

CHARACTERIZATION OF ACTIVE BIOMOLECULES FROM ENDOPHYTIC BACTERIUM *MAMMALIICOCCUS SCIURI* ISOLATED FROM *ANDROGRAPHIS PANICULATA* AND ITS ANTIMICROBIAL ACTIVITY AGAINST MULTI DRUG RESISTANT STRAINS

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Abstract–The unprecedented rise in the bacterial infections has led to the exploration of novel antibiotics from endophytic microorganisms. In the present study endophytic bacteria isolated from *Andrographis paniculata* were screened for their antibacterial activities. Out of nine endophytic bacterial isolates a single isolate APNS1 showed significant antimicrobial activity against *Bacillus sp.*, *Salmonella sp.*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and MRSA. The zone of inhibition was shown to be maximum against MRSA (16.6±0.5 mm), followed by *E. coli* (16.3±0.5 mm) and *P. aeruginosa* (16.3±0.5 mm). The selected endophytic bacterium was identified as *Mammaliicoccus sciuri* by 16s rRNA gene sequencing with NCBI accession number PQ169006. The FTIR analysis of the ethyl acetate extract has shown several peaks within a range of 4000 cm⁻¹ to 400 cm⁻¹. The bands later interpreted as aromatic compounds, saturated aliphatic compounds (with CH₂ and CH₃ stretch) and carboxylic acid group. Bioactive compounds such as n-hexadecanoic acid, malonic acid ester and beta-L- arabinopyranoside were identified through GC-MS analysis of the bacterial extract. These bioactive compounds with antimicrobial properties could be considered as potential ingredients for future pharmaceutical studies to develop novel antibiotics.

INTRODUCTION

Antibiotic resistance among pathogens has been a major concern for global healthcare. Based on statistical models, 1.27 million of the 4.95 million deaths that occurred in the year 2019 were caused by bacterial AMR (antimicrobial resistance). Main resistant pathogens associated with the deaths are *Escherichia coli*, MRSA, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* (Murray *et al.*, 2022). The bacterial resistance is mostly due to the production of inactive enzymes, lack of drug target or restricted ability of the antimicrobial agents to enter the cells due to active efflux pump (Zhang *et al.*, 2022). MDR (Multi Drug Resistant) strains have a tendency to become more virulent by transferring genetic material to other strains (Tiwari and Bae, 2020). Antibiotic resistance genes (ARGs) can horizontally transfer among

bacteria by mobile genetic elements (Von Wintersdorff *et al.*, 2016). Numerous studies have shown that antibiotic resistance genes in soil borne microbes can transfer to the plant roots and leaves (Chen *et al.*, 2018). Therefore, studying the endophytes for their antimicrobial activities is relevant and might lead to the discovery of novel antimicrobial compounds. This emphasizes how consuming medicinal plants encompassing endophytic bacteria could contribute to the antimicrobial activity to the resistance.

The current time period has seen an unprecedented rise in bacterial infections and resistance to the antibiotics which possess a great threat to the global health, food security and calls for immediate action (Read *et al.*, 2014, George, 2018). Since no noteworthy new antimicrobial compound has been discovered in recent times, it has become imperative to preserve the effectiveness of the currently accessible antibiotics (Iskandar *et al.*, 2022).

Most researchers have analysed antibacterial properties of plant extracts, essential oils (Gouda *et al.*, 2016). However, we cannot turn a blind eye to the issue of using the plant materials which could belong to the native or endangered plant species (Alvin *et al.*, 2014). Since plants with ethnomedicinal properties share a close relation with their endophytic population, those endophytes with potential medicinal advantages can be taken as possible candidate for the study. Endophytes not only produce medicinally and commercially important metabolites (Asaduzzaman, 2024) but also exhibit antimicrobial and anti-cancer properties (Damavandi *et al.*, 2023).

