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Extraction and identification of bioactive compounds from the mucus of a slug Laevicaulis alte

Suja P.S [1,4], Nija.C *[2,4] and Navinchandran.M [3,4]

***Corresponding author**.
Email: ID: nijadhas08@gmail.com

[1]Reg. No. 23113152192006, Research Scholar, ORCID ID 0009-0002-4200-3827, Department of Zoology, S.T.Hindu College, Nagercoil,
Tamil Nadu – 629002.
[2]Associate Professor and Head of the Department of Zoology, Women's Christian College, Nagercoil, Tamil Nadu – 629001. ORCID ID 0009-0003-0280-3325.
[3]Assistant Professor, Department of Zoology, S.T.Hindu College, Nagercoil, Tamil Nadu – 629002.
[4]Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, INDIA..

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ABSTRACT

Since ancient times, natural products have been crucial in maintaining human health because of their therapeutic benefits. The majority of prescribed drugs in affluent countries are extracted from mollusks. The emergence of drug resistance in human diseases against commonly used antibiotics has necessitated a search for novel antimicrobial molecules from a variety of sources. The land slug, Laevicaulis alte, is commonly seen in the Kanyakumari District. Its mucus is a sticky, elastic fluid with adhesive and lubricating properties. The biochemical characteristics of the land slug Laevicaulis alte mucus was studied, the results showed that the fat, protein, and carbohydrate content of slug mucus was substantially higher. Using GC-MS analysis, the bioactive components of slug crude mucus have been assessed, the majority of the compounds have antimicrobial properties.15 antimicrobial compounds extracted among these Octadecane (42.747), Tetrapentacontane (39.510),1-Heptacosanol (39.414) have high retention time. The identified compounds are known to have therapeutic benefits

KEYWORDS: Antimicrobial properties, Slug, Laevicaulis alte, Biochemical components, Bioactive compounds

INTRODUCTION

New indigenous medical systems like Siddha, Ayurveda, and Unani originated in India. Since ancient times, natural items have been a significant source of pharmaceuticals; currently, almost half of all effective medications come from natural sources. Since more than half of all contemporary clinical medications are derived from natural products, the hunt for natural products has produced novel candidates for drugs that are utilized to treat a variety of illnesses (Grace et al., 2011).

Snails and slugs belongs to the phylum Mollusca and class Gastropoda. Slugs are classified as snails without a shell, as opposed to snails, which have hard, calcareous shells covering their bodies (Barker, 2002; Ramzy, 2009). Slime, commonly referred to as mucus, is a sticky, elastic fluid with adhesive and lubricating properties that allows slugs and snails to adhere tenaciously to a range of surfaces. According to Hamalalnen et al. (2012) and South et al. (1992), mucus also keeps mollusks from becoming dehydrated and discourages potential predators from catching snails and slugs.

Snails and slugs, long valued in folk and traditional medicine, are now gaining attention in scientific research for their mucus properties. The biological and chemical characteristics of snail mucus are well detailed in the literature (Cilia et al., 2018); however, this does not occur in terrestrial slugs. Mucus from snails and slugs has been linked to general skin regeneration qualities for ages (Cilia et al., 2018).

When allergens bind to mast cells in the airway, the bioactive compound extracted from the slug causes the tracheal smooth muscle contraction to relax, which is caused by histamine (Jacob et al., 2013). Slugs need to defend themselves from a variety of predators and harmful bacteria that could deposit on their skin. A key factor in achieving this chemical protection is the use of natural products. It has already been shown that the slugs absorb and improve upon beneficial substances from their diet (Bogdanov et al., 2014, 2016).

The lectins mucopolysaccharide and glycoprotein are found in slug mucus (Deyrup Olsen et al., 1983). High levels of galactosamine and galactose were detected in the glycosaminaglycan of the Arion ater slug (Cottrellet al., 1994). Furthermore, Limacus flavus possesses domains that are homologous to the fibrinogen-related domain superfamily and likely contribute to blood coagulation. Pemberton (1970) observed that saline extracts from L.flavus contained nonspecific agglutinins for human erythrocytes. In the medical field, Li et al. created a surgical glue in 2017 using the mucus of the slug Arion subfucus...

There are two methods for producing small bioactive molecules: chemical synthesis or extraction from living things. Numerous species generate a wide range of natural products, some of which have biological function, from tiny peptides to chemical compounds. According to Alaish et al. (1996), natural bioactive compounds are created as chemical signals to regulate regular physiological functions like growth and differentiation.

Ulagesan and Kim, 2018 extracted a potent crude proteins from the land snail *Cryptozona bistrialis*, which was able to totally stop the growth of pathogenic fungi like *A. fumigatus, Candida albicans, Micrococcus luteus*, and *Pseudomonas aeruginosa*, as well as pathogenic bacteria like *Staphylococcus aureus, Micrococcus luteus, and Pseudomonas aeruginosa*.

In this study, biochemical components and bioactive compounds of terrestrial slug *Laevicaulis alte* have been evaluated using GC-MS techniques. The aim of this work focuses on the isolation and purification of bioactive compounds from the terrestrial slug *Laevicaulis alte* mucus. Literature survey revealed that not much work has been carried out in *Laevicaulis alte.* Hence, the *Laevicaulis alte* was chosen for the present study with well defined executable objectives, to identify the bioactive compounds present in the crude mucus of terrestrial slug *Laevicaulis alte.*

2. MATERIALS AND METHODS

2.1 Collection of Species

Specimens of *Laevicaulis alte* were brought to the lab from the Kanyakumari district.

2.2 Mucus Collection

Before the animals were put in a sterile petri plate to collect mucus, they were cleaned with distilled water. A 2% citric acid solution was used to collect mucus because it stimulates slugs to make more mucus. After collecting on the petri dishes, the mucus was scraped up with a spatula and refrigerated. This mucus is crude mucus.

