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Comparative Analysis of Bioactive Compounds in Invasive and Native Land Snail Species of Java, Indonesia

Meilisha Putri Pertiwi[1], **Ayu Savitri Nurinsiyah**[2], **Berry Juliandi**[1*], **Windra Priawandiputra**[1], **Johan Iskandar**[3]

[1]*Department of Biology, Faculty of Mathematics and Natural Science, IPB University, Bogor 16680, Indonesia*
[2]*Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Bogor 16911, Indonesia*
[3]*Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia*

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ABSTRACT

Land snails are part of Indonesia's rich biodiversity. Their mucus is widely used as a natural ingredient in pharmaceuticals and cosmetics. Despite this, research has primarily focused on a limited range of species, leaving the potential of many native land snail species unexplored. This indicates that there is still significant untapped potential in native Indonesian land snail species. This research aims to analyze the bioactive compounds in the mucus of native Indonesian land snails and compare them with those of invasive species. The research employed Liquid Chromatography-Mass Spectrometry (LC-MS) to identify biologically active substances in land snail mucus. The samples used were *Amphidromus palaceus* and *Lissachatina fulica* collected from Gunungkelir, Yogyakarta, and *Amphidromus perversus* from Gunungkidul, Yogyakarta, and Jember, East Java. The results revealed that *A. palaceus* exhibited the highest number of bioactive compounds, with 28 identified substances. The bioactive properties across all samples were categorized as antibacterial (16%), wound healing and anti-inflammatory (12%), anticancer (8%), neuron-related drugs (6%), anti-aging (4%), with the remaining activities related to liver-related drugs, antiviral, heart disease-related drugs, and kidney disease-related drugs. These results highlight that native species have significant potential for further research, enabling their application in medicine and cosmetics.

1. Introduction

Recent data indicate that around 170 of the 179 WHO member countries use alternative and complementary medicine (WHO 2019). Medicinal ingredients often originate from natural sources, including plants and marine invertebrates, such as those belonging to the phylum Mollusca (Coates and Nairn 2014; Dang *et al*. 2015). Mollusca are not limited to marine and terrestrial species, such as land snails and slugs. Due to their biologically active substances, which can be purified, synthesized, or developed as nutricosmeceutical ingredients in raw and semi-pure forms, they are increasingly recognized for their therapeutic potential (Ferdian 2020). In this case, land snails' biodiversity is reflected in their chemical composition and the production of various secondary metabolites (Ahmad *et al*. 2018). Mollusca parts widely used include the soft body, shell, mucus, or a combination thereof. The smooth body is traditionally burned and used as a medicine for asthma and ulcers (Husain and Wahidah 2019; Maharani *et al*. 2021).

* Corresponding Author
E-mail Address: bjuliandi@apps.ipb.ac.id

Meanwhile, land snail shells are ground into powder and used as a wound healer (Okoh *et al*. 2020).

The mucus is used for its anti-inflammatory properties, wound healing effects on the skin and teeth, and antimicrobial, anticancer, and antiaging benefits (Ulagesan and Kim 2018; Rosanto *et al*. 2022). This mucus contains a complex mixture of biochemically and pharmacologically active compounds unique to each species. In general, the mucus content of land snails is 90% water. It also contains mucin, a key component enriched with allantoin, antimicrobial peptides, enzymes, and glycoproteins (Trapella *et al*. 2018). Based on GC-MS and LC-MS analysis, the biologically active substances in land snails include achachin, mytimacin AF, sarcoplasmic calcium-binding protein, Methoxyphenyl-Oxime, 2-ethylacridine, Cyclotrisiloxane hexamethyl, and Thiophene (Zhong *et al*. 2013; EL-Zawawy and Mona 2021; Noothuan *et al*. 2021).

Most studies on the bioactive compounds of land snails have focused on *Lissachatina fulica* and *Helix aspersa* (Zhong *et al*. 2013; Aouji *et al*. 2023). Both species are non-native to Indonesia, with *L. fulica* (formerly *Achatina fulica*) classified as an invasive alien species. Other species studied, but less frequently, include *Eremina desertorum*, *Cryptozona bistrialis*, *Archachatina maginata*, and *Helix lucorum* (Dolashka *et al*. 2015; EL-Zawawy and Mona 2021). However, limited (almost none) information regarding the chemical compound composition of mucus from *Amphidromus* species is available. The *Amphidromus* land snails are characterized by their large shell, with heights ranging from 35-67 mm. The shell can be dextral or sinistral and varies in shape from very conical to ovoid, with thick shells displaying bright colours such as yellow, light green, whitish, or dark colours. The shell patterns are diverse, including monochrome, variegated, or with colour patterns, with lines or streaks, sometimes with spiral bands, and with or without additional variations. In the Indonesian Archipelago, *Amphidromus* species were recorded from Sumatra, Java, Kalimantan, Sulawesi, and their satellite islands, Bali and the Nusa Tenggara Islands in the southern part of Eastern Indonesia. Despite their wide distribution and unique features, their bioactive potential remains underexplored, making them a promising focus for further research (Dharma 2021).

