more likely to proliferate, which is gradually increasing and may emerge as the top cause of mortality by 2050, and treating infections can be difficult due to resistant microorganisms [1].

As drug-resistant pathogens and diseases continue to emerge, there is a need to investigate medicinal plants continuously for possible antimicrobial agents. Medicinal plants contain bioactive compounds that fight against drug resistance and improve the potency of existing treatments [3]. *Polyalthia longifolia,* also known as "Green Champa" or "Masquerade Tree" is a tall evergreen tree that is native to India and Sri Lanka. It has been used in traditional medicine to treat helminthiasis, diabetes, fever, skin conditions, and hypertension [4]. It has been discovered that the leaves and the roots contain phytosterols and monounsaturated fatty acids, which helps in lowering cholesterol [4]. The plant has traditionally been used to increase breathing, reduce blood pressure, stimulate and treat ailments such as uterine disorders, gonorrhea, leucorrhea, and menstrual issues [5]. According to earlier research, the most prevalent phytochemicals in *Polyalthia* plants include terpenes and alkaloids [6]. *Polyalthia* species also contains sterols,

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flavonoids, organic acidssteroids, phytosterols, resins, phenols, flavonoids, tannins, steroidal and cardiac glycosides, and saponins [6,7,8,9]. Methanolic extract of the leaf of *Polyalthia longifolia* exhibited bactericidal activity against *P. aeruginosa, E. coli, S. pneumoniae, S. aureus* and *C. difficile* and fungucidal activity against *A. niger, C. albicans, A. clavatus, , A. fumigatus, C. auris and C. tropicalis.* [10]

The study aims to investigate the phytochemical and antimicrobial properties of the extracts of the leaf of the Nigerian *Polyalthia longifolia* used in ethnomedicine.

II. MATERIALS AND METHODS

➢ *Plant Material Collection and Identification*

Polyalthia longifolia leaves were harvested in March 2025 from Veritas University, Abuja. The leaves were authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu and a specimen was deposited with voucher number NIPRD/H/7474. The leaves were weighed and washed with running distilled water. They were dried on a lab bench for two weeks at room temperature and without sunlight. Then, using an electric blender, the dried plant material was blended into powder.

➢ *Extraction of Dried Powdered Plant*

In a glass container, the dried powdered leaf of *Polyalthia longifolia* was cold macerated for 48 hours with intermittent shaking using 42.0 L of methanol. The extract was then filtered with a filter paper and glass funnel. A rotary evaporator set at 40 °C was used to evaporate the filtrate to dryness. A solid residue with dark green colouration was obtained. The crude extracted was spotted on a thin-layer chromatography (TLC) plate (an aluminum sheet pre-coated with silica gel) and using 1:5 ethyl acetate/ hexane as mobile phase, the best separation of components was observed, showing 5 spots.

➢ *Fractionation*

Aqueous methanol (50% H20 + 50% MeOH) was adde d into a 250 mL pre-weighed beaker containing the crude methanolic extract. After a few minutes of standing, the mixture was then extracted with hexane (2x 200 cm^3) using a separatory funnel to obtain the hexane fraction. 70 mL of $CHCl_2$ was added to the Aq. MeOH layer, and then extracted with 1 M HCl (2x100 cm^3) to obtain $CHCl_2$ layer and Aq. Acid-soluble layer. The $CHCl_2$ layer was also extracted with 1 M $NaHCO_3$ (2x100cm^3) to obtain the $CHCl_2$ layer fraction (neutrals) and the Aq. Layer, which was acidified with 1 M HCl, and then extracted with chloroform (2x 200 cm^3) to obtain the acid fraction. The Aq. Acid soluble layer was basified with $NH_{3(aq)}$ and then extracted with chloroform (2x250 cm^3) to obtain the base fraction (Figure 1). Each fraction was weighed after evaporating to dryness. TLC of the Aq. MeOH layer was done using an aluminum sheet pre-coated with silica gel and 1:5 ethyl acetate/hexane solvent system gave the best separation and showed 6 number of spots.