2.3 Estimation of Biochemical Components

Protein was estimated by using the procedure by Lowry et al., (1951), carbohydrate by Carroll et al., (1956) and Lipid by Frings et al., (1972).

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

A Shimadzu GC-MS-QP2010 gas chromatograph mass spectrometer equipped with a Rtx-5 fused silica capillary column (30 X0.25 mm, with 1 cm film thickness) and interfaced with a Turbo Mass quadrupole mass spectrometer was utilized to perform the GC-MS analysis of the material. The oven was set to rise from 1000°C to 3200°C at a rate of 1000°C per minute, with a 10-minute hold in between. At a flow rate of 1.0 mL/min, helium was employed as the carrier gas. The split ratio was 1:10, the injector temperature was 2500C, and the injection size was 1 µL neat. The mass spectra were obtained at 70eV with a mass scan range of 40-700 amu (atomic mass unit), while the interface and MS ion source were kept at 3200C and 2000C, respectively. Data handling was done using GC-MS solution software.

2.5 Identification of the components

The National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns, was used to interpret the mass spectrum. Unknown components' fragmentation pattern spectra were contrasted with those of known components that were kept in the NIST library. Each bio-component's relative percentage amount was determined by comparing its average peak area to the total area. The components of the test materials were identified by name, molecular weight, and structure.

3. RESULTS

3.1 Biochemical content of slug mucus

Table 1 shows the amounts of fat, protein, and carbohydrates in the mucus of the land slug *Laevicaulis alte*.

In order to conduct this study, the mucus of the terrestrial slug *Laevicaulis alte* was analysed for fat, protein, and carbohydrate content. The results were represented in milligrams per litre. (**Table.1**)

Table.1 Biochemical content of Slug mucus

Sample	**Biochemical Assays**		
Mucus	**Carbohydrate (mg/l)**	**Protein (mg/l)**	**Lipid (mg/l)**
	169.133 ± 0.057	114.234 ± 0.005	10.51 ±0.026

The result showed that the slug *Laevicaulis alte* mucus has high percentage carbohydrate content. It could be said that *Laevicaulis alte* mucus is a rich source of carbohydrates. The result also revealed that *Laevicaulis alte* mucus has a moderate amount of protein and low lipid value. (**Figure .1**)

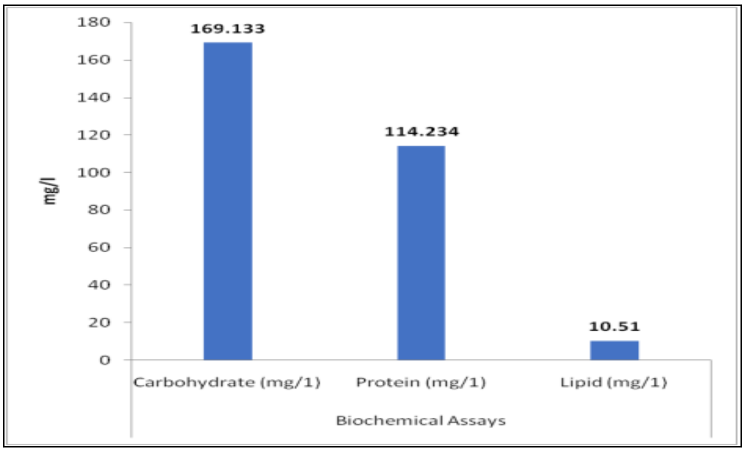


Figure.1 Biochemical content of Slug mucus

3.2 Bioactive compounds from slug crude mucus

There is a dearth of literature on the chemical components of *Laevicaulis alte* mucus. Through the use of GC-MS analysis, the bioactive compounds in *Laevicaulis alte* crude mucus was identified and confirmed. As a function of retention time, the gas chromatogram shows the relative concentrations of the various compounds that are eluted. The relative amounts of each component in the mucus are shown by the peak heights. For the GC-MS analysis of the active compounds in *Laevicaulis alte* chloroform extract of crude mucus was taken. 22 bioactive compounds were detected in the crude mucus (**Figure 2**), of which 15 compounds were with antimicrobial properties.

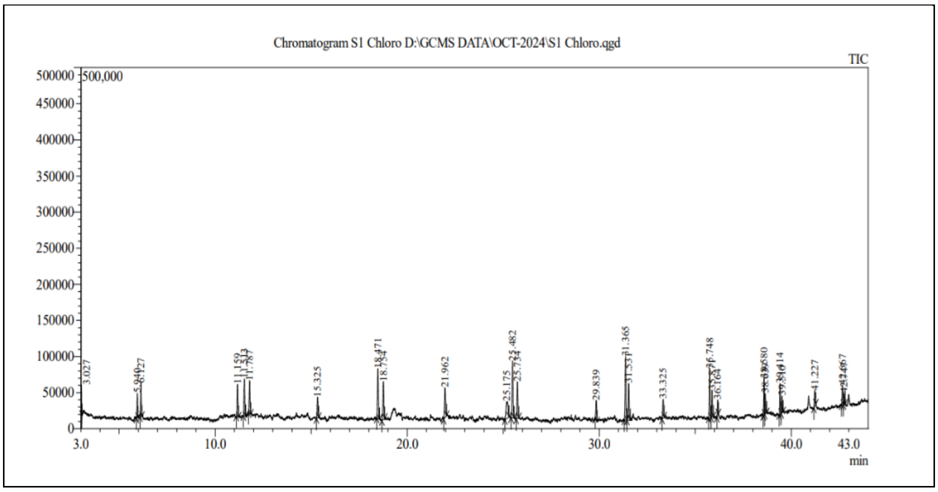


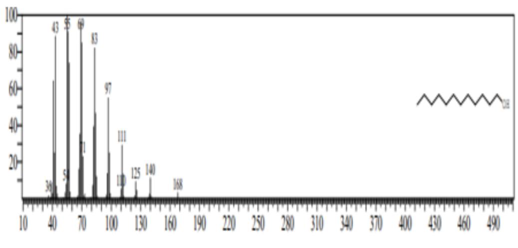
Figure 2. GC-MS chromatogram of *Laevicaulis alte* crude mucus