Endophytes reside in the plant tissues as symbionts without harming the host plant. They produce a variety of novel secondary metabolites which are responsible for plant growth promotion, increase agricultural yield and have tremendous biotechnological potential. Endophytes have been shown to contribute in host resistance by neutralizing invasive phytopathogens (Vega, 2018). Additionally, the production of chitinolytic enzymes by the endophytes aid in the management of solid seafood waste by degrading chitin (Thomas *et al.*, 2020). These characteristics have made endophytes a good choice for harnessing their potential in the industrial and agricultural sectors in a sustainable way. This led us to characterize biomolecules produced by endophytic bacteria isolated from the medicinal plant *Andrographis paniculata*. The plant also commonly called as “chiretta” or “bhui neem” is an erect annual herb belonging to the family acanthaceae with therapeutic applications. Terpenoid lactones and flavonoids isolated mostly from the aerial parts of the plant are responsible for many pharmacological activities (Kumar *et al.*, 2021). The presence of active metabolites such as alkane, diterpenes, ketones and aldehydes in the plant has rendered the plant with various medicinal applications such as anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiallergenic and antimalarial (Dai *et al.*, 2019, Mishra *et al.*, 2009). Andrographolide is one of the major bioactive compounds in the diterpene lactone category present in the plant and has wide range of medicinal applications. Though the medicinal plants produce huge number of metabolites, the current focus has been shifted to the bioactive secondary metabolites produced by their microbiome (Terhonen *et al.*, 2019). Not much data is available on bacterial endophytes isolated from *Andrographis*

paniculata from the region with antimicrobial properties. Therefore, it is essential to explore the plant microbiota for novel antibiotics and other aspects of environmental sustainability. Efforts to replace chemically produced compounds with naturally produced substances has recently gained traction. The chemical diversity in endophytes with medicinal properties have kindled the interest in researchers. Endophytes are known to produce antibacterial substances which have shown activity against multi drug resistant strains. Especially, bacterial endophytes interact closely with the host plant and live in a sheltered habitat with less competition for the nutrients (Reinhold-Hurek *et al.*, 2011). They produce different plant hormones as well as Indole-3-acetic acid (IAA), hence stimulating plant growth and enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase responsible for plant growth and stress tolerance (Glick *et al.*, 2014). They also produce novel antimicrobial volatile compounds thus antagonizing growth of other potential plant pathogens (Santoyo *et al.*, 2016). A number of formulations based on endophytic microorganisms have been developed for commercial purpose (Jayakumara *et al.*, 2020). Antibiotic resistance is a major risk to the global health and researching novel antimicrobial has become essential to tackle such threat.

As most endophytic researches on the plant are based on fungal endophytes, we have focused our work on isolation of bacterial endophytes from the plant in search of novel antimicrobial compounds. The primary aim of the investigation is to identify and characterize potential bioactive compounds with antibacterial properties from the endophytic bacteria isolated from *Andrographis paniculata*.

MATERIALS AND METHODS

Plant sample collection and processing for isolation of endophytic bacteria

Andrographis paniculata fresh twigs were collected from robust plants from a region in puri district, Odisha located at Lat 20.081119p Long 85.833405p in zipped polythene bags and washed with tap water in the lab followed by sterilized distilled water to remove the dust particles and surface microbes. Under aseptic circumstances, plant parts such leaves, stems, and petioles were chopped into pieces using a sterile blade. They were then immersed in 70% ethanol for two minutes and rinsed twice in sterile distilled water. After four

minutes of intermittent treatment in a 4% sodium hypochlorite (NaClO) solution, this procedure was repeated three times, each time being rinsed in sterile distilled water. To guarantee successful surface sterilization, the distilled water in the beaker was spread on a nutrient agar plate as control.

Isolation of endophyte and pure culture

After surface sterilization plant parts were inoculated on petri dish with different media such as water agar, nutrient agar and nutrient agar with 10% leaf extract. The culture plates were incubated at $34\pm 2^\circ\text{C}$ for 3-4 days. After the incubation period bacterial colonies grown closely to the lining of plant parts were collected and streaked on nutrient agar plates and again re-streaked to obtain bacteria pure culture. Pure cultures were secured on the agar slants and preserved at 4°C . Colony morphology of the endophytic bacteria isolated from *Andrographis paniculata* were observed and noted.

Screening of Bacterial Endophytes for antibacterial activities

Filter paper disc method

This method is also known as Kirby-Bauer method (Hudzicki, 2009). Broth cultures of the endophytic isolates were prepared by incubating for 24 hours at $34\pm 2^\circ\text{C}$. The broth culture centrifuged and the supernatant was collected by filtering through micro-filter. Small sterile paper discs of 6 mm size soaked with microbial broth supernatant placed on the Mueller-Hinton agar plates previously swabbed with test microorganisms. The plates were observed for clear rings around the paper discs after incubating at $34\pm 2^\circ\text{C}$ for 24 hours. The slightly clear rings around the discs shows the presence of zone of inhibition.

