



# Advancing the discovery of bioactive compounds, its extraction and identification from the underexplored mollusc, *Cipangopaludina lecythis* (W. H. Benson, 1836)

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## Abstract

The enormous diversity of molluscs has provided humans with food, colours, medicines and shells, among other resources. Molluscs have long been utilized in traditional medicine in several countries and they are a valuable source of medical supplies for many diverse communities worldwide. The purpose of this study was to identify and assess *Cipangopaludina lecythis* bioactive compounds in order to determine the nature of the primary ingredient that gives its medicinal properties. *C. lecythis* flesh and shell extracts using polar and lipophilic solvents were analyzed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS). This study provides the first chemical assessment of flesh and shell-opercula of *C. lecythis*. Chemical analysis of flesh and shell-opercula of *C. lecythis* clearly showed the presence of major compounds such as chitin, allantoin, linoleic acid, dihydrotachysterol, cyclotrisiloxane hexamethyl and 6-gingerol besides other minor compounds with bioactive properties of medicinal significance. Overall, this research provides good evidence that *C. lecythis* produce secondary metabolites with a variety of intriguing pharmacological characteristics. They have also long been a part of traditional medicine in many human cultural groups. Its usage by traditional practitioners to treat a range of human diseases is justified by the presence of numerous medicinally significant bioactive chemicals. However, more research on the bioactive compounds found in snails is necessary to standardize the extraction techniques for their detection, quantification and formulations, to validate their *in vivo* efficacy, and confirm their safety.

**Keywords:** Gastropods, Bioactive compounds, Traditional medicine, Shell, Opercula

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## Introduction

Many natural products have been identified and characterized; however, the primary obstacle to the development of natural products is the sourcing of natural products in the quantities needed for drug development (Berrue et al., 2011). Accordingly, research is needed to ensure the sustainable production of natural products, starting with an understanding of their biosynthetic origin. Since many of these molecules are not only novel but also complex and challenging to synthesize, one of the biggest obstacles facing researchers and pharmaceutical companies today in the production and development of successful natural products is the supply issue (Berrue et al., 2011). Alternative methods for providing adequate quantities of pharmaceutical compounds for clinical testing and commercialization include genetic modification or microbial fermentation, as well as aquaculture of molluscs producing medicinally significant bioactive compounds. Molluscs have become a significant source of many distinct secondary metabolites that may be utilized in the creation of novel medications to treat a variety of fatal, both communicable, and non-communicable illnesses. It is difficult to commercialize new medications, and during the phases of development and approval, economic viability for long-term production must be taken into account.

Molluscs are incredibly diverse animals that have given humans access to a variety of resources, including food, colours, medicines, and shells. After arthropods, molluscs make up the second biggest animal phylum on Earth, accounting for about 7% of all living things. An estimated 200,000 species exist (Bouchet, 2006), of these about 5,000 species are found in fresh water (Seddon, 2000). Because of their diversity and ability to adapt to a wide range of habitats, including freshwater, brackish water, the marine environment, and terrestrial regions, molluscs have developed a variety of lifestyles and feeding niches. In addition to being regarded as a delicacy and enjoyed in many cultures, molluscs are a rich source of chemicals with medicinal activity. Molluscs have been utilized for food and medical purposes since the beginning of time. Throughout history, molluscs have been a valuable source of medicinal products for numerous cultures worldwide. Molluscs are used in several traditional medicines from ancient Roman medicine, China, India, South Africa etc. (Bonnemain, 2005; Prabhakar & Roy, 2009). Many bioactive natural products derived from molluscs are being developed for use as pharmaceuticals. The majority of research on natural products derived from molluscs concentrates on marine

species, with few studies available regarding the natural products chemistry of freshwater and terrestrial molluscs.

Gastropods including slugs and snails make up the largest class of molluscs, accounting for over 80% of all species (Strong et al., 2008). Gastropods habitats are highly diverse and found in both freshwater and marine environments. Since, gastropods are highly biologically adaptive, it is possible that their secondary metabolism or chemistry is diverse. Gastropods have long piqued the interest of many researchers due to their intriguing biological activities and distinctive adaptations. Gastropods are frequently utilized as an affordable food source and in traditional medicine. The bioactive compounds of snails may find use in pharmaceutical and therapeutic research. Gastropods are also known for their high protein value, essential amino acids and low fat content. Gastropods, particularly snails, have long been utilized by numerous ethnic groups as food and medicine (Jadhav et al., 2023). Since ancient times, snails have been prized as delicacies, provided an inexpensive and sustainable source of animal protein in both developed and developing nations worldwide, and they are found throughout Asia and Africa (DeMarco et al., 2017). Gastropods particularly the snails may offer an affordable and sustainable source of animal protein for the people of developing nations.