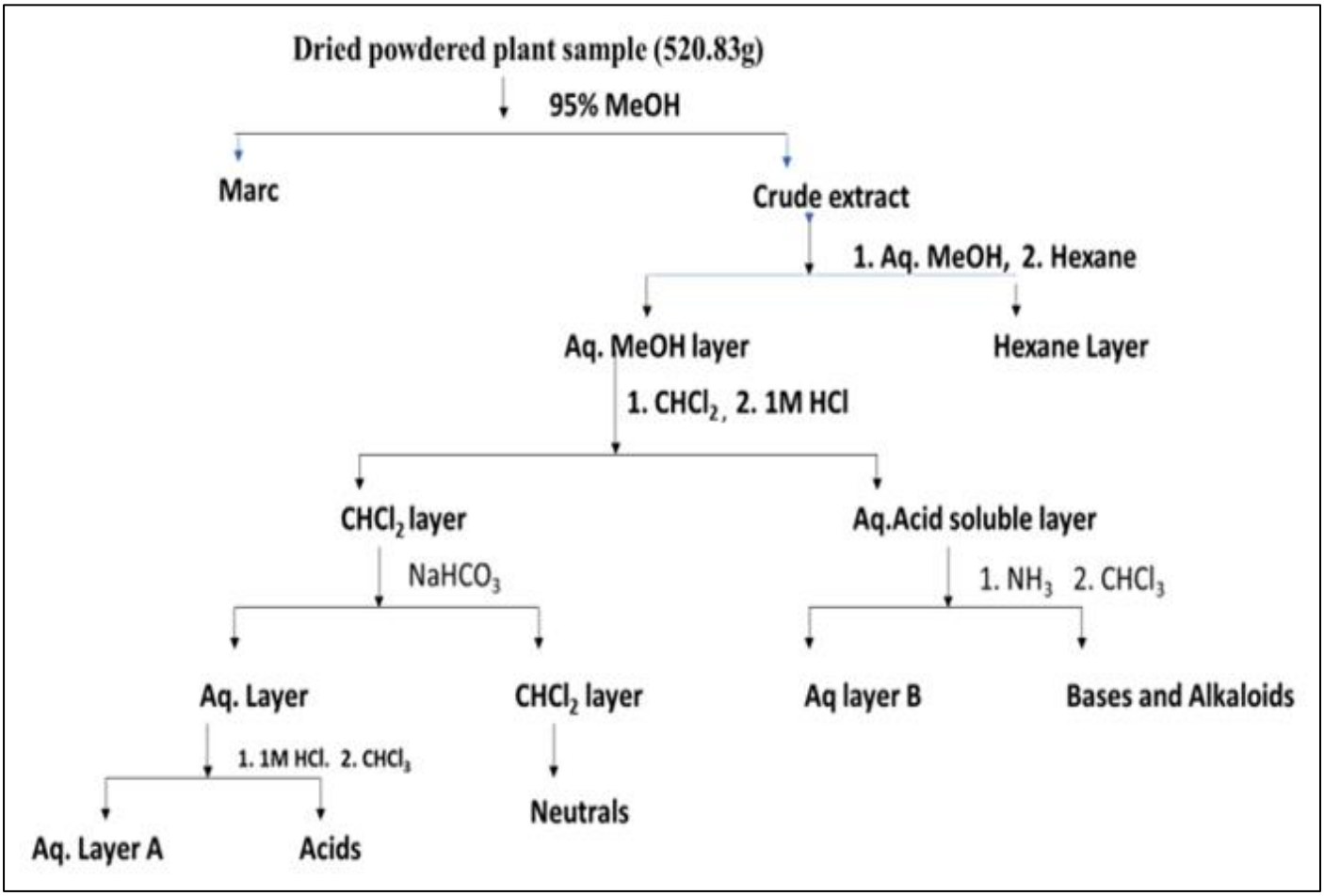

Fig 1 Scheme for Fractionation of the Crude Methanolic Extract of *Polyalthia longifolia*

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➢ *Preliminary Phytochemical Screening*

A preliminary qualitative phytochemical screening was performed on *Polyalthia longifolia's* crude methanolic extract. Using standard procedure [11], the crude extract was tested to identify eight secondary metabolites, including tannins, alkaloids, carbohydrates, saponins, sterols/steroids, flavonoids, volatile oils, and terpenoids.

➢ *Antimicrobial Screening*

The antimicrobial screenings, including sensitivity tests, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) were performed using a standard procedure[11].

➢ *Sensitivity Test for Determining Inhibitory Activity*

The sensitivity test was done by the agar well diffusion method . The standardised inocula of the bacterial and fungal isolates were streaked onto sterilised Mueller Hinton and Potatoe dextrose agar plates, respectively, with a sterile swab stick. Using a sterile cork borer, four wells were punched into each inoculated agar plate. The wells were properly labelled based on the concentrations of the extract prepared (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively). The extract (approximately 0.2 mL) was added to each well.

For about an hour, the inoculated plates with the extract were placed on the bench to allow the extract to permeate the agar.

Incubation of the Muller Hinton agar plates was done for 24 hours at 37°C. At room temperature, the potato dextrose agar plates were incubated for about 3 to 5 days.

After that, the incubated plates were checked for any signs of inhibition, which showed up as a clear zone of inhibition encircling the wells.

A clear ruler that was calibrated in millimetres was use d to measure these inhibitory zones' diameter.

➢ *Minimum Inhibitory Concentration (MIC) Determination*

Mueller Hinton Broth was employed as a diluent in the tube dilution method to determine the extracts' minimum inhibitory concentration.

In a test tube filled with Mueller Hinton broth, the extract was serially diluted to the lowest dose that inhibited each organism during the sensitivity test. In each tube containing the broth and extract, the standardised organisms were added. For twenty-four hours, the inoculated tubes were incubated at 37 °C. Using turbidity as a criteria, the tubes were inspected for growth at the conclusion of the incubation time. The minimum inhibitory concentration (MIC) was determined by taking the least concentration in the series that showed no growth or apparent sign.