3.3 Antibacterial compounds from slug crude mucus

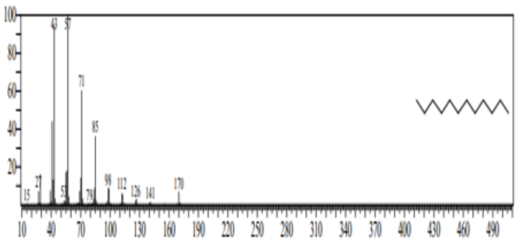
Table 2 enumerates the antibacterial compounds present in slug crude mucus. The compounds listed include various fatty alcohols such as 1-Dodecanol, 1-Tetradecanol, n-Pentadecanol,1-Hexacosanol and1-Heptacosanol; alkanes like Dodecane, Tetradecane, Hexadecane, Tetrapentacontane, and Octadecane; and other chemical structures including the aromatic compound O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl, the phenolic compound 2,4-Di-tert-butylphenol and cyclononasiloxane, octadecamethyl a siloxane compound. Compounds ranging from lighter ones such as dodecane (170 g/mol) to heavier molecules like cyclononasiloxane, octadecamethyl (666 g/mol) demonstrate the broad spectrum of molecular weights, showcasing the chemical diversity of mucus components that may possess antibacterial properties. **Figure 3** displays the mass spectrum and structure of each unique compounds.

Table.2 Antibacterial compound from slug crude mucus

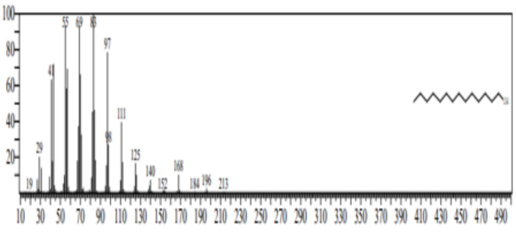
S. No	Retention time	Area %	Compound name	Compound nature	Molecular formula	Molecular weight
1	5.940	2.10	1-Dodecanol	Fatty alcohol	$C_{12}H_{26}O$	186
2	6.127	3.08	Dodecane	Alkane	$C_{12}H_{26}$	170
3	11.513	4.28	1-Tetradecanol	Fatty alcohol	$C_{14}H_{30}O$	214
4	11.787	3.87	Tetradecane	Alkane	$C_{14}H_{30}$	198
5	15.325	2.54	2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	206
6	18.471	6.60	n-Pentadecanol	Fatty alcohol	$C_{15}H_{32}O$	228
7	18.754	5.18	Hexadecane	Alkane	$C_{16}H_{34}$	226
8	25.175	4.89	Cyclononasiloxane,octadecamethyl	Siloxane	$C_{18}H_{54}O_9Si_9$	666
9	35.748	5.34	1-Hexacosanol	Fatty alcohol	$C_{26}H_{54}O$	382
10	38.580	3.89	O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl	Aromatic	$C_{28}H_{42}O_2$	410
11	39.414	3.08	1-Heptacosanol	Fatty alcohol	$C_{27}H_{56}O$	396
12	39.510	1.77	Tetrapentacontane	Alkane	$C_{54}H_{110}$	758
13	42.747	1.29	Octadecane	Alkane	$C_{18}H_{38}$	254