Amphidromus land snails represent just one example of the rich biodiversity found on Java and its surrounding islands. There are 263 species of land snails in Java, of which 104 are endemic or distributed only in Java and the surrounding islands (Nurinsiyah 2021). This suggests that there remains significant potential for biologically active substances in endemic land snails that have yet to be explored. The potential for biologically active substances from land snail mucus is closely related to its use in traditional medicine, especially in rural areas such as Gunungkelir Hamlet, Yogyakarta (Pertiwi *et al*. -in prep.). Evidence of land snail mucus in rural areas has been used for wounds, dry lungs, and toothache (Sudardi 2011; Prastikawati and Husain 2020; Lestiani 2022). In addition to these traditional applications, the biologically active substances found in land snail mucus represent a valuable resource for bioprospecting, with potential for developing novel therapeutic agents and other uses. Based on the points conveyed, this research is of significant importance. This study aims to examine the biologically active substance content in the mucus of native Indonesian land snails and compare it with that of invasive species to understand their therapeutic potential better.

2. Materials and Methods

2.1. Study Site

The samples of land snails were collected in Gunungkelir, Gunungkidul, and Jember. An overview of the locations is shown in Figure 1. The first location, Gunungkelir Hamlet, is in Jatimulyo Village, Kapanewon Girimulyo, Kulon Progo Regency, Yogyakarta. Its geographical coordinates are 7°45’10” South Latitude and 110°7’22” East Longitude. During the day, the average air temperature in Gunungkelir Hamlet is 27-29°C, while in the morning, the average is 21-23°C (Geomorfologi 2020). This hamlet also has natural potential in the form of beautiful Kelir cliffs. The potential natural wealth of Gunungkelir Hamlet makes this hamlet a suitable habitat for fauna. Jatimulyo Village, in particular, has earned the designation of a bird-friendly village, with Kulon Progo District ranking second in bird diversity in the region, hosting 227 bird species (Taufiqurrahman *et al*. 2015). In addition to birds, residents also breed goats, generating substantial manure up to six truckloads per day. Another potential fauna is the stingless bee, which lives naturally and is cultivated by locals in their home gardens (Kulonprogokab 2019). Moreover, land snails are abundant in the area across various substrates.

The second sampling location was the Gunungkidul Regional Conservation Area, DI Yogyakarta, with the

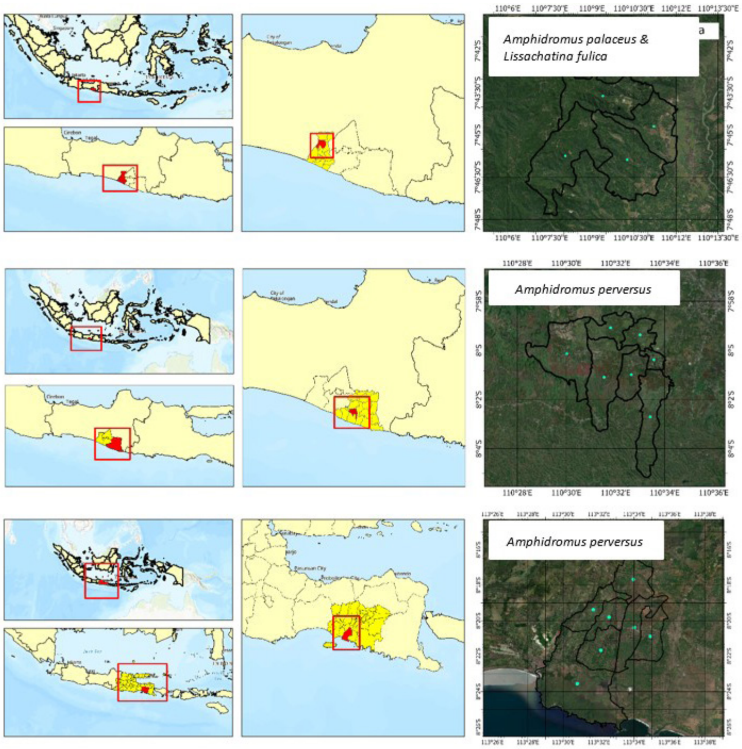

Figure 1. Land snail sampling location

geographical coordinates at 110 21' - 110 50' East Longitude and 7 46' - 8 09' South Latitude. The area is characterized by diverse vegetation such as teak, tamarind, duvet, melinjo, flamboyan, guava, mahogany, mango, lamtoro, and ketapang. In addition to its forested areas, the region is distinguished by extensive karst landscapes, including surface features such as dry valleys and lakes, subsurface (endokarst) formations such as underground rivers, caves, and their ornaments. The fauna in this forest consists of various wild animals, from large mammals to small mammals, reptiles, molluscs, and insects (BKSDAJOGJA 2022).

The final sampling location was the Watangan Puger Nature Reserve in Jember, East Java, geographically located at 8°24'12"S and 113°27'12"E. A river borders the area on one side and a production forest managed by Perum Perhutani on the other. The ecosystem is classified as a lowland tropical forest, comprising Moraceae, Fabaceae, and Myrtaceae species. Additionally, the reserve includes a coastal forest ecosystem dominated by sea waru, pandan, and nyamplung vegetation (BBKSDAJATIM 2024).