➢ *Minimum Bacteriocidal Concentration (MBC) Determination*

The findings from the MIC test were utilized to ascertain the MBC of the extract The test tubes that did not exhibit any turbidity (clear) in the MIC test were filled with a sterilised wire loop, and a loopful was streaked over sterile nutrient agar plates. For 18 to 24 hours, the plates were incubated at 37 °C. The plates were examined for the presence or absence of growth following the incubation time. This evaluation aimed to determine if the extract's antibacterial activity was bacteriostatic or bacteriocidal

➢ *FT-IR and GC-MS Analyses of Crude Methanolic Extract of P. longifolia*

FT-IR and GC-MS analyses were carried out on the crude methanolic extract of *Polyalthia longifolia* using a Nicolet iS10 FTIR Spectrophotometer equipped with Win-IR Pro Version software and a Perkin Elmer Turbo mass spectrometer (Norwalk, CTO6859, USA), respectively.

III. RESULTS AND DISCUSSION

➢ *Extraction and Fractionation*

A dark green coloration was obtained from crude extract with the highest yield (26.47 g). The result of cold methanol maceration from 520.83 g of dried leaves, produced a yield of about 5.08% relative to the plant material. Fractionation of the crude methanolic extract yielded hexane-soluble, acid, base, and neutral fractions in varying small amounts. The base fraction had the highest yield among the fractions (0.539 g, 2.036% of the crude), suggesting a higher presence of basic compounds, such as alkaloids. The acid and hexane fractions had nearly equal yields (~1%), while the neutral fraction yielded the least (0.233 g, 0.880%) (Table 1).

Most fractions were gummous in nature, though their color and shine differed: The hexane and neutral fractions resembled the crude extract in color (dark green). The acid and base fractions were yellowish with a shiny texture, indicating likely differences in compound composition and polarity. The fractionation procedure was successful in separating components based on polarity. The relatively high yield of the base fraction correlates with the phytochemical results showing alkaloid presence and with antimicrobial results where the base fraction showed strong activity. These differences in yield and appearance hint at varying concentrations and types of phytochemicals, which were later validated by antimicrobial screening and chemical analyses (GC-MS).

Table 1 Physical Features and Yields of Extracs from *Polyalthia longifolia* Leaves

Extractives	Yield (g) and % Relative to Crude	Colour and Consistency
Crude	26.47 (5.082)	Dark green gum
Hexane-soluble fraction	0.262 (0.989)	Dark green gum
Acid fraction	0.269 (1.016)	Light yellow shiny gum
Base fraction	0.539 (2.036)	Yellow shiny gum
Neutrals	0.233 (0.880)	Dark green gum

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➢ *Phytochemical Analysis*

The phytochemical analysis of the leaf showed that tannins, alkaloids, saponins, volatile oils, terpenoids, flavonoids, and steroids were present (Table 2). These phytochemicals are known for their broad spectrum of biological activities. Tannins possess antimicrobial and antioxidant properties by forming complexes with microbial proteins and enzymes [12]. Alkaloids exhibit antimicrobial action by disrupting DNA replication and microbial metabolism [13]. Saponins damage microbial membranes by interacting with sterols, causing increased permeability [14]. Volatile oils, or essential oils, exert antimicrobial effects by disrupting cell membranes and inhibiting microbial enzymes [15]. Terpenoids inhibit microbial growth by affecting membrane integrity and metabolic processes [16]. Flavonoids contribute to antimicrobial and antioxidant activities by interfering with nucleic acid synthesis and cell membrane function [17]. Furthermore, steroids have been demonstrated to possess anti-inflammatory properties and can influence the structural integrity of microbial cells [18]. The presence of these compounds in *P. longifolia* supports its traditional use in herbal medicine and provides a scientific basis for its observed antimicrobial activity.

Table 2 Phytochemical Analysis of the Crude Extract of *Polyalthia longifolia* Leaf

Metabolites	Results
Tannins	+
Alkaloids	+
Saponins	+
Volatile oils	+
Terpenoids	+
Flavonoids	+
Steroids	+

Key: (+) = Present

➢ *Antimicrobial Screening of Crude Extract and Fractions*

Antimicrobial screening of the crude extract and fractions of *Polyalthia longifolia* demonstrated potent antibacterial properties against bacteria of both gram-positive and gram-negative types, especially in the crude and neutral fractions.(Table 3). These fractions exhibited a concentration dependent inhibitory effect, with larger zones of inhibition observed at higher concentrations. The strongest antibacterial activity was found in the neutral fraction, with a zone of inhibition of 33 mm against *Staphylococcus aureus* at 100 mg/ml, closely comparable to the 37 mm zone produced by the standard antibiotic, Ciprofloxacin. Similarly, the neutral fraction was highly effective against *Salmonella typhi*, *Bacillus subtilis*, and *Escherichia coli*, having inhibition zones of 30 mm, 32 mm, and 28 mm, respectively (Table 3). The crude fraction also demonstrated strong antibacterial effects across the same organisms, though slightly lower than the neutral fraction. The hexane and base fractions displayed moderate antibacterial activity. Notably, the base fraction produced a high inhibition zone of 33 mm against *Salmonella typhi*, suggesting strong bactericidal action. In contrast, acid fraction exhibited minimal antibacterial activity, with little or no zones of inhibition, especially at lower concentrations.

