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Research Article

PHYTOCHEMICAL SCREENING, FT-IR, GC-MS CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *PANDANUS AMARYLLIFOLIUS* ROXB. LEAF EXTRACT

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ABSTRACT

The present study aimed to evaluate the presence of phytochemicals and antibacterial activity of the ethyl acetate extract of *Pandanus amarylifolius* leaves. Preliminary phytochemical screening revealed the presence of importance secondary metabolites such as phenols, alkaloids, flavonoids and some other bioactive compounds, which indicates the therapeutic potential of the plants. Fourier Transform Infrared (FT-IR) spectral analysis of ethyl extract of leaf depicted 12 functional groups peaks viz., Carboxylic acid (O-H), hydroxyl (O-H), alkane (C-H), Isothiocyanate (N=C=O) and Nitrile (CΞO). Gas chromatography and mass spectroscopy analysis leaf extracts exhibited 26 compounds, including β-Sitosterol, 1,2,3,6-Tetrahydrobenzylalcohol, β- Amyrone, 24-Noroleana-3,12-diene, Neophytadiene, Benzene diazonium, Phenol, Cyclohexanol, 4-Norursa-3,12-diene, Brasiliamide, 9-Octadecenoic acid, Epilupeol, 4-Benzyl-2-t-butyl-3-formyloxazolidine-4-carboxylic acid, Benzene, Z-7-Decen-1-yl acetate, 6,9,12-Octadecatrienoic, Butanoic acid, Cyclopentane, 2(5H)-Furanone, Propanamide, 2-Butenoic acid, Cyclopentasiloxane, 3-Methylsalicylic acid, Cyclohexasiloxane and 5-Tetramethylhexane. These phytochemicals revealed significant antibacterial activity. The highest inhibition zone was observed against *Staphylococcus aureus* (29.25±0.35 mm) and *Bacillus subtilis* (28.95±0.35 mm) at a concentration of 500 µg/ml. The gram-negative bacteria *Aeromonas hydrophila* (25.45±0.35) and *Klebsiella pneumonia* (24.60±0.14) exhibited moderate inhibition zones. In the present study, the ethyl acetate extract of *P. amaryllifolius* leaves confirms significant phytochemical and promising antimicrobial potential, which can be used as a natural therapeutic source.

Keywords: *Pandanus amaryllifolius*, β-Sitosterol, Isothiocyanate, *Bacillus subtilis*, *Klebsiella pneumonia*.

INTRODUCTION

Plant-based remedies are used as primary source of healthcare by approximatelyy 80% of the global population including both developed and developing countries. They are being considered as promising therapeutic sources for the drug. The diversity of natural products of medicinal plants makes them interesting objects of scientific investigations as potential leads to discover and develop new pharmacological bioactive compound (Nasim *et al*., 2022). These compounds contain a wide array of structurally diverse natural compounds that confer various pharmacological activities. Most of the naturally occurring compounds from medicinal plants are phenomenal active like phenolic, flavonoids (Roy *et al*., 2022), terpenoids (Masyita *et al*., 2022), saponins (Moses *et al*., 2014), steroids (Gadouche *et al*., 2022), and alkaloids exhibiting

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antimicrobial, antioxidant (Tian *et al*., 2019), antiviral (Parham *et al*., 2020), anti-inflammatory (Ayertey *et al*., 2021), and cytoprotective activities. *Pandanus amaryllifolius* is a tropical plant which belong to Pandanaceous family distributed in many parts of Southeast Asia including India, Thailand, Malaysia, Indonesia and Sri Lanka. The plant is known for providing plant coloring, flavoring and bioactive compounds which are used in making food additives and in pharmaceutical markets. The leaves smell of pandan fragrance which comes from 2-acetyl-1-pyrroline (2AP) similar to fragrant basmati rice (Bhatt et al., 2021). Its leaves are used as medicinal plant as antioxidant (Buddhakala *et al*., 2025), Antidiabetic (Chiabchalard *et al*., 2015), Anti-inflammatory (Buddhakala *et al*., 2025) , antimicrobial (Wahyuni *et al*., 2024), antihyperglycemic (Chiabchalard *et al*., 2015), anti-diabetic potential (Saenthaweesuk *et al*., 2016). The pandan prop root produces an alternate Bacterial cellulose (BC) that causes the unique function and bioactive compounds. A novel substrate for BC production will provide an alternative BC product that generates the unique functions and bioactivities which has not been found in the conventional substrate. The present study was to determine the secondary metabolites using FTIR spectra and GC–MS analysis and their potential of antimicrobial activity.

MATERIALS AND METHODS

Plant Material and Extract preparation

The sample plant material was obtained in Jamal Mohamed College, Tiruchirappalli, Tamil Nadu and India. The dried leaves collected were shade dried and ground into a fine powder using Soxhlet apparatus with the use of 6 hours of methanol. Whatman No.1 filtrates were used to filter the extracts and filtrates were concentrated under low pressure at 40º C using a rotary flash evaporator and stored at 4º C until they were used in phytochemical screening and pharmacological activity.

Phytochemical analysis of *P. amaryllifolius* leaf extract

Preliminary phytochemical analysis of ethyl acetate, acetone, ethanol and aqueous extracts was performed and the presence of reducing sugars, alkaloids, anthraquinones, glycosides, tannins, flavonoids, saponins and triterpenoids in the extracts were revealed (Dahiya *et al*., 2012).