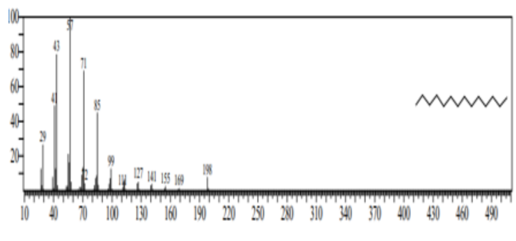


1-Dodecanol



Dodecane





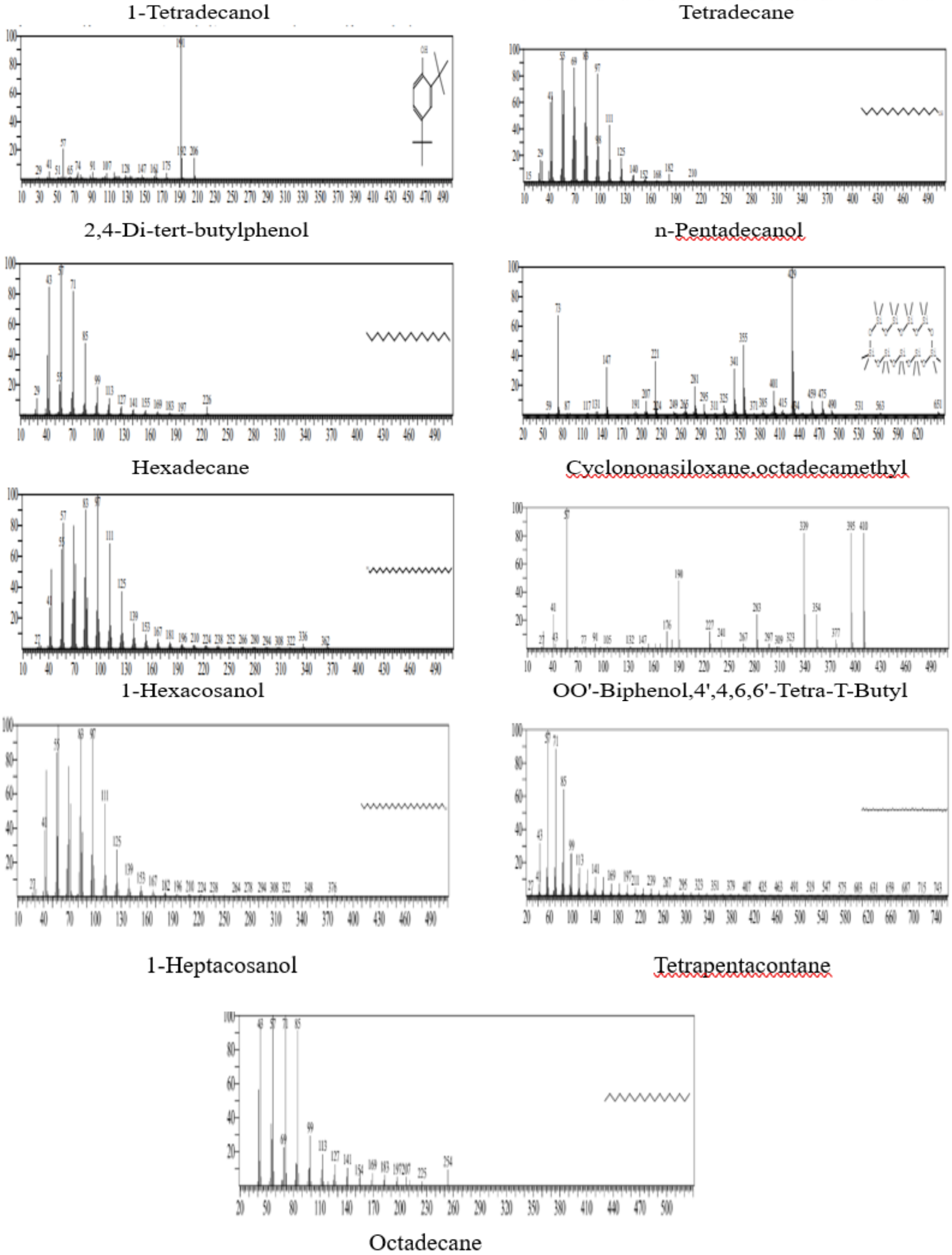


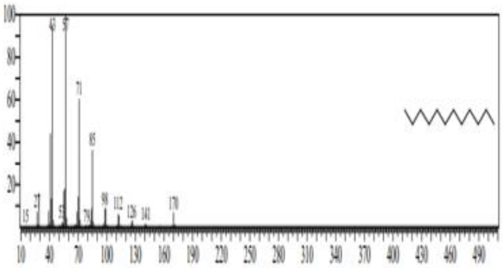
Figure 3. Mass fragmentation of the antibacterial compounds identified in the *Laevicaulis alte* crude mucus

3.4 Antifungal compounds from slug crude mucus

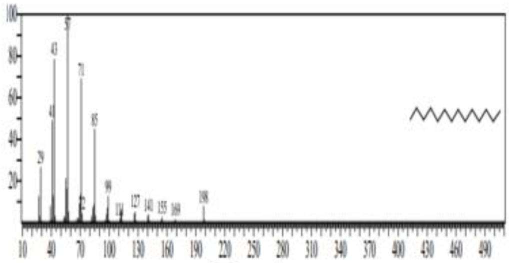
Various antifungal chemicals detected in slug crude mucus are listed in **table 3.** Alkanes like dodecane, tetradecane, eicosane, tetrapentacontane, and octadecane are among these compounds. 1-hexacosanol and 1-heptacosanol are fatty alcohols, phenols like 2,4-Di-tert-butylphenol, alkenes like 1-nonadecene, and aromatic compounds like O O'-Biphenol 4,4',6,6'-tetra-T-butyl. 2,4-Di-tert-butylphenol is considered one of the important antifungal compound. The molecular weights indicate a broad range of chemical types in the mucus that may have antifungal characteristics, ranging from lighter ones like dodecane (170 g/mol) to heavier ones like tetrapentacontane (758 g/mol). **Figure 4** displays the mass spectrum and structure of each unique compounds.

Table.3 Antifungal compounds from slug crude mucus

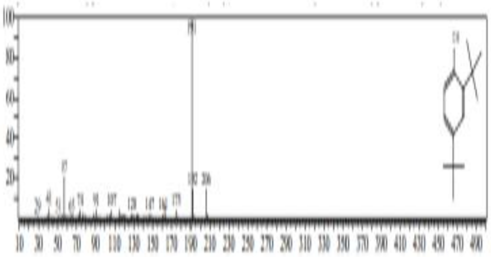
S.No	Retention time	Area%	Compound name	Compound nature	Molecular formula	Molecular weight
1	6.127	3.08	Dodecane	Alkane	$C_{12}H_{26}$	170
2	11.787	3.87	Tetradecane	Alkane	$C_{14}H_{30}$	198
3	15.325	2.54	2,4-Di-tert-butylphenol	Phenol	$C_{14}H_{22}O$	206
4	25.482	8.08	1-Nonadecene	Alkene	$C_{19}H_{38}$	266
5	25.734	4.79	Eicosane	Alkane	$C_{20}H_{42}$	282
6	35.748	5.34	1-Hexacosanol	Fatty alcohol	$C_{26}H_{54}O$	382
7	38.580	3.89	O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl	Aromatic	$C_{28}H_{42}O_2$	410
8	39.414	3.08	1-Heptacosanol	Fatty alcohol	$C_{27}H_{56}O$	396
9	39.510	1.77	Tetrapentacontane	Alkane	$C_{54}H_{11O}$	758
10	42.747	1.29	Octadecane	Alkane	$C_{18}H_{38}$	254