2.2. Mucus Collection

The land snail species from Gunungkelir are *Amphidromus palaceus* and *Lissachatina fulica*, while the species from Gunungkidul and Jember are *Amphidromus perversus*. Species identification referred to (van Benthem Jutting 1950) and (van Benthem Jutting 1952), the updated classification was based on molluscabase.org. The species *A. palaceus* and *A. perversus* are native to Indonesia (Mousson 1849), while *L. fulica* is an invasive alien species (Nurinsiyah and Hausdorf 2019) (Figure 2).

Land snails were collected by hand or with tools, such as long sticks. The collected snails were then placed in plastic containers. We selected snails of various species and large/adult sizes, ensuring they had no defects and intact shells. The snails were housed in a transparent cage measuring 15 × 15 × 7.5 cm, which had a lid with air holes for ventilation. Distinct cages were used for *Amphidromus* and *L. fulica* species. The base of the cages was filled with soil and sprinkled with eggshell powder to provide a source of calcium carbonate ($CaCO_3$). The snails' diet consisted of white mustard greens and cucumbers. Additionally, the cages were sprayed with water daily to maintain humidity and were cleaned regularly to remove dirt and leftover food.

The number of individuals required for mucus collection varies by species because of the differences in body size and mucus production. For *Amphidromus* species, approximately ± 20-25 individuals are required,

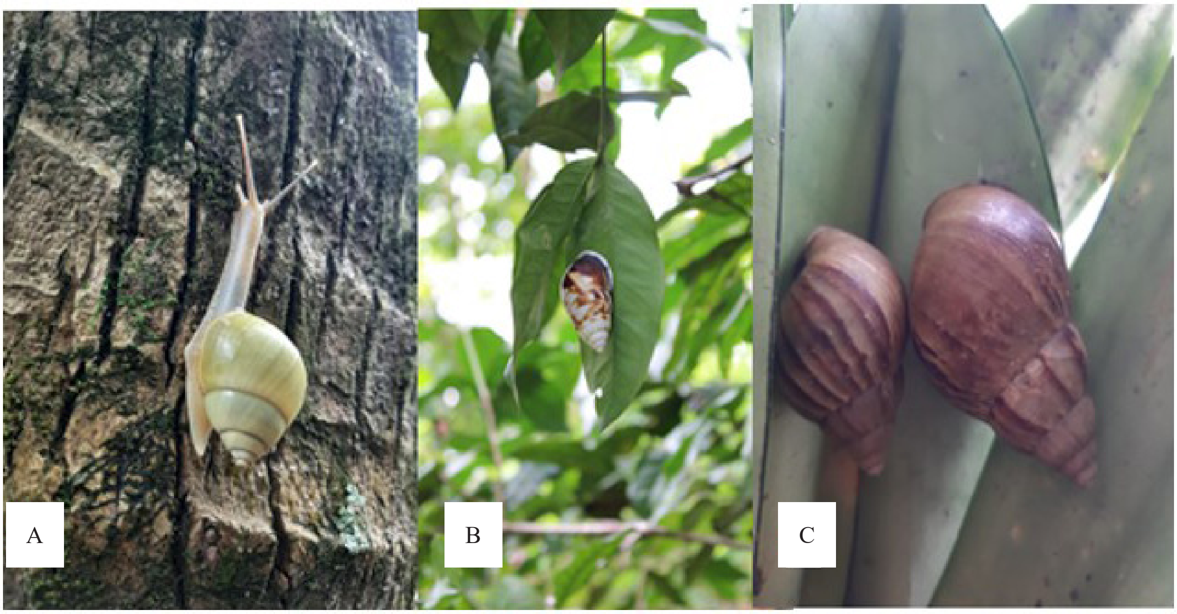

Figure 2. A. *Amphidromus palaceus*; B. *Amphidromus perversus*; C. *Lissachatina fulica*

while for *L. fulica* requires only ± 3-5 individuals. This discrepancy was caused by the smaller body size of *Amphidromus* which produce less mucus compared to *L. fulica*. Mucus collection was conducted naturally through a "milking" technique by stimulating the snail's soft body using a spatula (Pitt *et al*. 2015). The mucus secretion was collected into a vial and stored in a freezer or cooler box. On average, production from 4-5 *A. palaceus* individuals produces 2-3 ml, equivalent to the secretion from a single individual of *L. fulica*. The milking process took approximately 30-45 minutes and was conducted aseptically every 2 days for a duration of one month.

2.3. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

The bioactive compounds in land snail mucus were identifed using LC-MS/MS method. The analysis was conducted at the Advanced Laboratory, IPB University, Indonesia. A 10 mg powdered samples from each species was used for LCMS analysis. From which, 5 mg sample was filtered through a 0.2 μm PTFE membrane. The LC-MS/MS analysis was performed using a UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific, Accucore C18 column (100 × 2.1 mm, 1.5 m, ThermoScientific). The flow rate was maintaned at 0.2 mL/minute. The eluent gradient was water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), gradient 0-1 minute (5% B), 1-25 minutes (5-95% B), 25 -28 minutes (95%B), 28-33 minutes (5%B). The column temperature was 30°C with an injection volume of 2.0 μL. The analysis was performed within a molecular weight range of 100-1500 m/z using positive ionization mode. The database used for qualitatively identifying chemical contents in fractions was Compound Discoverer 3.2. Quantitative analysis was calculated from the peak area of the identified compounds.