FT-IR functional groups analysis of *P. amaryllifolius* leaf extract

The Fourier Transform Infrared (Shimadzu-119) analysis was performed at a Shimadzu-119 under the range 350 cm -1 to 4700 cm -1. The instrument was then left to warm up 30 min and calibrated with the inactive potassium bromide (KBr) and then analyzed. About 5 g of the *P. amaryllifolius* leaves were put in a mortar and crushed to fine powder. This sample was dried in an oven at 105º C in 30 minutes and then left to cool in a lidded crucible. Approximately, 1 mg of the dried sample was tipped to the FTIR mortar and the inert potassium bromide sample was added at a ratio of 1 to 50 times to the sample to KBr and ground to achieve the homogeneity. The FT-IR hand-press equipment was used to compress the mixture to form a translucent pellet. The pellet was loaded to the sample holder and attached into FTIR machine to be analyzed. FTIR spectrum was measured as transmittance versus the wavelength and result peaks were made in order to determine the character of functional groups that the sample contained (Mwangi *et al*., 2024).

GC–MS Analysis of *P. amaryllifolius* leaf extract

The extract of P. amaryllifolius plants were analyzed by means of a GCMS-QP (GCMS-QP 2010 Plus, Shimadzu). An ionization voltage of 70 eV and an injector temperature of 250 o C were used to carry out the analysis. The injector measured in split mode and with linear velocity of 36.5 cm/s and pressure of 57.5 kPa. The instrument operated in electron impact. About 1 µL of all the samples were injected into the system with the carrier gas helium (99.9% purity) flowing at 1 mL/min. The isothermal conditions were kept at 60º C within 5 minutes of time and thereafter, the temperature in the oven was raised at a rate of 10ºC/minute until 100ºC temperature was reached and 10 minutes later, the temperature was raised at a rate of 5º C/minute to reach a temperature of 270º C. The relative percentage of the respective components was calculated by comparing the mean peak area of the individual compounds to the total peak area. The compounds were identified through a comparison of mass spectra of the unknown components with the known compounds in the National Institute of Standards and Technology library (NIST-5), which offered information about the name of the compound, chemical formula, molecular weight, and structure of the compound (Nagaraj *et al*., 2023).

Antibacterial Activity of *P. amaryllifolius* leaf extract

The antibacterial effect was measured by disc diffusion technique against some chosen bacteria species which include *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Aeromonas hydrophila*. These isolates were taken in KAP Viswanathan medical college, microbiology department Tiruchirappalli, Tamil Nadu. The bacterial culture was placed in the petriplates in 2-3 cm of thickness with sterilized Nutrient Agar Medium. The medium petriplates were maintained at an aseptic condition and it was to check the contamination and also to solidify the petr. Micropipette (10 µl of extracts) was used to add each sterile disc separately with the positive control, which was the antibiotic disc (Bacterial antibiotic) of Chloramphenicol. The plates were incubated at 5º C to allow diffusion of the plant extracts and incubated at 35º C to allow incubation of 24 hours. Vernier caliper was used to measure the presence of the inhibition zones and this was recorded and taken to indicate presence of the antibacterial activity (Mostafa *et al*., 2018).

Statistical analysis

Results were calculated and expressed as Mean Standard deviation. P-value < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

A systematic qualitative phytochemical analysis of four different solvent extracts of *P. amaryllifolius* reveals the presence of bioactive compounds. The phytochemicals present in the four different extracts were determined using spot test method. The results reveal the presence of tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, alkaloids, anthraquinone, polyphenol and reducing sugar in the ethanol, ethyl acetate, aqueous and acetone extracts respectively. Among the four different solvents, Ethyl acetate extract performed well in the qualitative analysis of phytochemical studied. The Ethyl acetate extract possess Tannin, Saponin, Flavonoids, Steroids, Terpenoids, Alkaloids, Polyphenol were strongly present in the preliminary phytochemical analysis (Table 1). Many of the alkaloids have been found with several pharmacological activities including antimicrobial, analgesic, anti-inflammatory etc. Phenolic compounds are known for their bioactive properties which have been reported to show antimicrobial, anti-inflammatory, anticancer and antidiabetic activities (Kumar *et al*., 2025).

Table 1. Preliminary phytochemical analysis of *P. amaryllifolius* leaf extracts.

S. No	Phytochemicals	Ethanol extract	Ethyl Acetate extract	Aqueous extract	Acetone extract
1	Tannin	+	++	+	-
2	Phlobatannins	-	-	-	-
3	Saponin	+	+	++	-
4	Flavonoids	+	++	+	+
5	Steroids	-	++	-	+
6	Terpenoids	++	++	+	+
7	Triterpenoids	-	+	-	-
8	Alkaloids	+	++	-	-
9	Anthroquinone	+	++	++	-
10	Polyphenol	++	++	+	+
11	Reducing sugar	++	++	+	+