*Cipangopaludina* spp., a viviparid gastropod, is a common freshwater snail found in India, China, and numerous other South East Asian nations (Baghele et al., 2022; Zhao, 2011). This species lives in pools, lakes, streams, and other bodies of water, where it feeds on organic particles and microorganisms. Its fresh meat is traditionally eaten as food and medicine in China (Zhao, 2011) and by different ethnic groups in the states of northeast India (Tripathy & Mukhopadhyay, 2015). *C. chinensis* is one of the many edible mollusc species found in Asia, Africa, and Europe. The flesh of *C. chinensis* has been regarded as a delicious and delicate food in many countries for more than a millennium. Moreover, Chinese traditional medicine has long used *C. chinensis* to treat liver and diabetes disorders (Zhao, 2011). Numerous studies, mostly in China, have already been conducted on the bioactive compounds of *C. chinensis*. Another member of the Viviparidae family, *C. lecythis*, is used both as food and medicine (Zhao, 2011). *C. lecythis* has been used as food and traditional medicine by numerous ethnic groups in the northeastern part of India. However, the uses of *C. lecythis* for treating various medicinal conditions are not scientifically substantiated till date and little attention has been devoted to the bioactive compounds from *C. lecythis*. This study was pri-

marily aimed at preliminary screening of medicinally important bioactive compounds as there was no any earlier reports on the types of bioactive compounds present in *C. lecythis*. Hence, the purpose of this study was to identify and assess *C. lecythis*'s bioactive compounds in order to determine the nature of the primary ingredient that gives its medicinal properties. *C. lecythis* flesh and shell extracts using chloroform and methanol solvents were analysed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS). The presence of various medicinally important bioactive compounds justifies its uses for treating various ailments by traditional practitioners. Nevertheless, isolating and quantifying individual bioactive compounds and testing them for biological activity will undoubtedly produce positive results and aid in the discovery of new medications. This study provides the first report on the scientific validation of bioactive compounds from *C. lecythis*.