MIC and MBC results confirmed the antibacterial potential of the crude and neutral fractions, with low MBC values between 6.25 and 12.5 mg/ml and MIC values between 3.125 and 6.25 mg/ml.. These close MIC and MBC values suggest bactericidal effects. Conversely, the acid fraction showed high MIC and MBC values, or no measurable effect at all, indicating weak antibacterial potential (Table 4).

The neutral fraction exhibited particularly strong antibacterial effects, with inhibition zones reaching up to 33 mm against *Staphylococcus aureus*, surpassing results from earlier studies involving methanolic or aqueous extracts [19]. This suggests that the neutral, pH-balanced fraction may concentrate or preserve potent antibacterial compounds, a finding that has been rarely documented and merits further investigation.

Of significance, is the inactivity of the crude extract and fractions towards the fungi *Candida albicans* and *Aspergillus niger*. While earlier studies have reported antifungal effects of *P. longifolia* [20, 10], this inconsistent finding may be due to variation in soil composition and environmental circumstances. Furthermore, the base fraction demonstrated a notably high zone of inhibition (33 mm) against *Salmonella typhi*. This may point to the presence of basic or non-polar compounds particularly effective against *S. typhi*. Additionally, MIC and MBC values for the neutral and crude fractions were low, with MICs as low as 3.125 mg/ml, indicating potent bactericidal properties. These values are equal to or lower than previously reported figures [21], suggesting that the methods used in this study may yield more concentrated or bioavailable forms of active constituents.

Table 3 Inhibitory Activity of the Crude Extract and Fractions of *P. longifolia*

Zone of inhibition (mm) at different extract concentrations (mg/ml)																	
Test organisms	**crude**				**neutrals**				**hexane**				**base**				**acid**	**C**
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100 50 25 12.5	
S. aureus	27	25	21	19	33	29	27	24	30	25	23	21	23	20	18	16	18 15 12 -	37
B. subtillis	29	27	25	22	32	27	24	21	27	23	20	18	21	19	17	14	16 14 10 -	30
E. coli	26	23	21	18	28	26	23	20	22	20	17	15	22	19	16	15	- - - -	33

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S. typhi	28	25	22	20	30	27	24	22	28	26	22	22	33	29	26	22	-	-	-	-	35
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	46
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38

Key: (-) = No activity
C = Ciprofloxacin/Econazole (Control)

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) / Fungicidal Concentration (M.F.C) of the extracts

TEST ORGANISMS	MIC					MBC				
	Crude	Neutrals	Hexane	Base	Acid	Crude	Neutral	Hexane	Base	Acid
S. aureus	3.12	3.125	3.125	12.5	25	6.25	6.25	6.25	25	50
B. substilis	6.25	12.5	6.25	6.25	50	12.5	25	12.5	12.5	NIL
E. coli	3.125	6.25	12.5	25	_	6.25	12.5	25	50	_
S. typhi	3.125	6.25	12.5	6.25	_	6.125	12.5	25	12.5	_
C. albicans	-	–	–	–	–	-	–	–	–	–
A .niger	-	–	–	–	–	-	–	–	–	–

Key: (-) = Not determined for M.I.C/M.B.C
NIL= No M.B.C (The extract is bacteriostatic not bactericidal)

➢ *FT-IR and GC-MS Analyses of Crude Methanolic Extract*

The FT-IR spectrum of the crude methanolic extract of *Polyalthia longifolia* (Figure 2) provided valuable insight into the functional groups present in the plant extract.

Around 3300–3400 cm^{-1}, a considerable absorption band is observed, which suggests the existence of hydroxyl groups (–OH), which are common in alcohols, phenols, and flavonoids, confirming the results of the phytochemical screening that showed phenolic compounds and flavonoids were present. The absorptions band at 2825.3 cm-1 shows aliphatic C–H stretching vibrations, which suggests the presence of methyl (-C-H) groups while the band at 2918.5 cm-1 corresponds to that of the methylene groups (=C-H) typically found in phenols, flavonoids, and terpenoids.