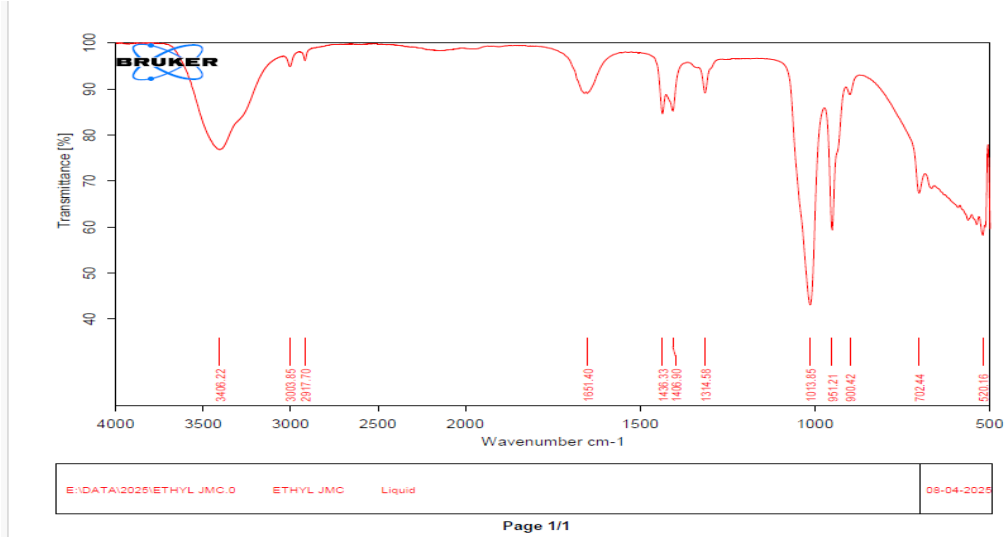

Figure 1. FTIR Spectrum of *Pandanus amaryllifolius* leaf extract

Table 2. Identification of functional groups using FTIR analysis of *Pandanus amaryllifolius leaf* extract.

S. No	Absorption (cm^{-1})	Peak Nature	Functional Group	Compound Class
1	3406.22	Weak/sharp	O-H Stretching	Alcohol
2	3003.85	Weak	O-H Stretching	Alcohol
3	2917.70	Strong	O-H Stretching	Alcohol
4	1651.40	Medium	C-H Stretching	Alkane
5	1436.33	Medium	C-H Stretching	Alkane
6	1406.90	Medium	C-H Stretching	Alkane
7	1314.58	Medium	C-H Stretching	Alkane
8	1013.85	Weak	CΞO Stretching	Nitrile
9	951.21	Weak	N=C=O Stretching	Isothiocyanate
10	900.42	Weak	N=C=O Stretching	Isothiocyanate
11	702.44	Medium/ Sharp	C=C Stretching	Conjugated Alkenes
12	520.16	Medium	C-H Bending	Alkane

The FT-IR spectrum was analyzed to identify the functional groups of the active components found in ethyl acetate extract of *P. amaryllifolius* leaf based on the peaks value in the region of IR radiation. When the leaf extract was passed into the FT-IR, the functional group of the components were separated based on its peak's ratio and absorption peak represented in the Table 2 & figure 1. The spectrum exhibited a weak absorption band at 3406.22 cm^{-1} and a weak band at 3003.85 cm^{-1}, along with a strong peak at 2917.70 cm^{-1}, which are indicative of O-H Stretching vibrations correspond to alcohol compounds. These compounds were widely reported in plant extracts and are known for their antioxidant and antimicrobial properties (Singh and Mendhulkar, 2015). A medium peak observed at 1651.40 cm^{-1},1436. 33 cm^{-1}, 1406.90 $cm^{-1}$and 1314.58 cm^{-1} revealed the presence of alkane compoundss, possibly related to C-H Stretching. These aliphatic hydrocarbons are commonly found in plant-derived extracts and are often associated with lipid components, waxes, and other non-polar phytoconstituents. Such compounds contribute to the structural integrity of plant tissues and may also exhibit biological aactivities, including antimicrobial effects (Mayuresh and Madhura, 2025). The peak at 1013.85 cm-1 (weak peak), suggests the presence of Nitrile compounds (CΞO Stretching). Weak absoption bands at 951.21 cm-1 and 900.42cm^{-1} are characteristics N=C=O Stretching vibrations, confirming the presence of Isothiocyanate functional groups. Isothiocyanates are well-known bioactive compounds derived from glucosinolate precursors and are widely reported for their strong antimicrobial, antioxidant, and anticancer aactivities (Kamal, *et al*., 2022). The peak at 702.44 cm-1 (Medium/ Sharp) is attributed to C=C Stretching vibrations of conjugated alkenes. A medium peak observed at 520.16.63cm-1 which stated the presence of Alkane compound corresponds to C–H stretching. These finding support earlier studies indicating that plants extract rich in diverse functional groups exhibit enhanced therapeutic potential (Kumar et al., 2024; Sasidharan *et al*., 2010).