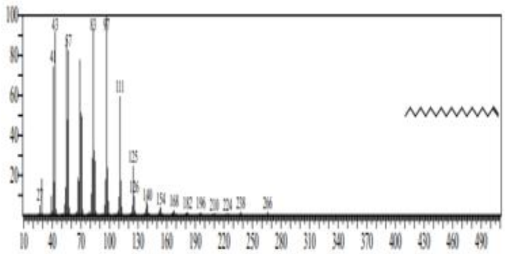
Dodecane



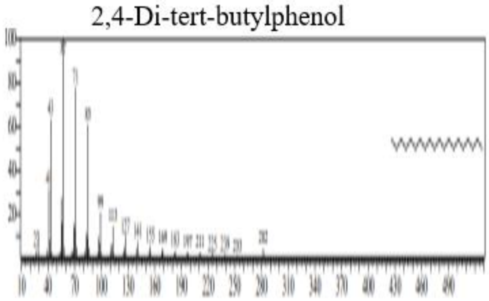
Tetradecane



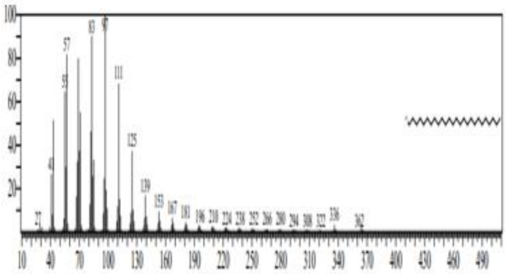
2,4-Di-tert-butylphenol



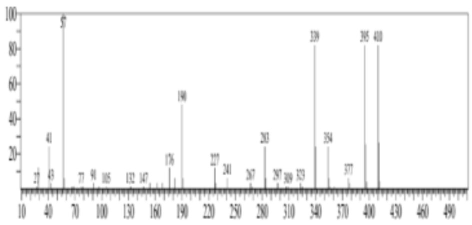
1-Nonadecene



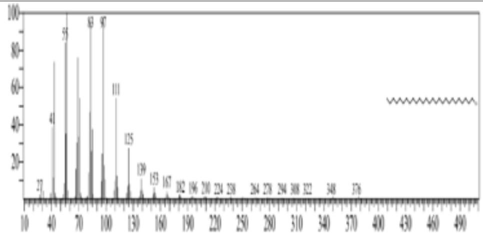
Eicosane



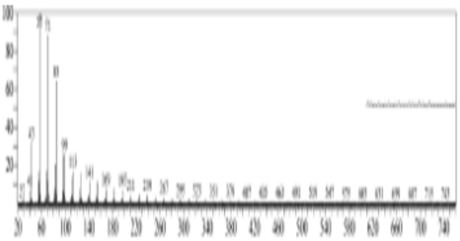
1-Hexacosanol



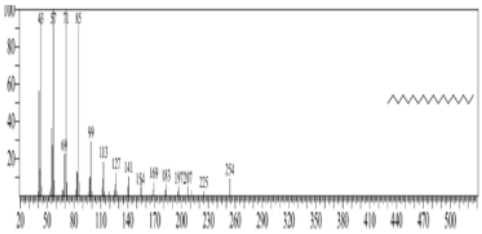
O O'-Biphenol,4',4,6,6'-Tetra-T-Butyl



1-Heptacosanol



Tetrapentacontane



Octadecane

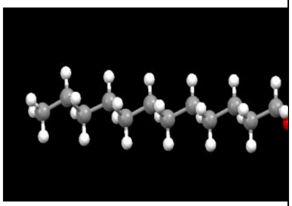
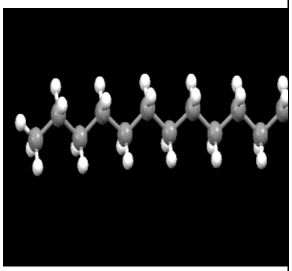
Figure 4. Mass fragmentation of the antifungal compounds identified in the *Laevicaulis alte* crude mucus

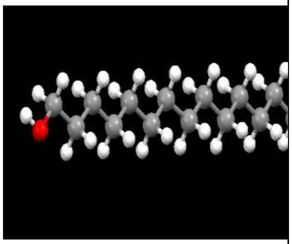
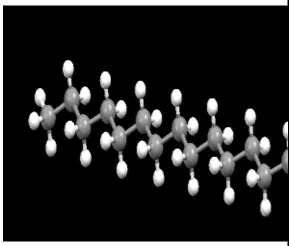
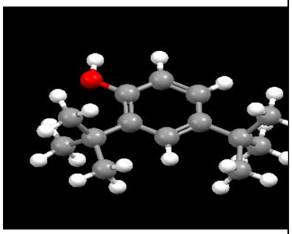
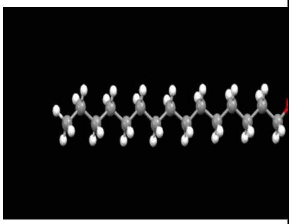
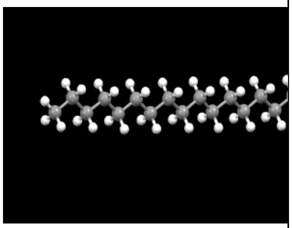
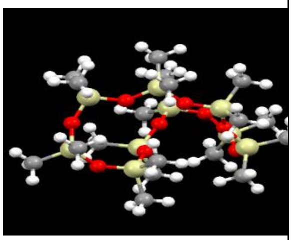
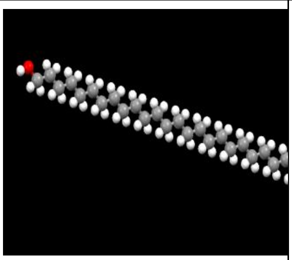
The 3D structure of antibacterial compounds in the crude mucus presented in **table 4.** 1-Dodecanol is a saturated fatty alcohol with a 12-carbon atom chain exhibits antibacterial activity. Dodecane is a straight-chain alkane with 12 carbon atoms has antimicrobial properties.1-Tetradecanol is a saturated fatty alcohol with a straight 14-carbon atom chain demonstrates antibacterial activities. Tetradecane is a straight-chain alkane with 14 carbon atoms exhibits antimicrobial properties. In 2,4-Di-tert-butylphenol, the phenol molecule has two tertiary butyl groups attached at the ortho and para positions displays antimicrobial activities.