3. Results

A total of 49 biologically active substances were produced from the collected land snail mucus. There were several substances that different species have in common. Based on Table 1, the number of biologically active substances is compared, as shown in Figure 3. The species *A. palaceus* contains the highest number of biological active substances totaling 28 compounds, followed by *L. fiulica* (16 compounds), *A. perversus* from Gunungkidul (13 compounds), and *A. perversus* from Jember (8 compounds) (Figure 3).

These 49 biologically active substances exhibit biological activities based on their characteristics. Supplementary Table 1 illustrates the variation of these activities. The majority of the biological activity observed in land snail mucus include antibacterial (16%), wound healing and anti-inflammatory (12%), anticancer (8%), neuron-related drugs (6%), antiaging (4%), the rest for drugs related to liver, antivirals, drugs related to heart disease, and drugs related to kidney disease can be seen in the Figure 4 below:

Table 1. Biological active substances from land snail samples

Compound name	Formula	*Amphidromus palaceus*		*Amphidromus perversus* (Gunungkidul)		*Amphidromus perversus* (Jember)		*Lissachatina fulica*	
		RT min	MW	RT min	MW	RT min	MW	RT min	MW
Betaine	$C_5H_{11}NO_2$	1.088	117.07884	1.105	117.07884	1.074	117.07891	1.079	117.079
1-Hexadecyllysophosphatidylcholine	$C_{24}H_{52}NO_6P$	20.495	481.35192	20.51	481.35207	20.492	481.35146	20.488	481.35378
DL-Stachydrine	$C_7H_{13}NO_2$	1.119	143.09433	1.136	143.09442	1.105	143.09447		
Choline	$C_5H_{13}NO$	1.067	103.0999	1.073	103.09984	1.041	103.09981		
Pipemidic acid	$C_{14}H_{17}N_5O_3$	1.421	303.13088			1.215	303.13085		
N-Acetyl-L-histidine	$C_8H_{11}N_3O_3$	2.297	197.07964					2.598	197.07985
Trigonelline	$C_7H_7NO_2$	1.099	137.04752					1.086	137.04759
Xanthine	$C_5H_4N_4O_2$	1.423	152.03317	1.435	152.03319				
Glutarylcarnitine	$C_{12}H_{21}NO_6$	2.237	275.13594						
L(-)-Carnitine	$C_7H_{15}NO_3$	1.078	161.10489						
NP-013088	$C_{23}H_{24}O_6$	21.705	396.15609						
Garcinone E	$C_{28}H_{32}O_6$	24.051	464.21844						
Garcinone C	$C_{23}H_{26}O_7$	18.11	414.16707						
g-Butyrobetaine	$C_7H_{15}NO_2$	1.096	145.11006						
Choline O-Sulfate	$C_5H_{13}NO_4S$	1.186	183.0564						
MFCD00133435	$C_{26}H_{52}NO_7P$	20.366	521.34734						
3-(1H-Imidazol-4-ylmethyl)-6-(1H-indol-3-ylmethyl)-2,5-piperazinedione	$C_{17}H_{17}N_5O_2$	5.056	323.13753						
2-linoleoyl-sn-glycero-3-phosphoethanolamine	$C_{23}H_{44}NO_7P$	18.566	477.28472						
(3R,4R)-4-[(3,4-dimethoxy-	$C_{21}H_{24}O_6$	23.418	394.14095						
phenyl)methyl]-3-[(4-hy-		1.136	159.08943						
droxy-3-methoxyphenyl)		1.568	149.04744						
methyl]oxolan-2-one		1.113	211.09552						
N-Acetylvaline	$C_7H_{13}NO_3$	1.108	260.17314						
N-Acetylimidoquinone	$C_8H_7NO_2$	1.568	131.03696						
Zalcitabine	$C_9H_{13}N_3O_3$	1.11	159.12581						
Carisoprodol	$C_{12}H_{24}N_2O_4$	1.423	244.15323						
Benzoyl cyanide	C_8H_5NO	7.454	212.10465						
Pregabalin	$C_8H_{17}NO_2$	1.469	267.0963						
N-{[(2R,4S,5R)-5-Ethyl-1-azabicyclo[2.2.2]oct-2-yl]methyl}-2-furamide	$C_{15}H_{22}N_2O_2$								
4-methyl-5-oxo-2-pentyl-2,5-dihydrofuran-3-carboxylic acid	$C_{11}H_{16}O_4$								
Adenosine	$C_{10}H_{13}N_5O_4$								
11-Aminoundecanoic acid	$C_{11}H_{23}NO_2$			1.207	201.17262	1.166	201.17262		
3-(1H-Imidazol-4-ylmethyl)-6-(1H-indol-3-ylmethyl)-2,5-piperazinedione									
Triphenylphosphine oxide	$C_{17}H_{17}N_5O_2$			5.005	323.13729	4.977	323.13719		
NP-016516	$C_{18}H_{15}OP$			14.505	278.0851				
DL-Carnitine	$C_{16}H_{24}O_4$			1.192	302.14726				
NP-000308	$C_7H_{15}NO_3$			1.107	161.10495				
L-(+)-Leucine	$C_{16}H_{12}O_6$			14.508	300.06731				
Hypoxanthin	$C_6H_{13}NO_2$			1.61	131.0945				
(±)13-HpODE	$C_5H_4N_4O$			1.43	136.03827				
O-ureido-D-serine	$C_{18}H_{32}O_4$					20.664	294.21883		
Cetrimonium	$C_4H_9N_3O_4$							0.953	163.05951