Bacteriocin like inhibitory substances production test

Bacterial isolates from *A. paniculata* that showed positive activity in the preliminary test were subjected to a test against *Salmonella sp.* (MTCC 1166), *Escherichia coli* (MTCC 433) and *Staphylococcus aureus* (MTCC 96) by the cross-streaking method on Muller-Hinton agar plates. The middle section of the agar plate was streaked with the chosen bacterial isolate perpendicularly, and incubated at $34\pm 2^\circ\text{C}$ for 48 hours. The bacterial culture mass was extracted from the plates entirely and were exposed to chloroform vapour for 30 minutes followed by UV radiation for 30 minutes to ensure the complete

killing of the microbes. After streaking a freshly cultivated broth of test microorganisms at a right angle to the cleared line of the endophyte, the plates were incubated for 24 hours at $34\pm 2^\circ\text{C}$ for observation.

MIC

MIC of the extract was determined by microbroth dilution method. 100 μl of sterilized MH broth added to ten wells of the microtiter plate followed by the addition of 100 μl extract to the first well and diluted serially. 20 μl broth culture of test microorganism with 1.5×10^8 cfu/ml added to each well. Well only with the extract and no test microorganism was taken as positive control. The plates incubated for 24 hours at $34\pm 2^\circ\text{C}$. 20 μl resazurin dye (0.015%) added to each well after incubation and left for 4 hours for colorimetric observation. To determine the MMC (Minimum Microbicidal Concentration), culture broth from each well streaked on MH agar plates and incubated at $34\pm 2^\circ\text{C}$ for 24 hours.

Extract preparation

The extraction was carried out following the method described by Balachandran (2012) with slight modification. The selected endophytic isolate was inoculated in 300 ml Mueller-Hinton broth and was incubated for 6 days at $34\pm 2^\circ\text{C}$. The broth culture was then centrifuged for 10 minutes at 8000 rpm. The collected supernatant was acidified and extracted with equal volume of ethyl acetate (1:1 v/v). The organic phase containing active metabolites was separated and evaporated to get it in a concentrated form. The crude extract was redissolved in Dimethyl Sulfoxide (DMSO) for further study and preserved at 4°C .

Agar Well Diffusion

The ethyl acetate extract of the selected isolate was tested against six test microorganisms *viz.* *Salmonella sp.* (MTCC1166), *Bacillus sp.* (MTCC297), *Escherichia coli* (MTCC433), *Staphylococcus aureus* (MTCC96), *Pseudomonas aeruginosa* (MTCC9800) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (MTCC7443) through agar well diffusion method. The MH agar plate surface was swabbed with the test microorganism culture broth. Using a cork borer, agar wells were made on the plates and 20 μl of the extract was added to three of the wells and DMSO was added to the control well. Incubated for 24 hours at $34\pm 2^\circ\text{C}$ and the zone of inhibition was measured.

Screening for biochemical activities

The selected isolate was tested for different biochemical activities such as protease, lipase (Ghodsalavi *et al.*, 2013), catalase (Malleswari and Bagyanarayana, 2013), oxidase, urease, simmons citrate, MR test, voges-proskauer test and triple sugar iron test.

Molecular Identification of the selected isolate

The bacterial identification was done by 16s rRNA gene sequencing through Sanger dideoxy sequence method. The rDNA fragment was amplified using high fidelity DNA polymerase. The amplification was performed using 5'-CCGTGTGTACAAGGCCCGG-3' reverse and 5'-GGATGAGCCCGCGGCCTA-3' forward primers. 94°C for 3 minutes of initial denaturation followed by 30 cycles of 94 °C denaturation for one minute, annealing at 50 °C for one minute, extension at 72 °C for two minutes, and final extension for 7 minutes at 72 °C. Sequencing and fragment analysis done with ABI 3130xl genetic analyser. The Basic Local Alignment Search Tool (BLAST) on NCBI was used to analyse the nucleotide sequences of the 16s rRNA gene. MEGA 12 software was employed for bootstrap analysis and phylogenetic tree was built using the neighbor-joining technique.