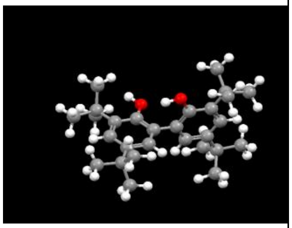
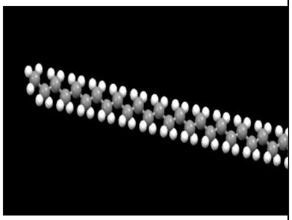
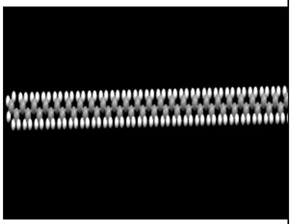
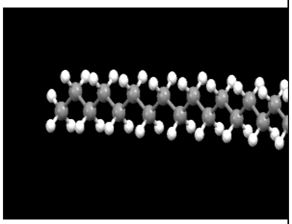
n-Pentadecanol is a long-chain fatty alcohol with 15 carbon atoms reveals antibacterial activity. Hexadecane is a straight-chain alkane comprising 16 carbon atoms shows antibacterial activities. Cyclononasiloxane, octadecamethyl, possesses a nine-membered ring structure consisting of cyclic frames with alternating silicon and oxygen atoms express antibacterial activities.1-Hexacosanol is a long-chain fatty alcohol containing 26 carbon atoms has antimicrobial activities. O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl is a biphenol with hydroxyl groups on each of its two benzene rings, connected by a single bond shows antimicrobial activities.

1-Heptacosanol is also a long-chain fatty alcohol, but with 27 carbon atoms displays antimicrobial activities. Tetrapentacontane is a straight-chain alkane with 54 carbon atoms shows antimicrobial properties. Octadecane is a straight-chain alkane with 18 carbon atoms reveals antimicrobial activities.

Table 4. 3D Structure of antibacterial compounds in the crude mucus

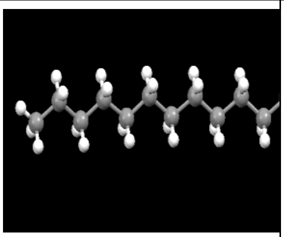
S. No	Compound name	Structure	Biological activity	References
1	1-Dodecanol		Antibacterial	Naoko Togashi et al.,2007
2	Dodecane		Antibacterial, antifungal	Padma et al.,2019

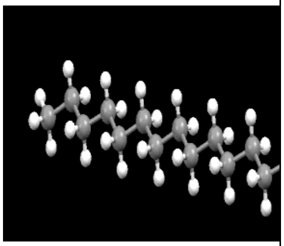
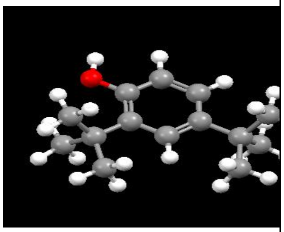
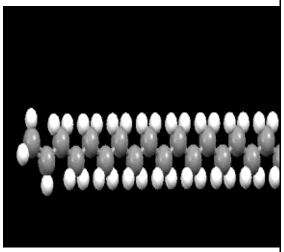
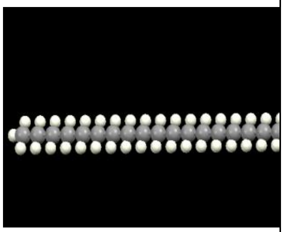
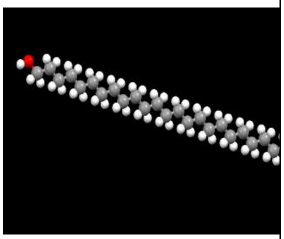
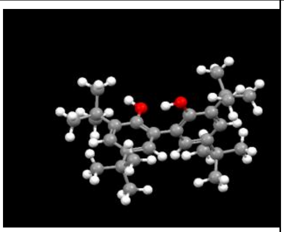
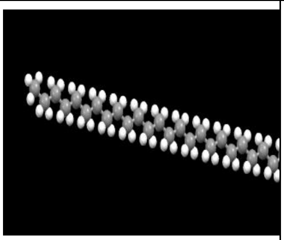
3	1-Tetradecanol		Anti-inflammatory, Cosmetics,antibacterial	medchemexpress.com, Thermofisher.com
4	Tetradecane		Antibacterial,antifungal	Zainab Said Nasr et al.,2022
5	2,4-Di-tert-butylphenol		Antibacterial, antifungal, anticancer	Vinatiorganics.com, Kai Fan et al.,2024, Sathuvan et al.,2012
6	n-Pentadecanol		Antibacterial, cosmetics	Kubo et al.,1995 Shell Global,2019
7	Hexadecane		Antibacterial, anti-oxidant	Yogeswari et al.,2012
8	Cyclononasiloxane,octadecamethyl		Antibacterial, anti-oxidant	Humaira Rizwana et al 2019, Kadri et al.,2011
9	1-Hexacosanol		Insecticidal,larvicidal,neurotoxic effect, antibacterial,anti-fungal, anti cancer	medchemexpress.com, Sriramya et al.,2017,Han et al.,2009, Wei&Bin, 2011