The chemical composition of the ethyl acetate extract of *P. amarylifolius* leaves was analyzed using Gas chromatography - Mass spectroscopy (GC-MS). The GC-MS chromatogram revealed the presence of multiple peaks, indicating a complex mixture of bioactive compounds. Each peak corresponds to a specific compound and identified based on their retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area %). A total of 26 compounds were identified in the extracts as presented in Table 3 and Figure 2. Among the detected compounds major cconstituents included β-Sitosterol (peak area 14.79 %), 1,2,3,6-Tetrahydrobenzyl alcohol (peak area 9.77%), β-Amyrone (peak area 5.71%), 24-Noroleana-3, 12-diene (peak area 5.48%), Neophytadiene (peak area 5.08%), Benzimidazolium (peak area 4.76%), Phenol (peak area 3.97%), Cyclohexanol (peak area 3.22%), (peak area 3.97%), 4-Norursa-3,12-diene (peak area 3.88%), Brasiliamide A (peak area 3.65%),9-Octadecenoic acid (peak area 2.67%), Epilupeol (peak area 1.94%), 4-Benzyl-2-t-butyl-3-f ormyloxazolidine-4-carboxylic acid (peak area 1.28%), Benzene (peak area 1.02%), Z-7-Decen-1-yl acetate (peak area 1.01%),6,9,12-Octadecatrienoic (peak area 0.98%), butanoic acid (peak area 0.96%), Cyclopentane (peak area 0.97%), (peak area 1.01%), 2(5H)-Furanone (peak area 0.93%), Propanamide (peak area 0.79%), 2-Butenoic acid (peak area 0.77%), Cyclopentasiloxane (peak area 0.74%), 3-Methylsalicylic acid (peak area 0.66%), Cyclohexasilox ane (peak area 0.64%), (peak area 0.93%) and 5-Tetra methylhexane (peak area 0.57%) which exhibited significant peak areas, suggesting their abundance in the extract. The presence of fatty acids and their esters indicates potential antimicrobial and anti-inflammatory ac tivities, while phenolic compounds are well known for their antioxidant properties. Additionally, the detection of terpenoids suggests possible pharmacological activities such as anticancer, antiviral, and anti-inflammatory effects. Compounds such as alkanes and hydrocarbons identified in the extract may contribute to the stability and structural

characteristics of plant metabolites. The occurrence of nitrogen-containing compounds and heterocyclic molecules further supports the presence of bioactive secondary metabolites with potential therapeutic applications. The GC-MS analysiss confirms that *P. amaryllifolius* leaf extract contains a wide spectrum of biologically active compounds. These findings are consistent with previous studies on medicinal plants, where GC–MS profiling revealed the presence of diverse Phytochemical responsible for various pharmacological activities (Hongal *et al*., 2024; Singh *et al*., 2023).

Sample Information

Analyzed by	: Admin
Analyzed	: 2/21/2025 2:03:17 PM
Sample Name	: RSK
Sample ID	: RSK
Vial #	: 6
Injection Volume	: 1.00
Data File	: E:\GCMS Data\RSK.qgd
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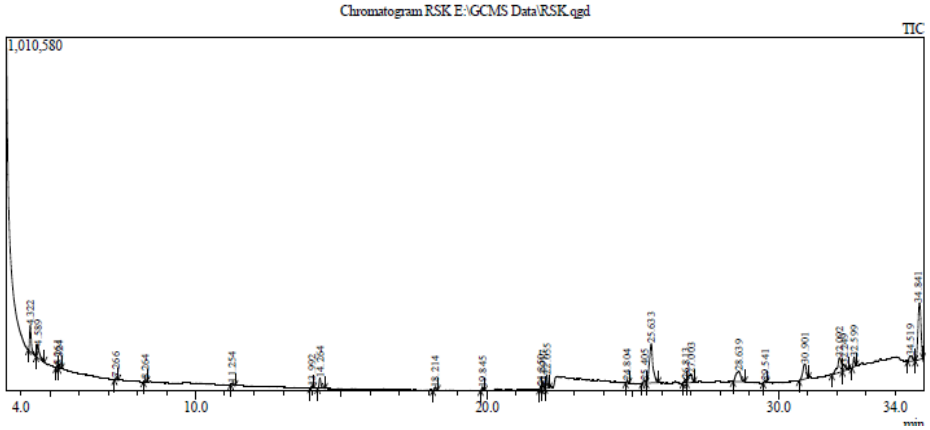
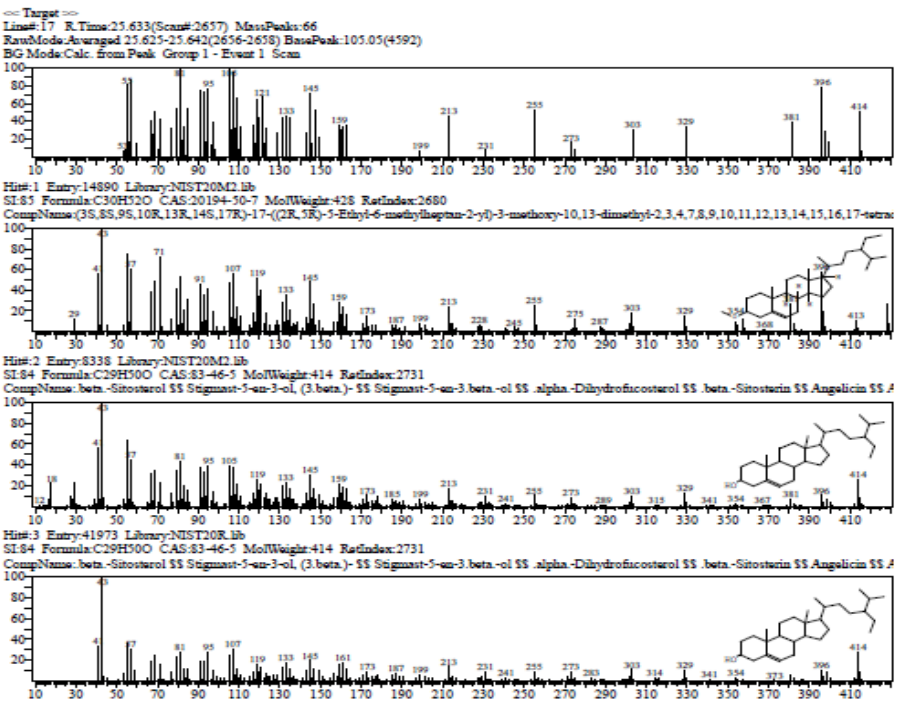
Chromatogram RSK E:\GCMS Data\RSK.qgd