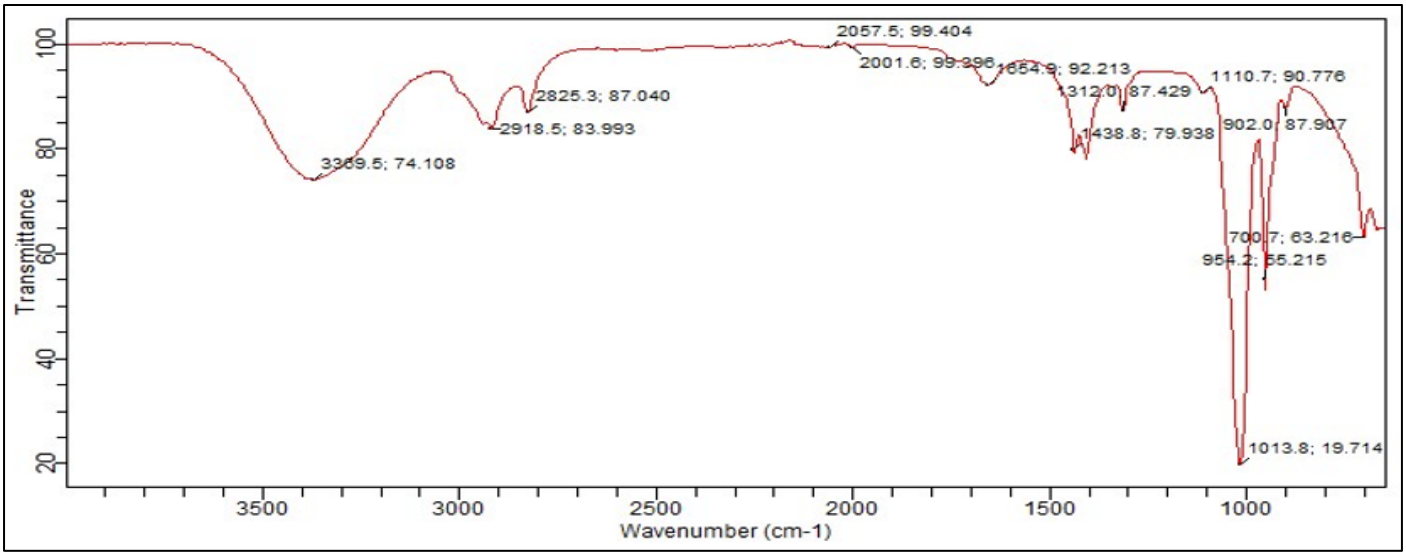

Fig 2 FT-IR Spectrum for Crude Methanolic Extract of *P. longifolia*

A sharp peak observed near 1700–1750 cm^{-1} indicates the presence of carbonyl (C=O) functional groups, consistent with the fatty acids such as oleic and palmitic acids identified in the GC-MS analysis. In the region around 1600–1650 cm^{-1}, peaks associated with C=C stretching vibrations point to the presence of aromatic compounds like flavonoids and other benzene derivatives.

The presence of strong and sharp band at 1013.8 cm^{-1} indicates the presence of the C-O functional group present in majority of the compounds identified in the physicochemical characterization.

The gas chromatogram of the crude extract P. longifolia revealed sixteen peaks based on a 1% area percentage and a 27% quality of characterisation (Figure 3). The sample's mass spectra were utilised to identify the structure and nature of the compounds by comparing them to standards from the database (Figure 4). The major compounds identified include: mesitylene, benzene,1,2,4-trimethyl, n-hexadecanoic acid, dibutyl phthalate, 1-Cyclohexene-1- acetaldehyde,2,6,6-trimethyl, 1,3,6,10-Cyclotetra-decatetraene,3,7,11-trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]-, oleic acid, octadecanoic acid, methyl kolavenate, farnesol formate, diisooctyl phthalate, benzamide, 2-fluoro-N-(2,4-dimethoxyphenyl),

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9,19-cyclolanostan-3-ol,acetate, (3.beta.), 5-ethylcyclopent-1-ene-1-carboxylic acid, naphthalene, decahydro-, 1,6-bis(methylene)-4-(1-methylethyl)-,(4.alpha., 4a.alpha.,8a. alpha.)- and 26-nor-5-cholesten- 3.beta.-ol-25-one (Table 5).

Compounds such as farnesol formate, 1,3,6,10-cyclotetradecatetraene, and methyl kolavenate are typical terpenoid structures known for their anti-inflammatory and antimicrobial activities [4]. Additionally, complex triterpenoids such as 26-nor-5-cholesten-3β-ol-25-one and 9,19-cyclolanostan-3-ol acetate represent steroidal backbones and confirm the presence of phytosterols, which have been associated with cholesterol-lowering and cytotoxic effects [5]. The identification of aromatic hydrocarbons and substituted benzene derivatives such as mesitylene and benzene-1,2,4-trimethyl suggests the presence of phenolic compounds, consistent with the phytochemical detection of flavonoids and tannins. While flavonoids were not directly named in the GC-MS output, the presence of methoxy-substituted benzamides, like benzamide, 2-fluoro-N-(2,4-dimethoxyphenyl), implies the presence of phenolic derivatives often seen in flavonoid structures [4]. Volatile constituents such as mesitylene, 1-cyclohexene acetaldehyde, and naphthalene derivatives account for the volatile oils detected in the phytochemical test. These compounds possess antimicrobial properties and are also associated with oxidative stress protection [5]. The nitrogen-containing compound (benzamide derivative) hints at alkaloid-like structures, reinforcing the ethnopharmacological relevance of the plant. Tannins, known for their astringent and antimicrobial effects, may not have appeared in the chromatogram due to their non-volatile nature but remain relevant based on the test results.