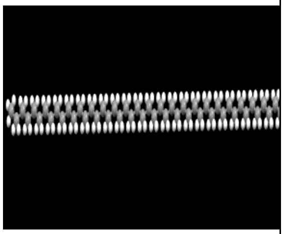
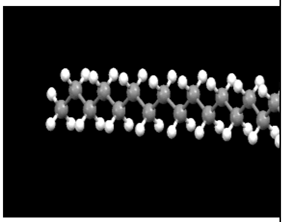
10	OO'Biphenol,4, 4',6,6'Tetra-T-Butyl		Antibacterial, Anti-fungal,anti-inflammatory	Poonam et al.,2021, Kai Fan et al., 2023,Irshad et al.,2021
11	1-Heptacosanol		Antibacterial, Anti-fungal,anti-oxidant	Mostafa et al.,2024, Eva Sanchez et al.,2021, Imada ,2005
12	Tetrapentacontane		Antibacterial, anti-fungal,anti-oxidant	Mahima et al.,2022, Abuzer et al.,2021, Zuhair et al.,2022
13	Octadecane		Antibacterial, anti-fungal	Xianfeng et al.,2020, Parismita et al.,2022

The 3D structure of antifungal compounds in the crude mucus presented in **table 5.** Dodecane is a straight-chain alkane with 12 carbon atoms has antimicrobial properties. Tetradecane is a straight-chain alkane with 14 carbon atoms exhibits antimicrobial properties. In 2,4-Di-tert-butylphenol, the phenol molecule has two tertiary butyl groups attached at the ortho and para positions displays antimicrobial activities.1-Nonadecene is a long-chain alkene with 19 carbon atoms has antifungal activity. Eicosane, containing 20 carbon atoms, is a straight-chain alkane exhibits antifungal activity.1-Hexacosanol is a long-chain fatty alcohol containing 26 carbon atoms has antimicrobial activities. O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl is a biphenol with hydroxyl groups on each of its two benzene rings, connected by a single bond shows antimicrobial activities. 1-Heptacosanol is also a long-chain fatty alcohol, but with 27 carbon atoms displays antimicrobial activities. Tetrapentacontane is a straight-chain alkane with 54 carbon atoms shows antimicrobial properties. Octadecane is a straight-chain alkane with 18 carbon atoms reveals antimicrobial activities.

Table 5. 3D Structure of antifungal compounds in the crude mucus

S. No	Compound name	Structure	Biological activity	References
1	Dodecane		Antibacterial, antifungal	Padma et al.,2019

2	Tetradecane		Antibacterial,antifungal	Zainab Said Nasr et al.,2022
3	2,4-Di-tert-butylphenol		Antibacterial, antifungal, anticancer	Vinatiorganics.com, Kai Fan et al.,2024, Sathuvan et al.,2012
4	1-Nonadecene		Anti-f ungal	Mansureh Ghavam et al.,2021
5	Eicosane		Anti-fungal	Meghashyama et al.,2024
6	1-Hexacosanol		Insecticidal,larvicidal,neurotoxic effect, antibacterial,anti-fungal, anti cancer	medchemexpress.com, Sriramya et al.,2017,Han et al.,2009, Wei&Bin, 2011
7	OO'Biphenol,4, 4',6,6'Tetra-T-Butyl		Antibacterial, Anti-fungal,anti-inflammatory	Poonam et al.,2021, Kai Fan et al., 2023,Irshad et al.,2021
8	1-Heptacosanol		Antibacterial, Anti-fungal,anti-oxidant	Mostafa et al.,2024, Eva Sanchez et al.,2021, Imada ,2005

9	Tetrapentacontan e		Antibacterial, anti-fungal,anti-oxidant	Mahima et al.,2022, Abuzer et al.,2021, Zuhair et al.,2022
10	Octadecane		Antibacterial, anti-fungal	Xianfeng et al.,2020, Parismita et al.,2022

4. DISCUSSION

For the past 30 years, antimicrobial peptides (AMPs) have been considered as a potential source for the creation of new antimicrobial drugs, against multi-resistant bacterial strains. Molluscs are employed in a variety of ways to isolate nutraceuticals from crude or semi-purified extracts (Dwek et al.,2001; Dolashka-Angelova et al., 2008).

In this study the biochemical estimate of *Laevicaulis alte* mucus indicate that the mucus contains 10.51±0.026 mg/l fat, 114.234±0.005 mg/l protein, and 169.133±0.057 mg/l carbohydrate. These result shows the significance of *Laevicaulis alte* mucus as a rich source of crude protein, aligning with previous research by Adeyeye (1966), Eruvbetine (2012), and Okon and Ibom (2012), which highlighted the high protein content in snail. Comparatively, the protein content in *Laevicaulis alte* mucus exceeds that of *Perronia virruculata,* which was found to contain 5.73 ± 0.98% fat and 59.42 ± 1.82% protein according to Solanki et al. (2017).

In the present study GC-MS analysis on the chloroform extract of crude mucus from *Laevicaulis alte* shows 22 bioactive compounds. The presence of bioactive chemicals in the giant African snail (*Archachantina marginata*), from which 26 compounds were extracted from the hemolymph, supports this conclusion (Lawal et al., 2015). Aishwarya Shetty and Pulikeshi M. Biradar (2024) discovered a number of bioactive substances in the tissue of *Eisenia fetida*, an epigeic earthworm. Of these, dodecane, tetradecane, hexadecane, octadecane and eicosane were identified to have antimicrobial activity.

Our study's results support Naoko Togashi et al. (2007)'s assertion that 1-dodecanol has antibacterial qualities. According to their findings, 1-dodecanol, a long-chain fatty alcohol, has strong antibacterial properties. Its capacity to damage bacterial cell membranes, which results in cell lysis and death, is what causes this activity. The dodecane extracted from slug mucus has antibacterial and antifungal activity. This aligns with Padma et al. (2019) who also reported these properties.