Table 1. Continued

Compound name	Formula	*Amphidromus palaceus*		*Amphidromus perversus* (Gunungkidul)		*Amphidromus perversus* (Jember)		*Lissachatina fulica*	
		RT min	MW	RT min	MW	RT min	MW	RT min	MW
DL-Citrulline	$C_{19}H_{41}N$							22.922	283.32378
Acetylcholine	$C_6H_{13}N_3O_3$							1.127	175.09548
Ethyl palmitoleate	$C_7H_{15}NO_2$							1.096	145.1102
DL-Phenylalanine	$C_{18}H_{34}O_2$							22.334	282.25578
1-(alpha-D-	$C_9H_{11}NO_2$							2.733	165.07889
Glucopyranosyluronosyl)-3-[(2S)-1-methyl-5-oxo-2-pyrrolidinyl]pyridinium	$C_{16}H_{20}N_2O_7$							8.328	352.127
RG1300000	$C_{18}H_{34}O_3$							23.042	298.25081
HIAA	$C_{10}H_9NO_3$							9.855	191.05821
Uracil	$C_4H_4N_2O_2$							1.073	112.02747
glutaral	$C_5H_8O_2$							1.12	100.05267
Creatine	$C_4H_9N_3O_2$							1.111	131.06952

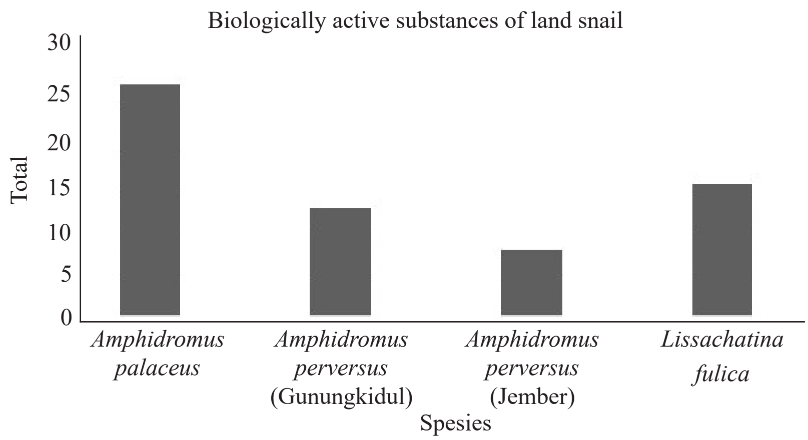

Figure 3. Comparison of the amount of biologically active substances from land snail mucus used in research

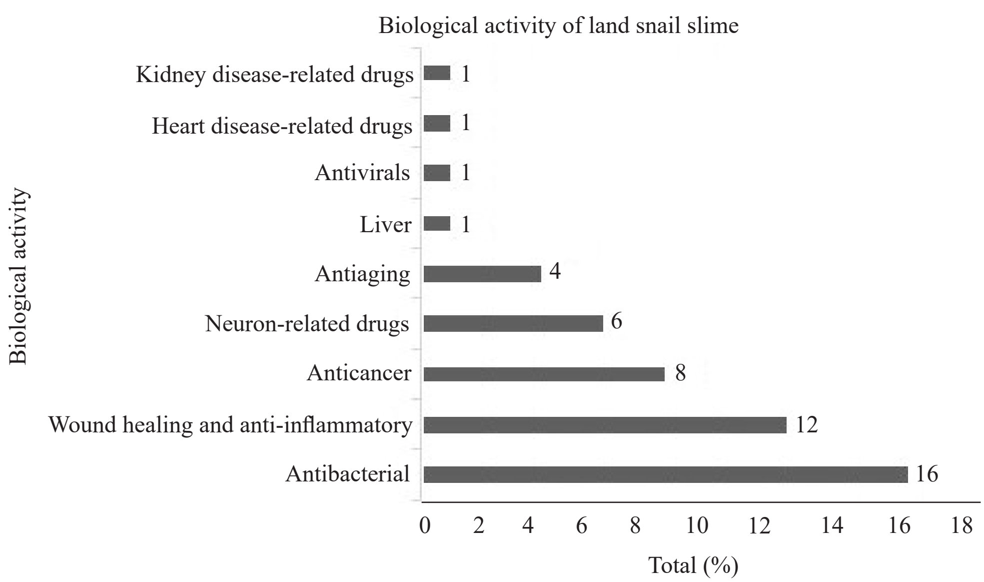

Figure 4. Summary of land snail slime biological activity

4. Discussion

LCMS results from land snails sample provided a total of 49 biologically active substances with unique compositions per species. These results also prove that each species can contain varying bioactive compounds. Land snails inhabit various types of vegetation, including crops, vegetables, and fruit trees, which results in a diverse range of food sources. Their digestive tracts contain four main genera of bacteria: *Acinetobacter*, *Aeromonas*, *Pseudomonas*, and *Enterobacter*. These bacteria exhibit varying abilities to digest cellulose, the primary component of plant cell walls (Lin *et al*. 2023). Differences in the absorption of nutrients from their food lead to variations in protein and peptide levels, which in turn produce different types of bioactive mucus. This was demonstrated in studies involving *C. aspersum*, where variations in protein and peptide levels were positively correlated with the biological activity of the mucus produced (Velkova *et al*. 2024).