## Materials and Methods

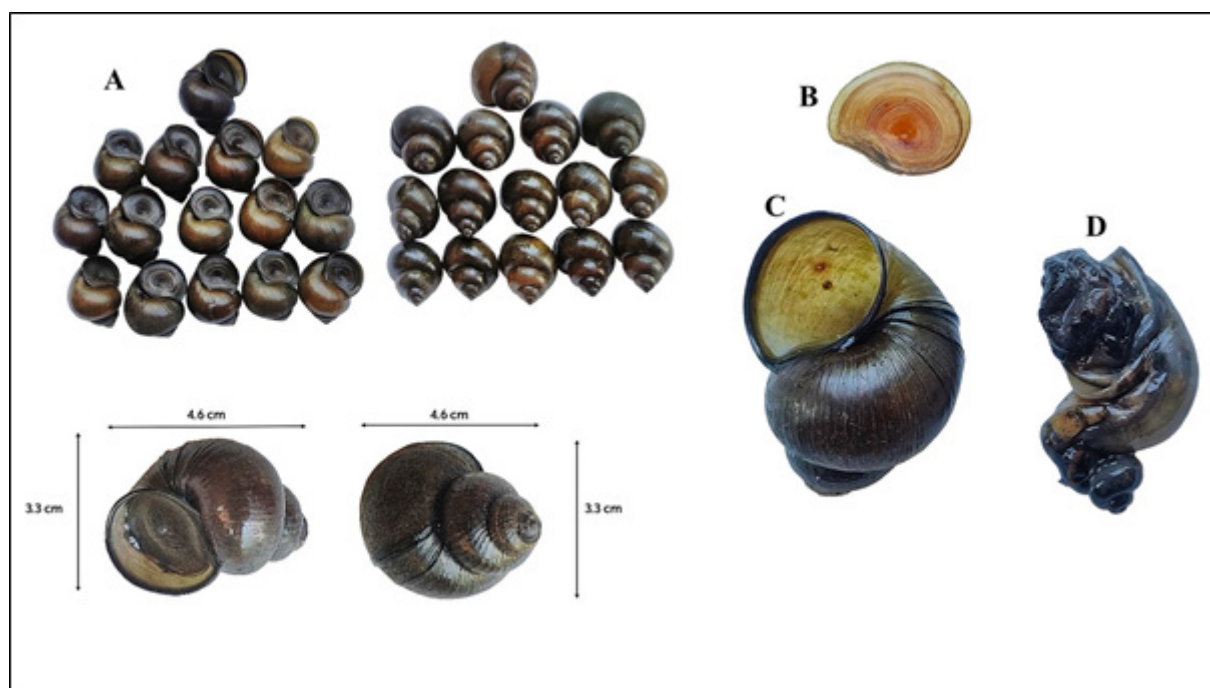
### Sample collection and dissection

Adult specimens of *C. lecythis* (Fig. 1A) were collected from

Waithou, Manipur, India during August 2023 and used for preparing sequential lipophilic (chloroform) and polar (methanol) solvent extracts. Snails were held in aerated water tanks for a maximum of 24 hours before processing. The shell of *C. lecythis* was removed by fracturing using a pressure applied at the point between the primary body whorl and spire. After removing the shell (Fig. 1C), opercula (Fig. 1B) were carefully separated from the snail flesh (Fig. 1D) using a sharp scalpel. The shell/opercula combined and flesh were used for LC-MS and GC-MS analysis.

### Preparation of lipophilic and polar shell-operculum and flesh extracts of *C. lecythis*

Shell-operculum and flesh tissue (10 g) of *C. lecythis* extracts were prepared using chloroform and methanol solvents (50 mL). Chloroform and methanol were HPLC grade (Sigma-Aldrich, St. Louis, MO, USA). The shell-operculum and flesh tissue from snails were homogenised using mortar and pestle in respective solvents and transferred to a glass vial. The solvent was decanted and replaced after every 2 h. This step was repeated for four times until the filtrate becomes colourless, with a final soak in solvent overnight in a fridge (Videocon 190



**Fig. 1. Freshwater snail, adult *Cipangopaludina lecythis* specimen (along with the scale measurement) and its different part used for bioactive compounds extraction. A, adult *C. lecythis*; B, opercula; C, shell; D, flesh of adult *C. lecythis*.**

L, Videocon, Mumbai, India) at 4 °C. The solvent extracts were combined and filtered using Whatman No.1 filter paper (Whatman, Buckinghamshire, UK). The resulting filtrate was dried under vacuum in rotary evaporator (Buchi, Flawil, Switzerland) at 40 °C and 150 mb pressure and then solvents were removed. Dried extract (0.5 g) was dissolved in respective solvent (5 mL) for transfer into already pre-weighed glass vials. The tissue extract was weighed before storing at –20 °C (Blue Star, India). The bioactive compound analysis of the snail extracts was performed by LC-MS using an Agilent 1260 Infinity II/LC-MSD iQ and volatile compounds of the snail extracts were separated using GC-MS Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a MS (5977B VL MSD, Agilent Technologies).

#### Liquid chromatography-mass spectrometry (LC-MS) analysis of *C. lecythis* extracts

*C. lecythis* chloroform and methanol extracts were analysed using LC-MS for the production of bioactive compounds. LC-MS analysis was undertaken using an Agilent 1260 Infinity II/LC-MSD iQ (Agilent Technologies) instrument according to the procedure described by Nongmaithem et al. (2017), using a 2.1 × 50 mm C18 column and gradient elution over 7 min with 5:95 acetonitrile/water with 0.1% formic acid to 95:5 acetonitrile/water with 0.1% formic acid. Mass spectrometry data were recorded 100–800 m/z in ESI+ and ESI- modes. The injection volume was 10 µl for all samples. Agilent ChemStation software was used to analyze the LC-MS data and predicted chemical formulae were used as input in the National Library of Medicine, USA when looking for matching bioactive metabolites. Statistical analysis was represented as mean with standard deviation in triplicate.

#### Gas chromatography-mass spectrometry (GC-MS) analysis of *C. lecythis* extracts

Volatile compounds from the snail extracts were separated using an Agilent 7890A GC (Agilent Technologies) equipped with a MS (5977B VL MSD, Agilent Technologies) according to the procedure described by Nongmaithem et al. (2017). The column temperature was programmed to increase from 40 °C (after a 5-min hold) to 120 °C at a rate of 3 °C/min (after a 2-min hold), and then to 250 °C at a rate of 8 °C/min with a 10-min hold. The total running time of GC was 60 min. Identification and interpretation of the bioactive compounds present were done using the library database of National Institute Standard and Technol-

ogy 2017 (NIST). Statistical analysis data were represented as mean with standard deviation in triplicate.