Furthermore, fatty acids such as oleic acid, n-hexadecanoic acid and octadecanoic acid were identified in high percentages. These fatty acids are known for their anti-inflammatory, antioxidant, and membrane-protective effects [22].

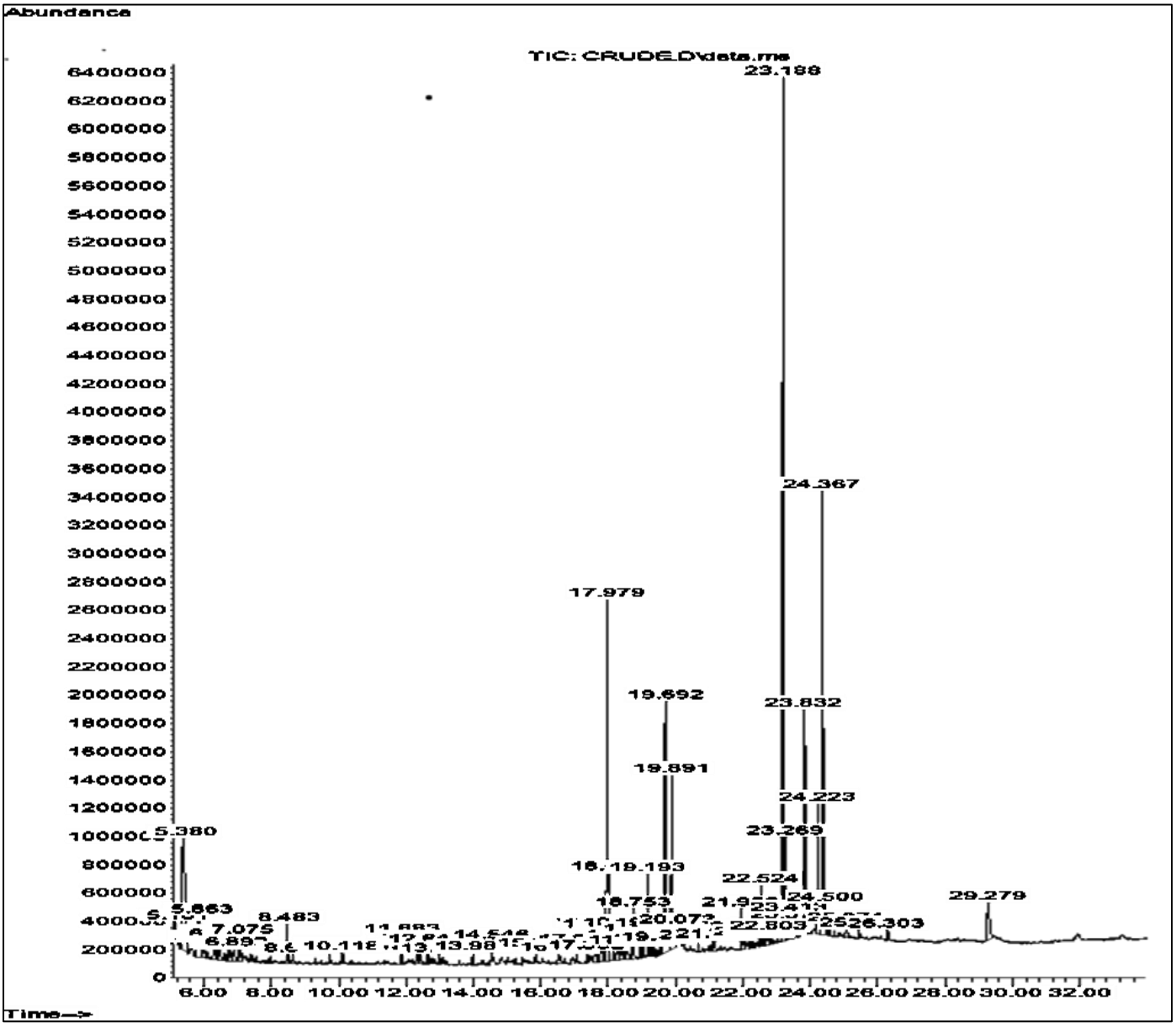