Additionally, environmental factors such as temperature, humidity, light intensity, and soil conditions can create unique bioactive compositions (EL-Zawawy and Mona 2021). Different temperature and humidity conditions can significantly impact land snails, which are sensitive to these changes. A prolonged dry season combined with high temperatures can cause considerable stress in land snails, leading to alterations in their metabolic response (Bailey *et al*. 1986; Staikou *et al*. 2016). This stress is further compounded by the presence of *Aeromonas* bacteria, which can hinder their ability to adapt to new environmental conditions (Lin *et al*. 2023). Consequently, this process of environmental adaptation may influence the production of bioactive compounds in land snails.

This study also shows several similarities in biologically active substances from different species. All *Amphidromus* species have four bioactives in common -although collected from different areas-, namely betaine, 1-hexadecyllysophosphatidylcholine, DL-stachydrine, and choline. The species *A. palaceus* and *A. perversus* (Gunungkidul population) both contain Xanthine, while *A. palaceus* and *A. perversus* (Jember population) share Pipemidic acid. The *A. perversus* populations from Gunungkidul and Jember shared two common bioactives, namely 11-Aminoundecanoic acid and 3-(1H-Imidazol-4-ylmethyl)-6-(1H-indol-3-ylmethyl)-2,5-piperazinedione. Furthermore, *A. palaceus* and *L. fulica* shared four bioactive compounds: betaine, 1-hexadecyllysophosphatidylcholine, N-Acetyl-L-histidine, and trigonelline. However, no other *Amphidromus* species have biologically active substances similar to those of *L. fulica*. This potentially caused by the difference in sampling locations i.e. variation in abiotic factors, food availability, and substrate.

Not only does the composition of bioactive substances differ betweene species, but within single organism, the contents can vary depending on the specific part of the body. Such variations have been studied in the shell (Latire *et al*. 2014), body (Li *et al*. 2014), mucus (Greistorfer *et al*. 2017), and hemolymph (Dolashka *et al*. 2015). Land snails are an invaluable source of several biologically active components (Trapella *et al*. 2018). This is similar to this study, in which variations in the biological activity of 49 biologically active substances were detected (Supplementary Table 2). The majority of the biological activity observed in land snail mucus include antibacterial (16%), wound healing and anti-inflammatory (12%), anticancer (8%), neuron-related drugs (6%), antiaging (4%), the rest for drugs related to liver, antivirals, drugs related to heart disease, and drugs related to kidney disease (Figure 3).

The defense mechanism of land snails rely on their innate immune system and the presence of biologically active substances. Land snails encounter many challenges in their environment, including bacteria, fungi, and other pathogens. However, land snails can still live because of the viscous nature of their mucus and its biochemically complex. This mucus serves multiple functions: facilitating movement, helps reduce water loss, defense mechanism against predators, and allows communication with land snails of the same species (Greistorfer *et al*. 2017). It has the highest biological activity as an antibacterial which inline as well with the findings of this study which reported an antibacterial rate of 16%. Active compounds in the mucus, such as Pipemidic acid and 4-methyl-5-oxo-2-pentyl-2,5-dihydrofuran-3-carboxylic acid contain antibacterial activity. The combination of acids in land snail mucus, such as acetic, citric, and lactic acids is responsible for its antibacterial properties (Cilia and Fratini 2018; Perpelek *et al*. 2021). Crude mucus extracts of land snails *Achatina achatina* and other *Achatina fulica* also have antimicrobial activity (Nantarat *et al*. 2019).

One of the traditional ethnozoological therapies for land snails is wound healing and inflammation. This aligns with the findings of this study which showed 12% or the second largest biological activity of land snail mucus was for wound helaing and anti-inflammatory. These properties are in the form of

1-Hexadecyllysophosphatidylcholine, Trigonelline, (±)13-HpODE, Cetrimonium, Ethyl palmitoleate, and HIAA. Inflammation can be described as the body's rapid response to disorders such as injury or infection, involving the isolation and removal of harmful stimuli including damages cells, chemical irritants and infections, as well as initiating tissue repair. The process is regulated sequence of spatial and temporal event in which cells and mediators collaborate to neutralize harmful stimuli and restore homeostasis. Although the inflammatory process helps eliminate stimulus damage, the prolonged process plays a significant role in an incomplete wound healing and the development of chronic wounds (Qian *et al*. 2016). For wound healing, snail mucus is effective in rats and mice without adverse effects on health (Song *et al*. 2021). The mucus of *Lissachatina fulica* can heal wounds twice as fast as an ordinary salt solution because of its glycosaminoglycan content. Likewise, in vitro experiments, HelixComplex containing glycolic acid and allantoin showed potential for wound healing (Trapella *et al*. 2018).