## Results and Discussion

This study provides the first chemical assessment of flesh and shell-opercula of *C. lecythis*. The chemical analysis of flesh and shell-opercula of *C. lecythis* clearly showed the presence of numerous compounds with bioactive properties of medicinal significance. For the flesh and shell-opercula of *C. lecythis*, the bioactive characteristics or uses along with their retention time, molecular formula, molecular weight, and concentration of each component using polar (methanol) and lipophilic (chloroform) solvents were displayed in Tables 1–3.

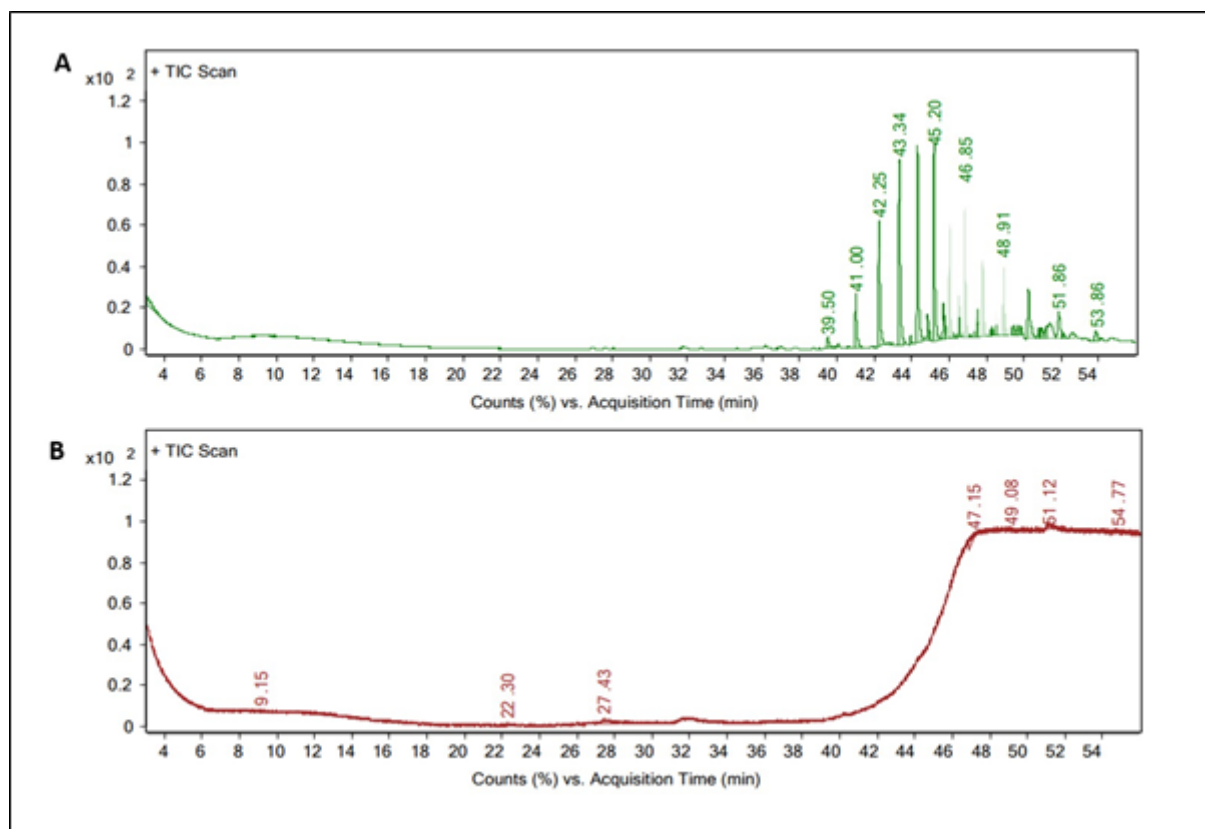
Our results clearly indicated that the major volatile compounds in the *C. lecythis* flesh contains 1-Octadecanol, chloroform extract (CE) cyclotrisiloxane, hexamethyl methanol extract (ME). The most prevailing volatile compounds in the flesh by the peak area obtained were identified as and hexamethylcyclotrisiloxane and 1-Octadecanol (Table 1 and Fig. 2). According to previous reports, hexamethylcyclotrisiloxane was reported to have antimicrobial, antioxidant, and anti-inflammatory properties (Ibrahim et al., 2022). These properties have also been reported from snails, *Eobania vermiculata*, *Theba pisana*, and *Monacha obstructa* (Sallam et al., 2009). Remarkably, hexamethylcyclotrisiloxane was the main compound found in *C. lecythis* flesh. There have been reports of the antibacterial, antifungal, and anti-larva properties of the bioactive compound, 1-Octadecanol (Falodun et al., 2009). Pringgenies et al. (2015) also reported the antibacterial property of 1-Octadecanol from the soft-bodied gastropod nudibranch, *Jorunna funebris*. Recently, it was also reported that snail extract could be used in treating wrinkled or burned skin (Noothuan et al., 2021). The biological properties of snail extracts are well recognized, and they are utilized in traditional medicine to treat a wide range of illnesses.