Fig 3 Gas Chromatogram for Crude Methanolic Extract of *P. longifolia*

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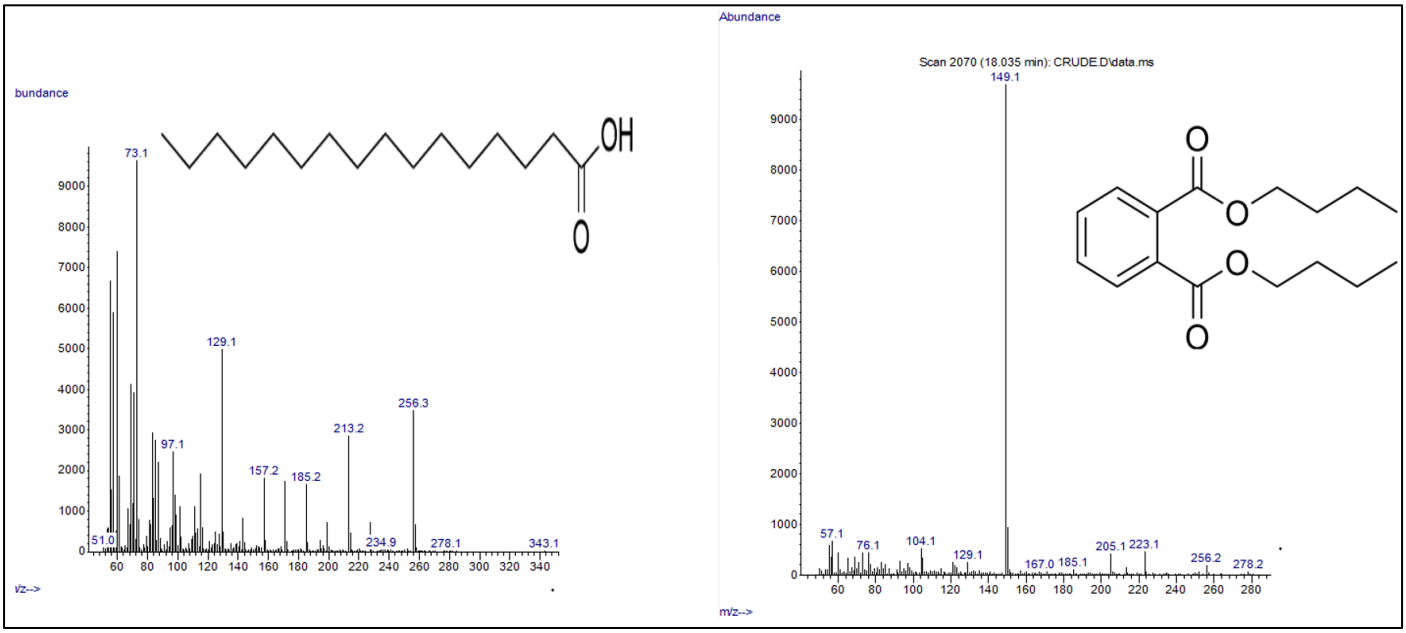
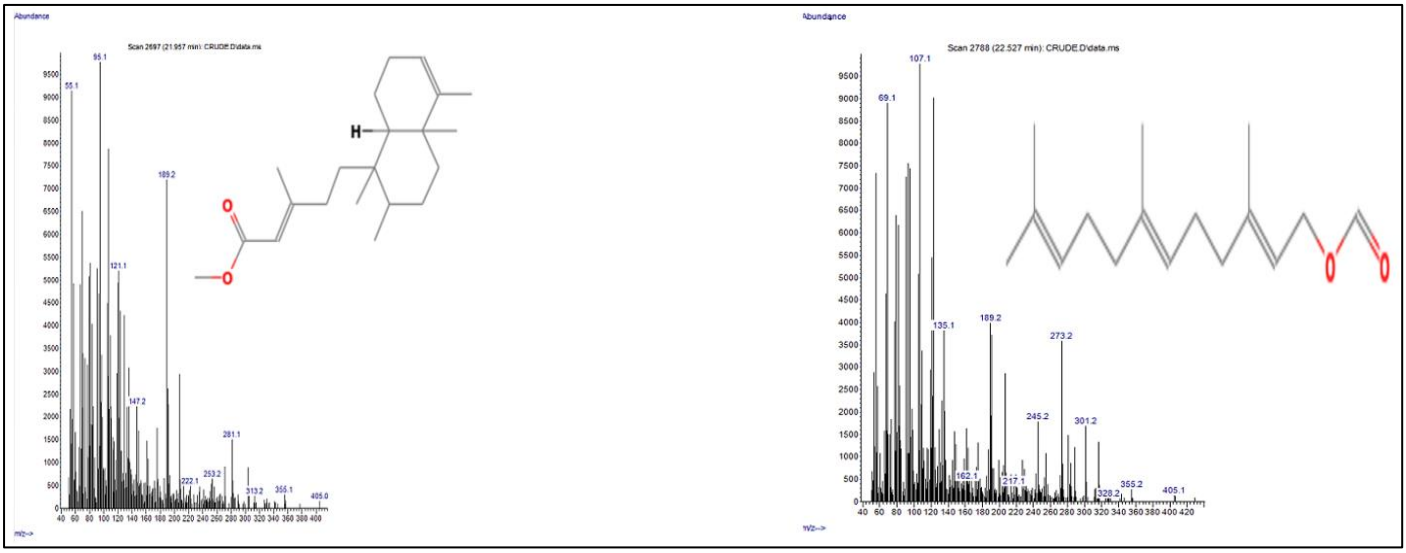
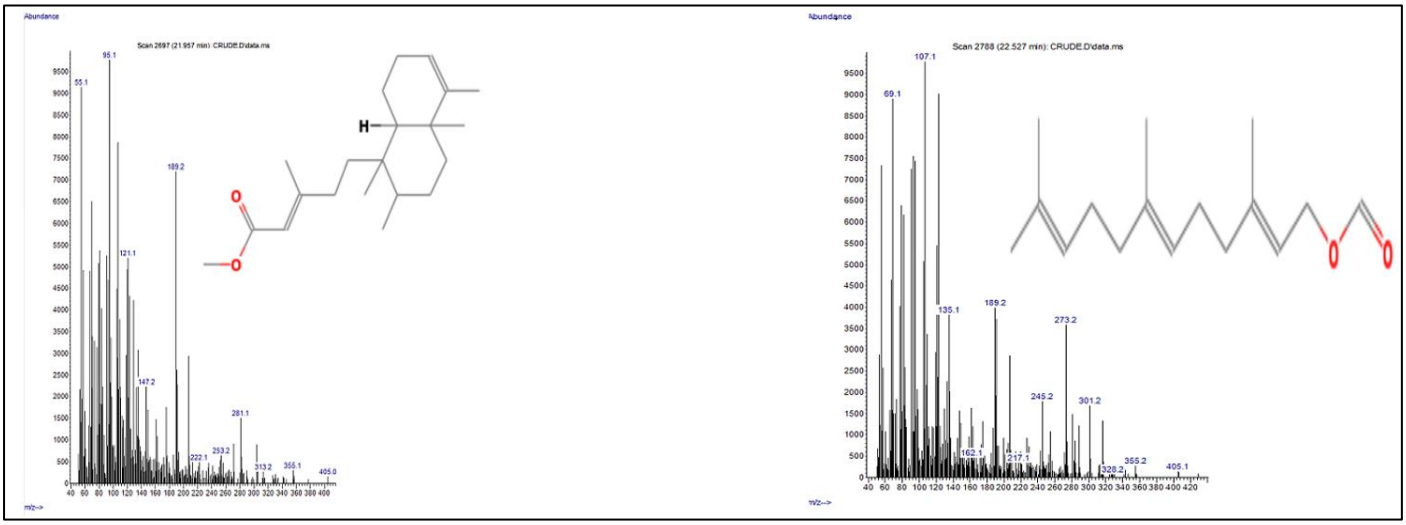