FTIR Spectroscopy

Analysis of the sample using Fourier Transform Infrared Spectroscopy (FTIR) was performed using a Thermo Nicolet-6700 FT-IR device to detect the presence of different functional groups in the bacterial extract. Before loading, the extract was completely dried for the analysis at the transmission mode from 400-4000 cm⁻¹. Different spikes obtained from the FTIR were noted and tallied with the chart for infrared absorption bands to know the presence of functional groups.

GC-MS analysis of the crude extract

The analysis was conducted by using Perkin Elmer, Model- Auto system XL with turbo mass equipped with Elite- 5MS (30 metres x 0.250 mm x 0.250 µm) separating column. The mass range was set to 20-620 amu. The oven temperature was set at 280 °C. The EI source temperature was set at 220 °C. The flow rate of the carrier Helium gas was set at 1ml/min. A 50-minute-long run was conducted and the mass spectrum of the peaks were compared with the spectrum of already investigated compounds in NIST database for the identification of the

compounds. The number of hits with reverse search, molecular weight and molecular formula were recorded.

RESULTS AND DISCUSSION

Sample collection and processing for isolation of pure culture

A total of 9 bacterial endophytes were isolated, six from stem, one from the leaf, and two from the petiole. All the isolates were sub-cultured and maintained in slants. The colonization frequency was found to be higher in stem (18.75%) followed by leaf and petiole (6.25%). Five bacteria colonies were pigmented. The isolate APNS2 showed swarming colonies with rapid and collective movement across the surface.

Activity of isolated endophytes against test microorganisms

In the initial antibacterial screening four out of nine isolates showed activity against *Salmonella sp.*, *Escherichia coli* and *Staphylococcus aureus*. Only one isolate, i.e APNS1 showed positive BLIS activity by inhibiting the growth of all test microorganisms upon cross streaking. The lowest MIC value of the bacterial extract was calculated 0.75 mg/ml. against *E. coli* and *S.aureus*. It reveals that the selected isolate produces bacteriocin like substance which is in fact responsible for the antimicrobial activity and is water soluble in nature. Antibacterial substances also known as bacteriocin are produced by ribosomal or non-ribosomal enzymatic systems with varying size and structure (Cotter *et al.*, 2013). Due to their diverse molecular targets and lethality,

Table 1. Biochemical characteristics of APNS1 (*M. sciuri*)

Biochemical tests	Result
Lipase	-
Protease	+
Catalase	+
Oxidase	+
Urease	-
Simmon's citrate	-
Methyl red	+
Voges Proskauer	-
TSI	
Glucose	+
Sucrose	-
Lactose	-
Gas	-
H ₂ S	-

bacteriocins are known to have inhibitory effects on wide range of multi drug resistant bacteria (Simons *et al.*, 2020). The crude extract of the isolate showed antibacterial activity against all six test microorganisms (Table 2) which is relevant to the study and proves its potential to have broad spectrum antibacterial substances. The maximum zone of inhibition shown against MRSA (16.6±0.5 mm) followed by *E. coli* (16.3±0.5 mm) and *P. aeruginosa* (16.3±0.5 mm). The selected isolate was later tested for other biochemical activities and showed positive result for catalase, oxidase, protease and methyl red (MR) test. In the gram staining the organism was found to be gram-positive cocci.

Table 2. Zone of Inhibition shown by bacterial extract

Test microorganisms	Zone of Inhibition (mm)
<i>E. coli</i>	16.3±0.5
<i>Salmonella sp.</i>	14.6±0.5
<i>Bacillus sp.</i>	12
<i>S. aureus</i>	12.3±0.5
<i>P. aeruginosa</i>	16.3±0.5
MRSA	16.6±0.5