CAPSTONE BIOSCIENCES

TRIBIOTECH INSTRUMENT FACILITY



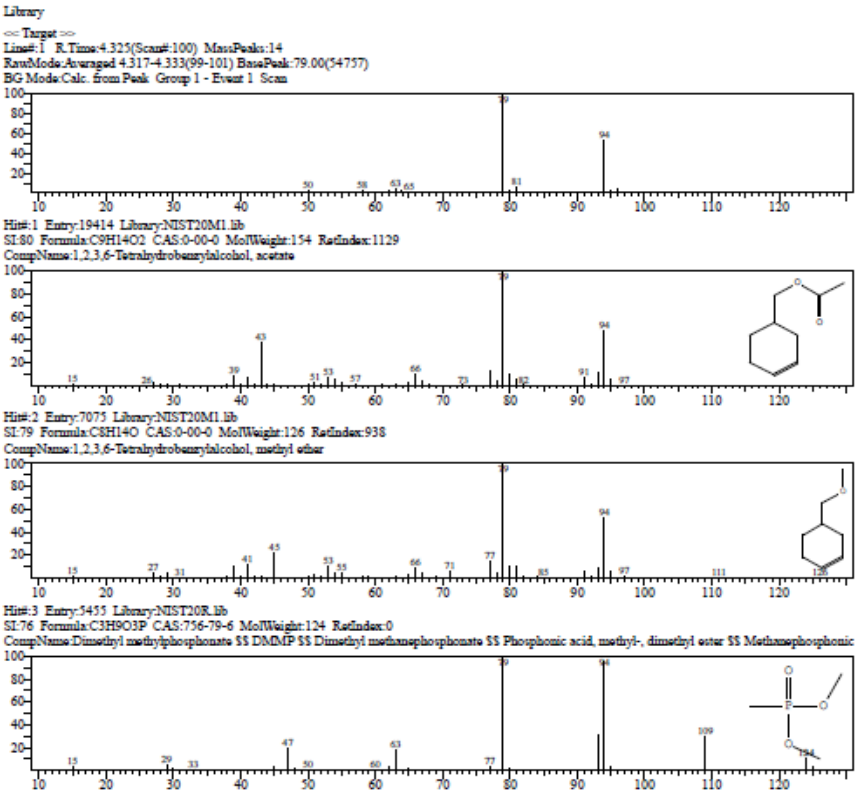


Figure 2. GC-MS analysis of *Pandanus amaryllifolius* leaf extract.

Table 3. Detection of phytoconstituents using GC-MS analysis of *P. amaryllifolius* leaf extracts.

Peak	Retention Time	Area%	Height	Height %	Molecular formula	Molecular weight	Name of the Bioactive compound
1	4.322	5.91	73422	9.77	$C_9H_{14}O_2$	154	1,2,3,6-etrahydrobenzylalcohol
2	4.589	3.88	35741	4.76	$C_6H_4N_2O$	120	Benzenediazonium
3	5.261	0.46	6990	0.93	$C_5H_6O_2$	98	2(5H)-Furanone
4	5.324	0.44	7282	0.97	C_7H_{14}	98	Cyclopentane
5	7.266	0.24	4284	0.57	$C_{10}H_{22}$	86	5-Tetramethylhexane
6	8.264	0.35	5594	0.74	$C_{10}H_30O_5Si_5$	370	Cyclopentasiloxane
7	11.254	0.32	4821	0.64	$C_{12}H_{36}O_6Si_6$	444	Cyclohexasiloxane
8	13.992	0.34	4982	0.66	$C_{14}H_{24}O_3Si_2$	296	3-Methylsalicylic acid
9	14.264	2.59	29814	3.97	$C_{14}H_{22}O$	206	Phenol
10	18.214	0.64	7698	1.02	$C_{11}H_{16}S$	180	Benzene
11	19.845	0.48	7218	0.96	$C_5H_{10}O_2$	102	Butanoic acid
12	21.85	0.49	7566	1.01	$C_{12}H_{22}O_2$	184	Z-7-Decen-1-yl acetate
13	21.917	1.51	20102	2.67	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid
14	22.055	2.78	38155	5.08	$C_{20}H_{38}$	278	Neophytadiene
15	24.804	0.37	5946	0.79	$C_{16}H_{15}F_6N_3O_2$	395	Propanamide
16	25.405	0.83	7370	0.98	$C_{12}H_{25}Cl$	204	6,9,12-Octadecatrienoic
17	25.633	19.58	111143	14.79	$C_{29}H_{50}O$	414	β-Sitosterol
18	26.813	0.97	9601	1.28	$C_{17}H_{23}NO_4$	305	4-Benzyl-2-t-butyl-3-formyloxazolidine-4-carboxylic acid