The main bioactive component found in the shell-opercula of *C. lecythis* extract was dihydrotachysterol (<sup>ME</sup>) (Table 2). A number of additional minor bioactive volatile compounds was also found (Table 2 and Fig. 3). Dihydrotachysterol, a synthetic hydrogenated analog of vitamin D is required for normal bone mineralization, calcium and phosphate absorption and utilization, and for enhancing calcium deposition (Cordy, 1977). Light catalyzes the rearrangement of ecdysterone in the enol form to penta-hydroxycholecalciferol or hexahydroxy-dihydrotachys-

**Table 1. Chemical constituents of *Cipangopaludina lecythis* flesh extract using gas chromatography-mass spectrometry (GC-MS)**

Compounds	Molecular formula	Retention time (min)	Major ion m/z	Area % compound $\pm$ SD <sup>1)</sup>	Bioactive properties or uses	References
Eicosane <sup>CE</sup>	C <sub>20</sub> H <sub>42</sub>	36.09	281.1; 261.1	1.5 $\pm$ 0.29	Anti-inflammatory, analgesic, and antipyretic and antifungal	Ahsan et al. (2017)
1-Octadecanol <sup>CE</sup>	C <sub>18</sub> H <sub>38</sub> O	45.86	295.3; 279.3	11.33 $\pm$ 0.45	Antibacterial, antifungal, anti-larval	Falodun et al. (2009)
Octacosanol <sup>CE</sup>	C <sub>28</sub> H <sub>58</sub> O	47.07	292.2; 281.1	4.83 $\pm$ 0.55	Anti-fatigue, anti-hypoxia, antioxidant, Anti-inflammatory, antitumor	Zhou et al. (2022)
Octadecane <sup>CE</sup>	C <sub>18</sub> H <sub>38</sub>	47.47	295.3; 286.0	4.2 $\pm$ 0.24	Unknown	ND
Cyclohexylmethyl octadecyl ester <sup>CE</sup>	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub> S	48.23	285.1; 277.1	5.39 $\pm$ 0.36	Unknown	ND
Heiicosane, 5-methyl <sup>CE</sup>	C <sub>22</sub> H <sub>46</sub>	48.29	292.3; 281.2	3.94 $\pm$ 0.25	Unknown	ND
2-Azido-2,4,4,6,6,8,8-heptamethylnonane <sup>CE</sup>	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub>	49.40	284.1; 269.0	4.76 $\pm$ 0.17	Unknown	ND
Eicosane, 2,4-dimethyl <sup>CE</sup>	C <sub>22</sub> H <sub>46</sub>	49.49	295.3; 287.1	3.88 $\pm$ 0.23	Unknown	ND
Nonyl tetraicosyl ether <sup>CE</sup>	C <sub>33</sub> H <sub>68</sub> O	50.39	295.3; 278.2	3.38 $\pm$ 0.37	Unknown	ND
2-(1-Methylcyclohexyloxy)-tetrahydropyran <sup>CE</sup>	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>	50.89	295.3; 281.1	3.80 $\pm$ 0.30	Unknown	ND
2-methylhexacosane <sup>CE</sup>	C <sub>27</sub> H <sub>56</sub>	50.92	291.1; 281.1	2.92 $\pm$ 0.14	Antibacterial and antifungal	Chakraborty et al. (2022)
Dotriacontane, 1-iodo <sup>CE</sup>	C <sub>32</sub> H <sub>66</sub> I	51.06	290.1; 275.2	3.88 $\pm$ 0.29	Unknown	ND
2-Methyl-cis-7,8-epoxymonadecane <sup>CE</sup>	C <sub>30</sub> H <sub>60</sub> O	51.31	291.1; 281.1	3.21 $\pm$ 0.39	Unknown	ND
Eicosane, 9-cyclohexyl <sup>CE</sup>	C <sub>27</sub> H <sub>52</sub>	52.08	291.1; 281.1	3.09 $\pm$ 0.07	Antioxidant, laxative, antimicrobial, antitumor, anti-nociceptive	Mohamad et al. (2018)
Nonyl tetraicosyl ether <sup>CE</sup>	C <sub>33</sub> H <sub>68</sub> O	52.39	292.2; 278.1	3.016 $\pm$ 0.15	Unknown	ND
Nonahexacontanoic acid <sup>CE</sup>	C <sub>60</sub> H <sub>138</sub> O <sub>2</sub>	52.41	294.3; 278.1	2.93 $\pm$ 0.20	Unknown	ND
Heiicosyl pentafluoropropionate <sup>CE</sup>	C <sub>28</sub> H <sub>43</sub> F <sub>5</sub> O <sub>2</sub>	52.59	294.3; 278.0	2.34 $\pm$ 0.37	Unknown	ND
Heiicosyl heptafluorobutyrate <sup>CE</sup>	C <sub>28</sub> H <sub>43</sub> F <sub>7</sub> O <sub>2</sub>	52.71	295.3; 279.3	1.5 $\pm$ 0.19	Unknown	ND
Cyclotetrasiloxane, octamethyl <sup>ME</sup>	C <sub>8</sub> H <sub>24</sub> O <sub>4</sub> Si <sub>4</sub>	22.22	281.0; 207.0	1.6 $\pm$ 0.24	Hair conditioning agent, antiseptic, antimicrobial, and skin-conditioning	Mary & Giri (2016)
2,6-Difluorobenzoic acid, 4-nitrophenyl ester <sup>ME</sup>	C <sub>13</sub> H <sub>7</sub> F <sub>2</sub> NO <sub>4</sub>	27.18	207.1; 191.1	1.40 $\pm$ 0.31	Unknown	ND
4-Chlorobutyric acid, cyclohexylmethyl ester <sup>ME</sup>	C <sub>11</sub> H <sub>19</sub> ClO <sub>2</sub>	39.85	283.0; 266.9	0.35 $\pm$ 0.07	Unknown	ND
Cyclotrisiloxane, hexamethyl <sup>ME</sup>	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si <sub>3</sub>	46.88	295.0; 281.0	5.52 $\pm$ 0.39	Antimicrobial	Ibrahim et al. (2022)
2-Methyl(dipyrrolo[1,2-a:2'1'-c]pyrazine-3-carbonitrile <sup>ME</sup>	C <sub>12</sub> H <sub>9</sub> N <sub>3</sub>	53.50	285.0; 271.0	4.73 $\pm$ 0.21	Unknown	ND
1,4-Benzenediol[2,3,5,6-tetrafluoro-bis(butyl)ether] <sup>ME</sup>	C <sub>18</sub> H <sub>18</sub> F <sub>4</sub> O <sub>2</sub>	55.50	285.1; 270.9	3.83 $\pm$ 0.20	Unknown	ND