Identification of the bacterial isolate

Total base nucleotide analysis for 16s rRNA gene sequence was done through bootstrap analysis using neighbor-joining technique to construct the phylogenetic tree (Fig. 1). The BLAST analysis showed 99% similarity with *Mammaliococcus sciuri* strain GH-02 16s rRNA gene. The gene sequence was submitted to the gene bank with NCBI accession number PQ169006. In record *Staphylococcus sciuri* has been recently reclassified as *Mammaliococcus sciuri* (Madhaiyan *et al.*, 2020). *Mammalii coccussciuri* is a gram positive and coagulase negative cocci normally found on the skin of the animals and humans (Madhaiyan *et al.*, 2020). They are also found in the soil microbiota (Varier *et al.*, 2025). In a similar research endophytic *Staphylococcus sp.* has been found to be one of the most abundant genus in *A. paniculata* (Kumari *et al.*, 2023). *S. sciuri* isolated from the medicinal plant *Peganum harmala* L. has been reported to have shown antagonistic activity against MRSA (Bibi, 2017). In a study *Staphylococcus sciuri* isolated from food chicken was found to contain biosynthetic gene cluster which encodes the peptide *micrococcin P1*, a type of bacteriocin classified as a thiopeptide that

targets bacterial ribosome thus inhibiting protein synthesis (Fernández-Fernández *et al.*, 2025). In a report *Mammalii coccussciuri* from strawberry stolon was found to produce antifungal compounds and also had salt tolerance potential (Alijani *et al.*, 2019). *M. sciuri* from kiwi fruit reported as the dominant group in the total isolated endophytic bacteria and possessed significant antinematode potential (Banihashemian *et al.*, 2022).

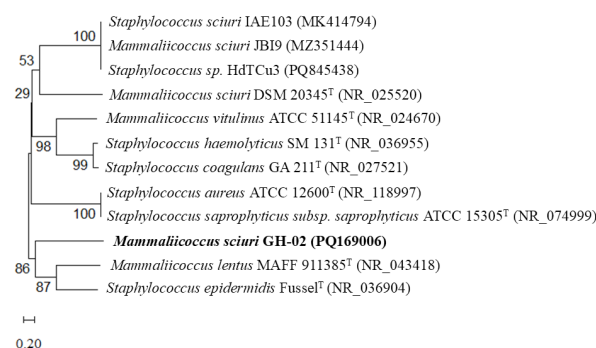


Fig. 1. Phylogenetic tree constructed with 16s rRNA gene sequence of *Mammaliococcus sciuri* GH-02 (APNS1) using neighbor-joining method (MEGA 12). The analysis consists of 12 nucleotide sequences. Positions containing gaps and missing data were removed. Numbers at each node indicates bootstrap value.

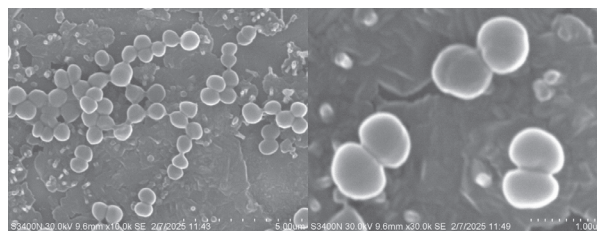


Fig. 2. Scanning electron microscopy of APNS1 (*M. sciuri*) showing gram positive cocci cell dynamics and cell splitting

FTIR

The FTIR analysis (Fig. 3) of the ethyl acetate extract of APNS1 showed an IR spectrum with several peaks within a range of 3909.94 cm^{-1} to 405.82 cm^{-1} (Table 3). The interpretation of the bands showed the presence of compounds such as carboxylic acids, saturated aliphatic compounds (with CH_2 and CH_3 stretch) and aromatic compounds with disubstituted benzene rings.

Analysis of the extract using GC-MS

Peaks with more than 2% area were picked and identified by comparing mass spectra in NIST database. The compounds found are listed in Table

4. A total of six compounds with higher percentage area were identified. 2-Butanol, 3-methyl showed highest percent area (35.81%) with a retention time of 6.09 minutes. 2-Butanol, 3-methyl is normally used as an intermediate to manufacture other chemicals (Almasi and Mohebbifar, 2024). The next compound in the list i.e. N- Hexadecanoic acid has antioxidant, antibacterial (Ganesan *et al.*, 2024) and anti-inflammatory (Aparna *et al.*, 2012) properties. The GC-MS analysis of the fatty acid composition of the biosurfactant from *Staphylococcus sciuri subsp. rodentinum* strain SE1 has shown the presence of hexadecanoic acid (30.97%) as a major fatty acid

with a retention time of 26.89 minutes (Sharma *et al.*, 2022). In the GC-MS analysis, beta-L-arabinopyranoside was found in the extract which has previously been reported from the plant *Parkia timoriana* and has anti-inflammatory and anti-cancer properties (Ralte *et al.*, 2022). The compound also has been found to be one of the major bioactive compounds in *Eranthemum indicum* and has medicinal properties (Nonglang *et al.*, 2022).