Research indicates that 1-Tetradecanol, a compound extracted from slug mucus has antibacterial properties. According to MedChemExpress, this compound has shown promising potential in combating bacterial infections. The compound tetradecane, which has been isolated from slug mucus, demonstrates remarkable antibacterial and antifungal properties. This observation is supported by the study conducted by Zainab Said Nasr et al. (2022), which thoroughly investigates the bioactive potential of tetradecane. Ogunlesi et al. (2010) further corroborates these findings, highlighting tetradecane's efficacy against both Gram-positive and Gram-negative bacteria.

The compound 2,4-Di-tert-butylphenol, isolated from slug mucus, exhibits notable antibacterial and antifungal properties. A natural substance called 2,4-di-tert-butylphenol can harm bacterial species by preventing them from sensing quorums, lowering the release of virulence factors, and preventing the production of biofilms (Rashmi Mishra et al 2020). With an EC50 value of 0.087 mmol/L, 2,4-di-tert-butylphenol demonstrated potent antifungal activity against *Ustilaginoidea virens*. 2,4-DTBP may cause fungal cell death by destroying *U. virens'* cell wall, cell membrane, and cellular redox equilibrium. This has been proven with scanning electron microscopy, fluorescence staining, and biochemical experiments (Kai Fan et al.2023).Vinatiorganics.com claims that 2,4-di-tert-butylphenol has antibacterial qualities.

The results of this investigation are consistent with those of Kubo et al. (1995), who discovered that n-pentadecanol prevents the growth of a number of microbial strains. According to Yogeswari et al. (2012), isolated hexadecane in this investigation has significant bactericidal effects. The antibacterial qualities of cyclononasiloxane and octadecamethyl are highlighted in the work by Humaira Rizwana et al. (2019). It is especially efficient against marine *Bacillus cereus*, a pathogen that is known to cause food borne diseases. Bacterial cell lysis and death result from this compound's disruption of the cell membrane.

The chemical 1-Nonadecene, that was obtained from slug mucus for this study, exhibits antifungal action. As stated by El-Sakhawy et al. (1998), 1-Nonadecene has antifungal action against *Candida albicans*. The study by Meghashyama Prabhakara Bhat et al. (2024) provides compelling evidence for the antifungal properties of eicosane extracted from slug mucus.1-Hexacosanol, a long-chain fatty alcohol isolated from slug mucus, has demonstrated significant antimicrobial properties. The bactericidal properties of 1-hexacosanol were highlighted in a study by Sriramya Grade et al. (2017), which showed its effectiveness against various bacterial strains. Additionally, Han et al. (2009) investigated the antifungal activity of 1-hexacosanol and found it to be effective against several fungal pathogens.

Slug mucus contains O O'-Biphenol, 4,4',6,6' 'Tetra-T-Butyl, which have demonstrated promising antimicrobial qualities. In their investigation of O O'-Biphenol, 4,4', 6,6'Tetra-T-Butyl's antibacterial qualities, Poonam Ratrey et al. (2021) discovered that it was efficient against a variety of bacterial strains. The antifungal capabilities of O O'-Biphenol, 4,4', 6,6'Tetra-T-Butyl were investigated by Kai Fan et al. (2023) who found that they significantly inhibited a variety of fungal infections.

An important development in the study of natural antimicrobial compounds is the identification of 1-heptacosanol, which is derived from slug mucus and displays antibacterial features. Eva Sanchez-Hernandez et al. (2021) study proved that 1-heptacosanol had antifungal qualities. Furthermore, by demonstrating the bactericidal qualities of 1-heptacosanol, the study by Mostafa H. Baky et al., (2024) built upon these discoveries.

A long-chain hydrocarbon called tetrapentacontane was obtained from slug mucus and shown to have good antibacterial qualities. Tetrapentacontane exhibits strong antifungal action against a range of fungal infections, according to Abuzer Ali et al. (2021). The method of action most likely entails rupturing the integrity of the fungal cell membrane, which results in cell lysis and death. Tetrapentacontane also demonstrates antibacterial qualities against a variety of bacterial species, according to Mahima Sharma et al. (2022).

The antifungal and antibacterial qualities of octadecane extracted from slug mucus are highlighted by Parismita Borgohain et al. (2022) and Xianfeng Wang et al. (2020) both of which are consistent with our findings. Likewise, our investigation confirmed the antibacterial activity of octadecane and tetradecane from Spirulina sp., as documented by Nazemi et al. (2010). Additionally, our research validates Guo et al. (2008) findings of these compounds' antifungal capabilities against *Candida albicans.*

Our research confirms the antimicrobial abilities of chemicals produced from slug mucus, 1-Dodecanol, Dodecane, 1-Tetradecanol, Tetradecane, 2,4-Di-tert-butylphenol,1-Nonadecene, Eicosane, n-Pentadecanol, Hexadecane, Cyclononasiloxane,octadecamethyl,1-Hexacosanol, O O'-Biphenol,4,4',6,6'-Tetra-T-Butyl, 1-heptacosanol, tetrapentacontane and octadecane. These discoveries create new opportunities for the development of natural antimicrobial medicines and provide the groundwork for future studies to better understand and utilize the therapeutic potential of these substances.

5. CONCLUSION

Findings of this study demonstrated that *Laevicaulis alte* mucus include a sizable amount of easily accessible proteins; as a result, they may be useful in the creation of novel medicines to combat pathogenic microorganisms that are resistant to multiple drugs (MDR).

Using GC-MS analysis, the current study has found and verified the existence of bioactive components in the terrestrial slug *Laevicaulis alte*. It can therefore be regarded as a valuable source of natural products for human therapy and may also be used in a variety of medications. Future research requires the analysis of the extract its bioactive components, the identification of the responsible bioactive compounds, and their biological activity. This also provided a new insight towards the development of good candidates for pharmaceutical and bioactive natural products.

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