19	27.003	4.7	24226	3.22	$C_{14}H_{23}NO$	221	Cyclohexanol
20	28.639	6.74	29171	3.88	$C_{29}H_{46}$	394	4-Norursa-3,12-diene
21	29.541	0.39	5759	0.77	$C_{14}H_{24}O_2$	224	2-Butenoic acid
22	30.901	7.34	42882	5.71	$C_{30}H_{48}O_2$	424	β-Amyrone
23	32.092	8.2	41190	5.48	$C_{29}H_{46}$	394	24-Noroleana-3,12-diene
24	32.249	2.34	17283	2.3	$C_{23}H_{33}NO_4$	387	Phenanthrenepropanenitrile
25	32.599	2.17	27396	3.65	$C_{25}H_{28}N_2O_6$	452	Brasiliamide A
26	34.519	2.3	14566	1.94	$C_{32}H_{52}O_2$	468	Epilupeol

Table 4. Phytoconstituents and their Biological activities of *P. amaryllifolius* leaf extract.

S. No	Molecular formula	Name of the Bioactive compound	Biological activities of the Compound
1	$C_9H_{14}O_2$	1,2,3,6-Tetrahydrobenzylalcohol	Antibacterial and antifungal properties (Sarzyński *et al*., 2024)
2	$C_6H_4N_2O$	Benzenediazonium	Antileishmanial activity (Singh *et al*., 2020)
3	$C_5H_6O_2$	2(5H)-Furanone	anti-inflammatory, antitumor, antimicrobial, antioxidant, analgesic, antituberculosis, antiulcer activity (Khabibrakhmanova *et al*., 2023; Rossi *et al*., 2017; Kayumov *et al., 2020*)
4	C_7H_{14}	Cyclopentane	Antiviral activity (Smee *et al*., 2001)
5	$C_{10}H_{22}$	5-Tetramethylhexane	5-Tetramethylhexane
6	$C_{10}H_{30}O_5Si_5$	Cyclopentasiloxane	Cosmetic products (De Castro *et al*., 2025)
7	$C12H_{36}O_6Si_6$	Cyclohexasiloxane	Cosmetic products (Ko *et al*., 2024)
8	$C_{14}H_{24}O_3Si_2$	3-Methylsalicylic acid	Anti-inflammatory drugs, analgesics (Almasirad *et al*., 2014)
9	$C_{14}H_{22}O$	Phenol	Antioxidant activity (QuirósSauceda *et al*., 2017)
10	$C_{11}H_{16}S$	Benzene	Antifungal activity, anti-inflammatory activity, antitumor activity, antioxidant activity (Zain-Alabdeen *et al*., 2022)
11	$C_5H_{10}O_2$	Butanoic acid	Flavor additive to impart fruit-like aromas (Liu *et al*., 2026) immunomodulatory activity (Gerunova *et al*., 2024)
12	$C_{12}H_{22}O_2$	Z-7-Decen-1-yl acetate	Sex pheromone compound (Rasmussen *et al*., 1997)
13	$C_{19}H_{36}O_2$	9-Octadecenoic acid	Anti-inflammatory and antioxidant properties (Muzahid *et al*., 2022)
14	$C_{20}H_{38}$	Neophytadiene	Analgesic, anti-oxidant, anti-microbial, anti-cancer, anti-malarial, insecticidal, and neuroprotective properties, (Rajeswaran *et al*., 2025)
15	$C_{16}H_{15}F_6N_3O_2$	Propanamide	Antiproliferation (He *et al*., 2021)
16	$C_{12}H_{25}Cl$	6,9,12-Octadecatrienoic	Anti-inflammatory, and anti-oxidative properties (Parvathi *et al*., 2022)
17	$C_{29}H_{50}O$	β-Sitosterol	Anti- inflammation, anti-cancer effects (Nandi *et al*., 2024)
18	$C_{17}H_{23}NO_4$	4-Benzyl-2-t-butyl-3-formyloxazolidine-4-carboxylic acid	Antimicrobial activity (Vlasov *et al*., 2024)
19	$C_{14}H_{23}NO$	Cyclohexanol	Cyclohexanol (antimicrobial, antibacterial, antifungal, anticancer, antiviral, anti-inflammatory (Mahyavanshi *et al*., 2017; Ullah *et al*., 2022)

20	$C_{29}H_{46}$	4-Norursa-3,12-diene	Antimicrobial activity (Ullah *et al*., 2022)
21	$C_{14}H_{24}O_2$	2-Butenoic acid	Antifungal properties (Uguen *et al*., 2021)
22	$C_{30}H_{48}O$	β-Amyrone	Antimicrobial agent (Han *et al*., 2022)
23	$C_{29}H_{46}$	24-Noroleana-3,12-diene	Analgesic, anti-inflammatory, and antioxidant activity (Jiko *et al*., 2024)
24	$C_{23}H_{33}NO_4$	1-Phenanthrenepropanenitrile	Antimicrobial, spasmolytic, anti-inflammatory (Kovács *et al*., 2008)
25	$C_{25}H_{28}N_2O_6$	Brasiliamide A	Antibacterial activity and cytotoxic activity (Paluka *et al*., 2020)
26	$C_{32}H_{52}O_2$	Epilupeol	Anti-inflammatory compound, (Sánchez-Ramos *et al*., 2023)