Another potential biological activity observed in this study is anticancer activity, accounting for 8% of the identified effects. Oxidative stress triggers the formation of reactive oxygen species (ROS) in human body cells. This process is associated with the onset of factors that accelerate the emergence of various diseases, such as cancer, neurodegenerative, cardiovascular diseases, diabetes mellitus, and many other pathologies. The endogenous antioxidant system captures ROS and free radicals to maintain balance in the body. The primary function of antioxidants depends on the power of reducing oxidative damage (Jelic *et al*. 2021). The antioxidant activity of allantoin has been demonstrated in the mucus of *Cornu aspersum* (Kostadinova *et al*. 2018), while the increased antioxidant properties were observed in the mucus of *Eremina desertorum* during in vitro experiments (Atta *et al*. 2021). In line with these findings, the addition of two *Achatina fulica* mucus protein fractions can reduce the viability of breast cancer cells (MCF-7) (E-kobon *et al*. 2016). Similarly with Ouar *et al*. (2017), *Helix aspersa* Müller extract has anticancer activity against breast cancer cells (Hs578T). This study further highlights the anticancer potential of compounds such as DL-Stachidrine, NP-013088, Garcinone E, 2-linoleoyl-sn-glycero-3-phosphoethanolamine, which requires further investigation.

In this study, 6% of the biologically active substances identified from land snail mucus were associated with neuron-related drugs. These active substances include Acetylcholine, Choline, and Pregabalin. The mucus extract of *Helix aspersa* significantly improved cognitive deficits in behavioural tests carried out by male Wistar rats. Additionally, AChE (acetylcholinesterase) has inhibitory activity, a critical target in treating Alzheimer's (Tancheva and Lazarova 2022). The neurological implications of specific components in the mucus, including neuroprotective properties and potential contribution to neurodegeneration, represent a promising avenue for research into neurological disorders and therapeutic strategies. For example, its potential antioxidant effects are thought to treat impaired cognitive brain function and improve cardiovascular protection. It is suspected that there is a protein with a molecular weight of 24-27 kDa belonging to the glutathione peroxidases family, is responsible in reducing oxidative stress and prevents neurodegeneration (Tsvetanova *et al*. 2020). Other findings suggest that allantoin has therapeutic potential for cognitive dysfunction observed in Alzheimer's disease. The neuroprotective effects of natural exogenous antioxidants are now seen as a persuasive therapy for nerve loss because they can fight and neutralize free radicals. The molecular mechanisms of neurodegeneration remain largely unknown, and effective therapies are not currently available, so further research is needed.

The cosmetics and skin care industries are also harnessed the potential benefits of gastropod mucus. Compounds such as hyaluronic acid and glycoproteins found in the mucus are known for their moisturizing and smoothing effect on the skin, making them valuable additions to beauty products formulations. Land snail is particularly valued for its anti-aging properties, and its integration into skincare products has gained significant attention and adoption in the cosmetic industry (Dolashka *et al*. 2015). The current study highlights the antiaging and moisturizing potential of bioactive compounds such as betaine and cetrimonium, which accounted for 4% of the identified activities. Natural ingredients are the primary source of antioxidants, including vitamins, carotenoids and phenolic compounds from plants, animal proteins and peptides. These antioxidants are considered a safe product and widely applied in the pharmaceutical and cosmetic industries. Using the mucus secretion of *Helix aspersa* for 3 months significantly improved photoaging's clinical and histological signs, an affect attributed to its glycosaminoglycan (Tribo-Boixareu *et al*. 2009). Mucus from *L. fulica* is used as a cosmetic ingredient (Nguyen *et al*. 2020), but more studies are needed on its biological properties.

Natural products are widely regarded as the primary source of bioactive pharmaceutical components used in drug development. These substances have several advantages over synthetic products including easy to obtain, cost effective to produce, and require minimal use of resources. Among natural products, land snails are a valuable source of viscous secretory mucus and attract attention because of their characteristics. This study found that the native species *A. palaceus* has a greater quantity of biologically active compounds compared to the invasive species *L. fulica*, which is commonly used in research. Although *L. fulica* has a significant potential despite being present in smaller amounts than *A. palaceus*, native land snail species may offer even greater opportunities for utilization. The various active substances contained in land snail mucus have much potential for medicine and cosmetics. Therefore, land snail mucus has gained global attention for its therapeutic and cosmetic uses. It can increase their economic value. Implementing a circular economy involves multiple stakeholders who maintain a responsible approach and conduct environmental risk assessments. However this study also identifies active compounds whose identities and potential remain unknown and need to be discovered. Further molecular dynamics studies are needed to understand its mechanism of action, which could enrich the benefits of land snail biodiversity in Indonesia. Effective conservation efforts are essential to guaranteeing the sustainable use of our invaluable natural resources. By prioritizing these initiatives, we can protect our environment for future generations while fostering a balanced coexistence with nature.