n-Hexadecanoic Acid Dibutyl Phthalate



Methyl Kolavenate Farnesol Formate



Diisooctyl Phthalate 9,19-Cyclolanostan- 3-ol, Acetate, (3.beta.)

Fig 4 MS Spectra and Structures of some Components of the Crude Methanolic Extract of *P.longifolia*

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Table 5 The GC-MS Analysis of the Crude Methanolic Extract of *P. longifolia,* Showing its Major Compounds

Name	Retention Time (mins)	Area(%)	Quality	Molecular Weight(g/mol)	Molecular Formula
Mesitylene	5.380	6.23	97	120.19	C_9H_{12}
Benzene,1,2,4-trimethyl	5.863	2.05	64	120.19	C_9H_{12}
n-Hexadecanoic acid	17.979	8.92	99	256.43	$C_{16}H_{32}O_2$
Dibutyl phthalate	18.037	2.29	96	278.34	$C_{16}H_{22}O_4$
1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl	18.753	1.13	43	166.26	$C_{11}H_{18}O$
1,3,6,10-Cyclotetra-decatetraene,3,7,11-trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]-	19.193	1.56	70	272.5	$C_{20}H_{32}$
Oleic acid	19.692	9.07	99	282.46	$C_{18}H_{34}O_2$
Octadecanoic acid	19.891	4.41	99	284.47	$C_{18}H_{36}O_2$
Methyl kolavenate	21.956	1.09	58	318.49	$C_{21}H_{34}O_2$
Farnesol formate	22.524	1.37	45	250.37	$C_{16}H_{26}O_2$
Diisooctyl phthalate	23.188	16.71	91	390.6	$C_{24}H_{38}O_4$
Benzamide, 2-fluoro-N-(2,4-dimethoxy phenyl)	23.269	2.73	45	275.27	$C_{15}H_{14}FNO_3$
9,19-Cyclolanostan-3-ol,acetate, (3.beta.)	23.832	5.88	27	470.80	$C_{32}H_{54}O_2$
5-Ethylcyclopent-1-ene-1-carboxylic acid	24.223	3.19	30	140.18	$C_8H_{12}O_2$
Naphthalene,decahydro-1,6-bis(methylene)-4-(1-methylethyl)-, (4.alpha., 4a.alpha., 8a. alpha.)-	24.367	9.24	38	204.35	$C_{15}H_{24}$
26-Nor-5-cholesten-3.beta.-ol-25-one	29.279	1.88	95	386.60	$C_{26}H_{42}O_2$

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IV. CONCLUSION

In conclusion, these findings demonstrate the potential of *P. longifolia* as a natural source of antibacterial compounds and offer scientific support for its traditional usage in herbal medicine. However, the extracts showed no activity against Aspergillus niger and Candida albicans, indicating a narrower spectrum of antifungal potential.

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