<sup>1)</sup>The total Area % for the compounds presented here does not add up to 100% due to the presence of other unidentified compounds in the extracts. CE, chloroform extract; ME, methanol extract; ND, not detected.



**Fig. 2. Gas chromatography-mass spectrometry (GC-MS) chromatograms of *Cipangopaludina lecythis* flesh extracts. A, chloroform extract; B, methanol extract.**

terol, and has been previously reported that ecdysterone plays a role in the shell calcification of snails (Whitehead & Saleuddin, 1978). Dihydrotachysterol, which has been found in the shell and opercula of *C. lecythis*, might have originated from the environment or from food sources.

Chitin and 6-gingerol were among the major compounds identified in the flesh using LC-MS methanol extract (Table 3). Based on the peak area obtained, chitin was the most prevalent compound in the flesh (Fig. 4). It is interesting to note that chitin was found in the flesh of *C. lecythis* rather than in the shell (Fig. 4). Chitin has been reported to be present in the snail foot muscle of *Charonia tritonis*, a giant triton snail, and *Chrysomallon squamiferum*, a scaly-foot snail (Zhang et al., 2021). The mandible of *Helix pomatia* was found to contain chitin, according to Tóth & Zechmeister (1939), and the snail's gut contained the chitin-degrading enzyme chitinase. According to Yonezawa et al. (2016), chitin in mollusc shells was known to be crucial to the calcification of the shell. Chitin, a natural polysaccharide, is frequently present in the arthropods, including crabs, shrimp,

and insects, as well as in the shells of bivalves, but it is less common in the shells of snails. Chitin is recognized for possessing special biochemical qualities, non-toxic, biocompatible, and biodegradable biopolymer with potential uses in medicine. Chitin is also found to be utilized in tissue engineering, wound dressings, antimicrobial agents, anti-aging cosmetics, and vaccine adjuvants (Ali-Komi & Hamblin, 2016).