The bioactive compound with an area of 3.92% in the GC-MS table could be interpreted as an aminobenzyl alcohol with a carboxylic acid group as the base peak 91 in the mass spectra is due to Tropilium ion which means the molecule has to

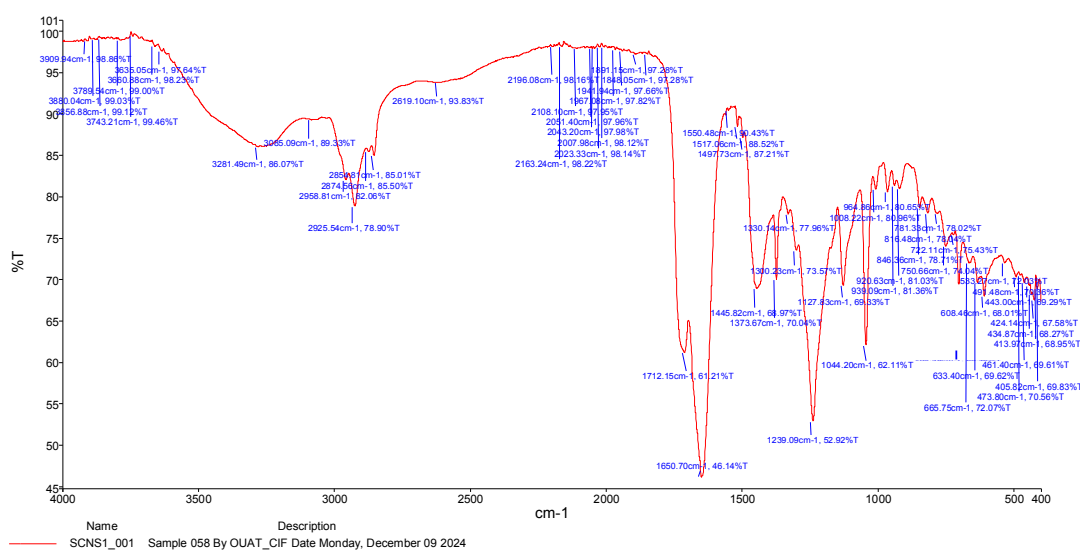


Fig. 3. Fourier transform infrared spectroscopy (FTIR) spectrum of APNS1 extract with different wave numbers within a range of 4000 cm^{-1} to 400 cm^{-1}

Table 3. Functional groups present in the extract of APNS1 (*M. sciuri*) detected through FTIR

Wave number(cm^{-1})	Assignments	Interpretation of the band
3500 cm^{-1} - 3085 cm^{-1}	-OH broad	Presence of hydroxyl group
2958 cm^{-1}	Asymmetric CH_3 stretch	Presence of CH_3
2925 cm^{-1}	Symmetric CH_3 stretch	
2874 cm^{-1}	Asymmetric CH_2 stretch	Presence of CH_2
2854 cm^{-1}	Symmetric CH_2 stretch	
1712 cm^{-1}	C=O stretch	Carboxylic acid group (not ketone or aldehyde as -OH stretch is also observed)
1650 cm^{-1}	C=C stretch	3 bands of aromatic stretch
1445 cm^{-1}		
1373 cm^{-1}		
1239 cm^{-1}		
1127 cm^{-1}	Fingerprint region	
1044 cm^{-1}	C-O stretch	Carboxylic acid group
750 cm^{-1}	C-H bend	Aromatic (The compound could be an aromatic carboxylic acid having CH_3 and CH_2 substitution)
722 cm^{-1}		
665 cm^{-1}		