The antibacterial activity of the eethyl acetate extract of *P. amaryllifolius* leaves was evaluated against selected patho genic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Aeromonas hydroph ila*, using disc diffusion method. The results were assessed based on the diameter of the zone of inhibition (mm) and compared with a standard antibiotic used as a positive control (Chloramphenicol). The leaf extract exhibited varying degrees of antibacterial activity against all tested microorganisms. Among the tested strains, the highest zone of inhibition was observed against *B. subtilis* (29.25±0.35) and *B. subtilis* (28.95±0.35) at 500 µg/ml, that indicating strong susceptibility of Gram-positive bacteria to the extract. A moderate inhibitory effect was observed against Gram-positive bacteria, *A. hydrophila* (25. 45±0.35) and *K. pneumoniae* (24.60 ± 0.14) at 500 µg/ml. The positive control (Chloramphenicol) exhibited a significantly larger zone of inhibition against all tested organisms (Table 5 & figure 4 and 5), confirming the validity and sensitivity of the assay. However, the appreciable activity shown by the plant extract suggests the presence of potent antibacterial compounds. The present study of antibacterial activity can be correlated with the presence of various phytoconstituents identified through FT-IR and GC-MS analyses. Functional groups such as phenols, alkaloids, alcohols, amines, and sulfur-containing compounds are known to contribute to antimicrobial activity by disrupting microbial celll walls, altering membrane permeability, and inhibiting essential enzymatic pathways (Taher at al., 2023). Phenolic compounds, in particular, can denature proteins and interfere with microbial metabolism, leading to cell death. The higherr antibacterial activity observed against Gram-positive bacteria, particularly *S. aureus*, may be due to the relatively simple structure of their cell wall, which lacks an outer membrane. This structural feature allows easier penetration of bioactive phytochemical into the bacterial cell, resulting in effective inhibition. In contrast, Gram-negative bacteria such as *K. pneumoniae* and *A. hydrophila* possess an additional outer membrane composed of lipopolysaccharides, which acts as a permeability barrier and reduces the uptake of antimicrobial agents (Timoszyk *et al*., 2022; Silhavy *et al*., 2010) . These findings agree with previous studies on medicinal plants, where leaf extracts demonstrated significant antibacterial activity against both Gram-positive and Gram-negactive bacteria, although with varying degrees of effectiveness (Kamaraj *et al*., 2012). The results suggest that *P. amaryllifolius* leaf extract could serve as a potential source of natural antibacterial agents.

Table 5. Antibacterial Activity of *P. amaryllifolius.*

S. No	Name of the test organism	Zone of inhibition (mm) Mean ± SD				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	Positive Control (PC)
1.	*Staphylococcus aureus*	29.25±0.35	25.35±0.21	23.35±0.21	19.25±0.35	30.10±0.14
2.	*Bacillus subtilis*	28.95±0.35	24.25±0.35	22.45±0.35	18.25±0.35	29.10±0.14
3.	*Klebsiella pneumoniae*	24.6±0.14	21.35±0.21	20.25±0.35	16.35±0.49	28.25±0.35
4.	*Aeromonas hydrophila*	25.45±0.35	22.25±0.35	21.25±0.35	16.35±0.49	28.35±0.49