This study also found that linoleic acid, which is commonly present in snails and slugs, has anti-inflammatory qualities (Özçiçek et al., 2023). Essential fatty acids such as linoleic acid play a crucial role in maintaining nerve blood flow by being a structural component of the phospholipids that make up neuronal membranes (Zhu et al., 1994).

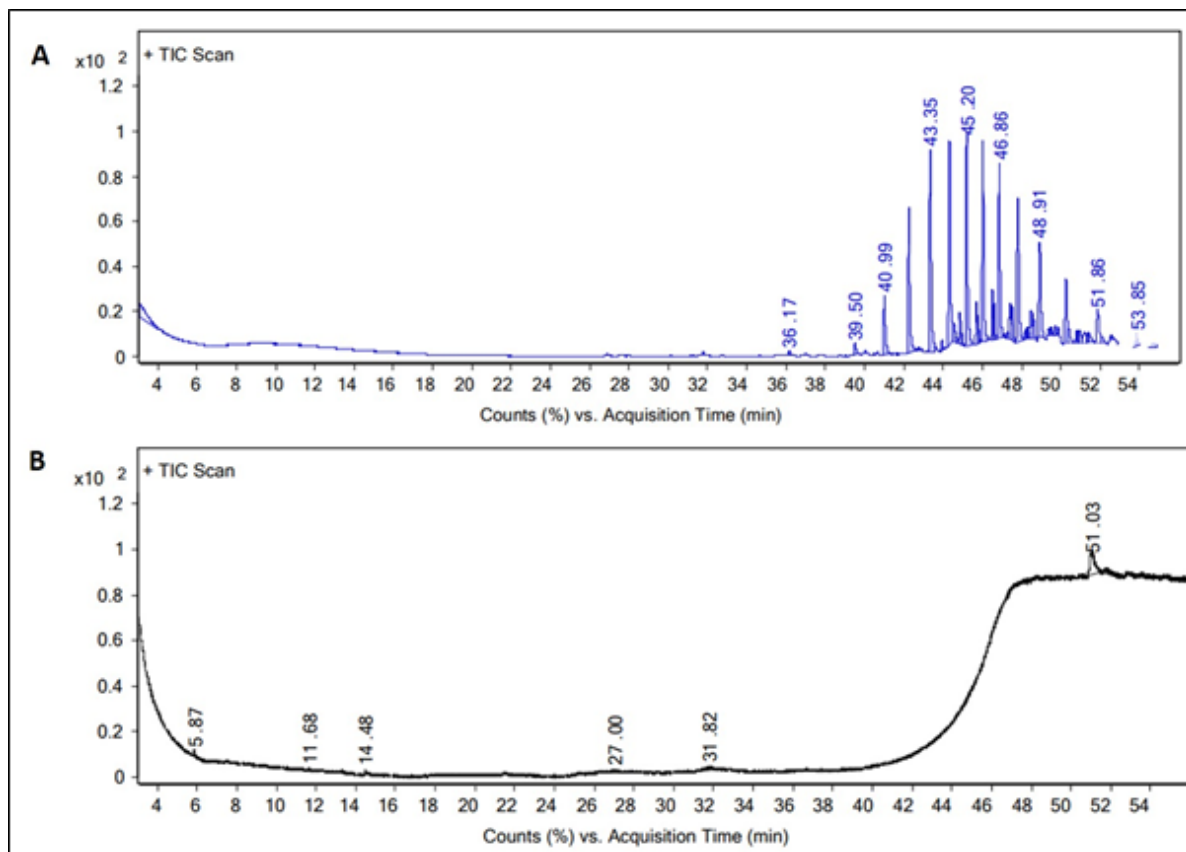
This investigation also unveiled 6-gingerol, another significant bioactive substance from the flesh of *C. lecythis*. 6-gingerol from the snail *Achatina fulica* was also reported by Ajisafe & Raichur, (2023), and their findings suggested that 6-gingerol may be useful for cartilage tissue engineering and therapeutic repair. The mucus secretion from *A. fulica* exhibited

**Table 2. Chemical Constituents of *Cipangopaludina lecythis* shell-opercula extract using gas chromatography-mass spectrometry (GC-MS)**