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Supplementary Materials

Supplementary Table 1. Variations in biological activity of land snail mucus biological active substances

Compound name	Activities
Betaine	skin moisturizert, anti aging, anti skin irritation (Cho *et al*. 2017; Burnett *et al*. 2018)
1-Hexadecyllysophosphatidylcholine	Wound healing agent/anti-inflammation (Ceng *et al*. 2009)
DL-Stachydrine	anticancer (Zeng *et al*. 2023)
Choline	muscle and brain damage prevention (Zeisel & da Costa 2009; Wallace *et al*. 2018)
Pipemidic acid	antibacterial (Shimizu *et al.* 1975; Lavorgna *et al*. 2019)
N-Acetyl-L-histidine	Mengurangi risiko katarak (Remo *et al*. 2014; Baslow & Guilfoyle 2015)
Trigonelline	Wound healing agent/anti-inflammation (Li *et al*. 2024;
Xanthine	kidney disease biomarker (Korsmo *et al*. 2024), medicine bitterness sensor (Zhao *et al*. 2024)
Glutarylcarnitine	Biochemical markers of GA type 1 (Tortorelli *et al*. 2005)
L(-)-Carnitine	heart medicine and weight loss (Pekala *et al*. 2011), energy metabolism and increase
NP-013088	stamina (Vecchio *et al*. 2021)
Garcinone E	anticancer (Nittayananta *et al*. 2024)
Garcinone C	anticancer (Xu *et al*. 2017; Wei *et al*. 2023)
g-Butyrobetaine	antitumor (Xia *et al*. 2018; Chen *et al*. 2020)
Choline O-Sulfate	proatherogenic to Carnitine (Koeth *et al*. 2014)
MFCD00133435	amyloid formation inhibitor (Hagihara *et al*. 2012)
3-(1H-Imidazol-4-ylmethyl)-6-(1H-indol-3-ylmethyl)-2,5-piperazinedione	unknown unknown
2-linoleoyl-sn-glycero-3-phosphoethanolamine	anticancer (Luan *et al*. 2019), collagen induction (Ding *et al*. 2014)
(3R,4R)-4-[(3,4-dimethoxyphenyl)methyl]-3-[(4-hydroxy-3-methoxyphenyl)methyl] oxolan-2-one	Treating liver fibrosis (Lv *et al*. 2024), antivirus (Shukla *et al*. 2024)
N-Acetylvaline	antimicrobes (Arinbasarova *et al*. 2017), colorectal cancer biomarkers (Lin *et al*. 2016)
N-Acetylimidoquinone	substance mediator (Hinson *et al*. 1979; Holownia *et al*. 1998)
Zalcitabine	antiviral agent (Schooley *et al*. 1996; Paintsil & Cheng 2009)
Carisoprodol	pain relief (Kumar & Dillon 2015; Soprano *et al*. 2020)
Benzoyl cyanide	benzoylation reagent (Prasad *et al*. 2005)
Pregabalin	therapy of neuropathy pain (Freynhagen *et al*. 2021; Toth 2013)
N-{[(2R,4S,5R)-5-Ethyl-1-azabicyclo[2.2.2]oct-2-yl]methyl}-2-furamide	unknown
4-methyl-5-oxo-2-pentyl-2,5-dihydrofuran-3-carboxylic acid	antibacterial (Sheng *et al*. 2019)
Adenosine	antibacterial (Sangsuwan *et al*. 2024)
11-Aminoundecanoic acid	Biopolimer (Martino *et al*. 2014; Costa *et al*. 2023)
3-(1H-Imidazol-4-ylmethyl)-6-(1H-indol-3-ylmethyl)-2,5-piperazinedione	Unknown
Triphenylphosphine oxide	Esterification of chemical compounds (Jia *et al*. 2018)
NP-016516	Unknown
DL-Carnitine	fatty acid synthesis (Haeffner & Privett 1975)
NP-000308	Unknown
L-(+)-Leucine	muscle mass nutrition (Martinez-Arnau *et al*. 2019; Ely *et al*. 2023)
Hypoxanthin	intermediate metabolites (Lee *at al*. 2018; Fujiwara *et al*. 2022)
(±)13-HpODE	anti-inflammation (Wolff *et al*. 2019), gene expression inducer for detoxification (Faizo *et al*. 2021)
O-ureido-D-serine	antitubercular agent (Kumagai *et al*. 2010)
Cetrimonium	wound healing agent (Pratt *et al*. 2024), antibacterial (Dong *et al*. 2019), Skin cleansing & exfoliation (Misar *et al*. 2022)

Supplementary Materials

Supplementary Table 1. Continued

Compound name	Activities
DL-Citrulline	antimicrobes (Reddy & Ranganathan 1983), biomarker of artritis reumatoid (Babos *et al*. 2013)
Acetylcholine	neuromodulator (Picciotto *et al*. 2012; Halder & Lal 2021)
Ethyl palmitoleate	antimicrobes (Huang *et al*. 2010), anti-inflammation (Hasdemir *et al*. 2019)
DL-Phenylalanine	antidepressant (Beckmann *et al*. 1977), anticoagulant (Rizk *et al*. 2023)
1-(alpha-D-Glucopyranosyluronosyl)-3-[(2S)-1-methyl-5-oxo-2-pyrrolidinyl]pyridinium	Unknown
RG1300000	Unknown
HIAA	anti-inflammation (Oluwagbemigun *et al*. 2023), biomarker of neuroendokrin tumor (Ewang-Emukowhate *et al*. 2023),
Uracil	hypertrophic cardiomyopathy therapy (Desai & Braunwald 2024; Rehan *et al*. 2024)
glutaral	antibacterial (Sehmi *et al*. 2016), desinfectant (Jara *et al*. 2013)
Creatine	energy supplement (Cooper *et al*. 2012; Kreider *et al*. 2017)