The values are expressed as Mean ± Standard deviation. P-value < 0.05

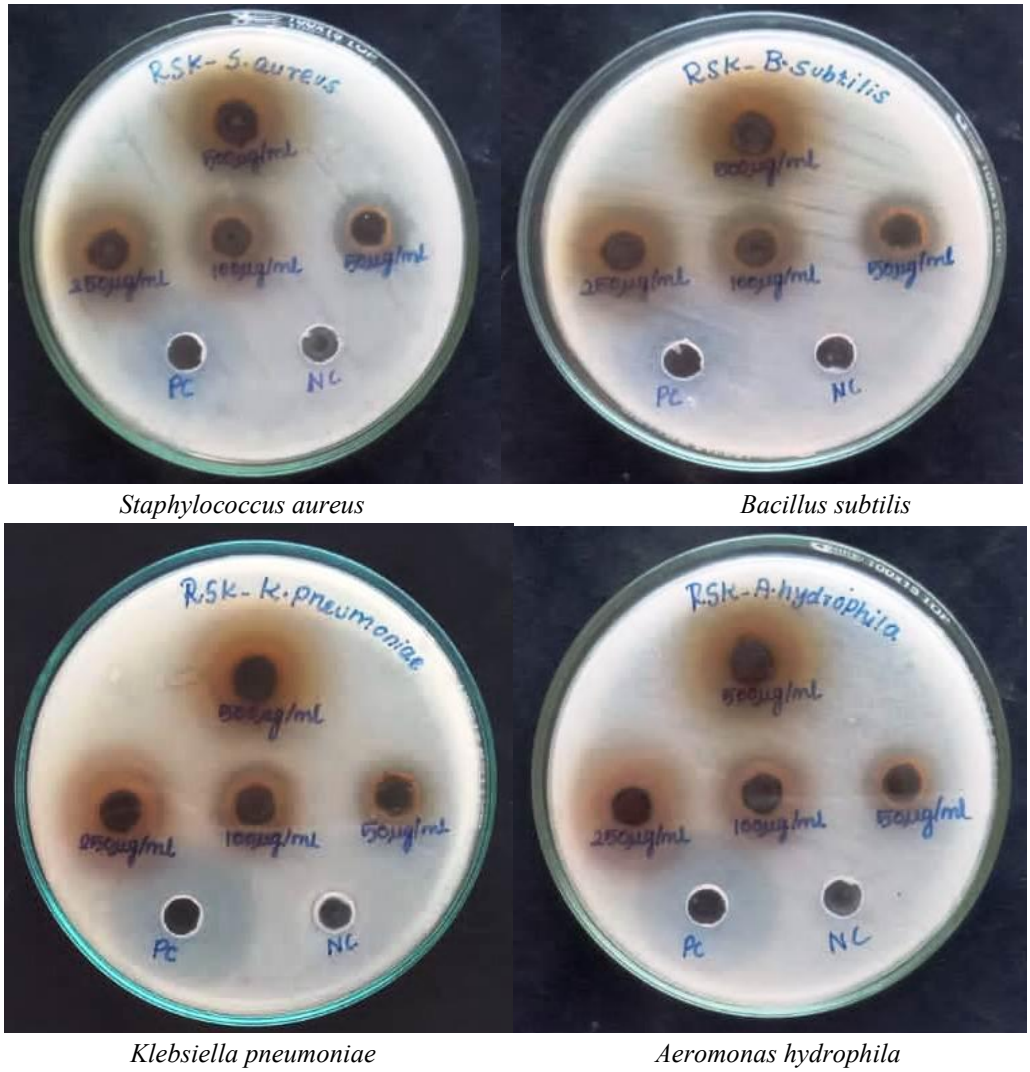


Figure 3. Antibacterial activity of *Pandanus amaryllifolius* leaf extract.

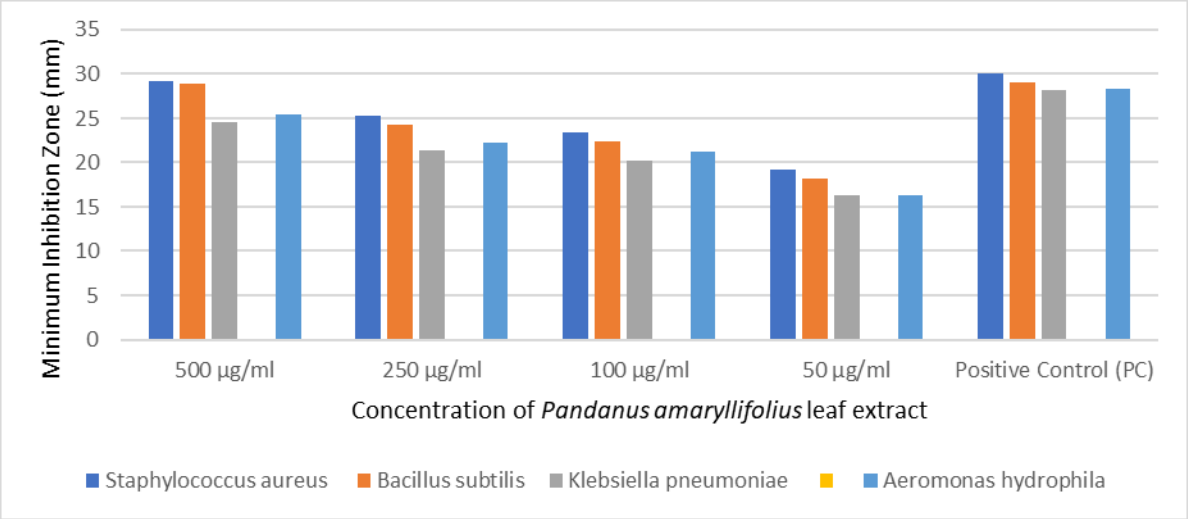


Figure 4. Graphical expression of Antibacterial activity of *Pandanus amaryllifolius* leaf extract.

CONCLUSION

The present study comprehensively evaluated the phytochemical composition FTIR, GC-MS and antibacterial potential of the ethyl acetate extract of *Pandanus amaryllifolius* leaves. Preliminary phytochemical screening revealed the presence of secondary metabolites, including tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, alkaloids, anthraquinone, polyphenol and reducing sugar, indicating the therapeutic potential of the plant. FT-IR spectral analysis identifying characteristic functional groups such as hydroxyl (O–H), alkane (C–H), alkene (C=C), alcohol (O–H), amine (C–N), sulfoxide (S=O) , nitrile (C≡N), and isothiocyanate (N=C=O). These functional groups are commonly associated with b biologically active molecules and play a crucial role in determining the pharmacological properties of plant extracts. GC–MS analysis revealed a complex profile of chemical constituents, including hydrocarbons, fatty acids, esters, phenolic compounds, and other secondary metabolites. The presence of these compounds highlights the chemical diversity of the extracts and provides insight into its potential biological activities. Many of the identified compounds are known to possess antimicrobial, antioxidant, and anti-inflammatory properties. The antibacterialal activity assay demonstrated that the leaf extract exhibits significant inhibitory effects against both Gram-positive and Gram-negative bacteria, namely *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *A.hydrophila*. The extract showed comparatively higher activity against Gram-positive bacteria, which may be attribute to differences in cell wall structure. The observed antibacterial effect is likely due to the synergistic.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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