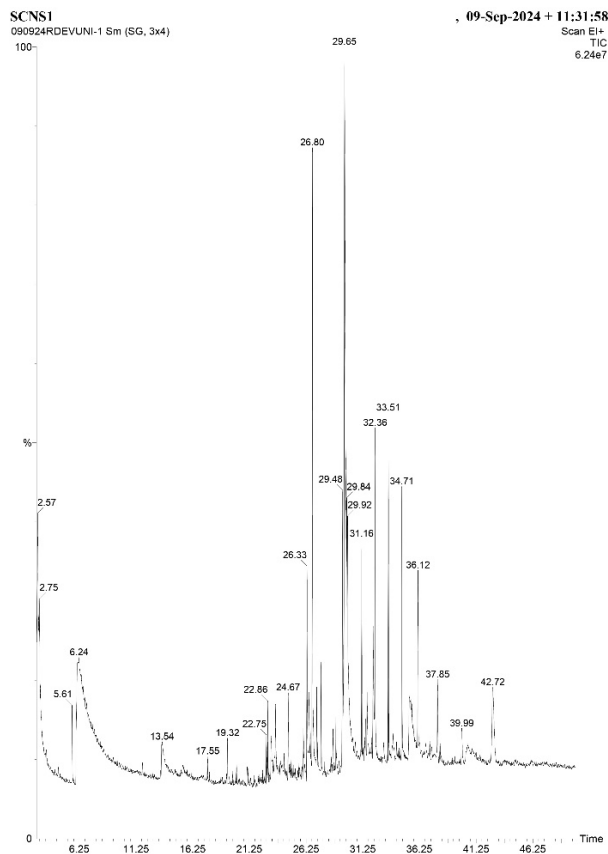


Fig. 4. GC-MS chromatogram of ethyl acetate extract of the bacterial isolate APNS1 showing different peak area

have a benzene ring attached to a $-CH_2$ group. The IR data also confirms the presence of $-COOH$ group. Aminobenzyl alcohol can react with other compounds to form various N-heterocycles such as quinolines and quinazolines through catalytic system (Doraghi *et al.*, 2024). These compounds have broad range of pharmaceutical applications such as antibacterial, antimalarial, anticancer, anti-inflammatory (Ajani *et al.*, 2022) and antiviral (Zhao *et al.*, 2023). *Staphylococcus sciuri* has been found to produce acetic acid, butyl ester (Zahra *et al.*, 2019) whereas; in the study it is found to be producing malonic acid, dihydroxy diisobutyl ester. Malonic acid esters are known to have antimicrobial properties (Jacob *et al.*, 2020). It has also been isolated from the extract of saffron floral waste (Yousuf *et al.*, 2017). According to a report, *Mammali cocussciuri*, which was isolated from raw goat milk was found to be the most powerful bacteriocin-producing and bile-tolerant microbe among the six isolates exhibiting probiotic potential (Naqqash *et al.*, 2022). A few strains of *Staphylococcus sciuri* are capable of producing surfactin and can act as agent for bioremediation (Sharma *et al.*, 2022).

CONCLUSION

The endophytic isolate APNS1 from *Andrographis paniculata* showed antibacterial properties and was

Table 4. Compounds present in the extract of APNS1 (*M. sciuri*) identified by GC-MS

Sl No.	Compound name	Molecular formula	MW	RT (min)	Area %	Molecular structure	Chemical nature
1	2-Butanol, 3-methyl	$C_5H_{12}O$	88	6.090	35.818		Alcohol
2	N- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.808	6.692		Saturated long-chain fatty acid
3	Beta-L-arabinopyranoside, methyl	$C_6H_{12}O_5$	164	29.484	2.920		Glycoside
4	Hexadecane	$C_{16}H_{34}$	226	33.51	3.01		Alkane hydrocarbon
5	Aminobenzyl alcohol-3-carboxylic acid	$C_8H_9NO_3$	167	35.392	3.926		Aromatic amine
6	Malonic acid, dihydroxy diisobutylester	$C_{11}H_{20}O_6$	248	42.740	3.329		diisobutyl ester

identified as *Mammalii coccussciuri* with NCBI accession number PQ169006. The novelty of the study lies in determining the effectiveness of the isolated compounds from the endophytic bacteria against drug resistant strains. Bioactive compounds like n-hexadecanoic acid, malonic acid ester and beta-L- arabinopyranoside identified by FT-IR and GC-MS analysis from the extract reported to have antibacterial properties. These bioactive compounds could be taken as promising antibacterial agents with further research to exploit their biotechnological potential for application in pharmaceutical and cosmetic industries. Further analytical chromatographic techniques and relevant methods should be employed to determine the relevant bioactive compound accountable for the antimicrobial activity.

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Conflicts of interest All the authors declare no conflict of interest.

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