Compounds	Molecular formula	Retention time (min)	Major ion m/z	Area % compound $\pm$ SD <sup>1)</sup>	Bioactive properties or uses	References
Eicosane <sup>CE</sup>	C <sub>20</sub> H <sub>42</sub>	36.10	261.1; 253.2	2.42 $\pm$ 0.27	Anti-inflammatory, analgesic, and antipyretic and antifungal	Ahsan et al. (2017)
Heicosane <sup>CE</sup>	C <sub>21</sub> H <sub>44</sub>	39.43	281.1; 267.2	3.34 $\pm$ 0.42	Unknown	ND
Pyran-4-carboxylic acid, 4-(4-methoxyphenyl)-tetrahydro <sup>CE</sup>	C <sub>13</sub> H <sub>16</sub> O <sub>4</sub>	44.51	293.1; 282.0	9.05 $\pm$ 0.14	Unknown	ND
Sulfurous acid, cyclohexylmethyl octadecyl ester <sup>CE</sup>	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub> S	48.20	292.2; 283.0	3.03 $\pm$ 0.16	Unknown	ND
Eicosane, 2,4-dimethyl <sup>CE</sup>	C <sub>22</sub> H <sub>46</sub>	48.27	295.3; 279.3	4.95 $\pm$ 0.14	Unknown	ND
2-Thiopheneacetamide, N-(2-ethylhexyl) <sup>CE</sup>	C <sub>14</sub> H <sub>23</sub> NOS	49.41	289.1; 281.1	2.94 $\pm$ 0.15	Unknown	ND
Cyclohexane, docosyl <sup>CE</sup>	C <sub>28</sub> H <sub>56</sub>	50.39	292.2; 279.3	1.58 $\pm$ 0.35	Unknown	ND
1-Eicosene <sup>CE</sup>	C <sub>20</sub> H <sub>40</sub>	50.69	296.3; 281.1	2.03 $\pm$ 0.21	Unknown	ND
2-Methylhexacosane <sup>CE</sup>	C <sub>27</sub> H <sub>56</sub>	50.90	295.3; 281.1	1.42 $\pm$ 0.33	Antibacterial and antifungal	Chakraborty et al. (2022)
22-Tricosenoic acid <sup>CE</sup>	C <sub>23</sub> H <sub>44</sub> O <sub>2</sub>	51.40	291.1; 273.0	4.22 $\pm$ 0.25	Unknown	ND
Docosyl octyl ether <sup>CE</sup>	C <sub>30</sub> H <sub>62</sub> O	52.58	292.3; 279.2	1.69 $\pm$ 0.22	Unknown	ND
1-Dodecanol, 2-octyl <sup>CE</sup>	C <sub>20</sub> H <sub>42</sub> O	54.50	293.2; 278.3	1.33 $\pm$ 0.28	Unknown	ND
Hexane, 3,3-dimethyl <sup>ME</sup>	C <sub>8</sub> H <sub>18</sub>	14.44	127.1; 113.0	1.01 $\pm$ 0.12	Unknown	ND
Butanoic acid, 2-methyl-, 1,2-dimethylpropyl ester <sup>ME</sup>	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	21.36	207.0; 126.0	2.99 $\pm$ 0.31	Unknown	ND
Nonane, 5-(1-methylpropyl) <sup>ME</sup>	C <sub>13</sub> H <sub>28</sub>	26.79	253.0; 281.1	3.95 $\pm$ 0.17	Unknown	ND
Oxirane, 2-methyl-3-propyl-, cis <sup>ME</sup>	C <sub>6</sub> H <sub>12</sub> O	26.96	155.0; 130.1	0.92 $\pm$ 0.14	Unknown	ND
Fumaric acid, pent-4-en-2-yl propyl ester <sup>ME</sup>	C <sub>12</sub> H <sub>18</sub> O <sub>4</sub>	27.25	207.0; 191.1	4.56 $\pm$ 0.38	Unknown	ND
1,4-Benzenediol, 2,3,5,6-tetrafluoro-, bis(butyl) ether <sup>ME</sup>	C <sub>14</sub> H <sub>18</sub> F <sub>4</sub> O <sub>2</sub>	32.71	281.0; 207.1	1.59 $\pm$ 0.21	Unknown	ND
Cyclotrisiloxane, hexamethyl <sup>ME</sup>	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si <sub>3</sub>	42.33	281.0; 267.0	3.31 $\pm$ 0.40	Antioxidant and antidiabetic	Ibrahim et al. (2022)
Octacosanol <sup>ME</sup>	C <sub>28</sub> H <sub>58</sub> O	48.09	292.3; 284.0	1.82 $\pm$ 0.36	Anti-fatigue, antioxidant, anti-inflammatory, antitumor	Zhou et al. (2022)
Eicosane, 2,4-dimethyl <sup>ME</sup>	C <sub>22</sub> H <sub>46</sub>	48.29	294.4; 282.1	2.54 $\pm$ 0.24	Unknown	ND
Dotriacontane, 1-iodo <sup>ME</sup>	C <sub>32</sub> H <sub>65</sub> I	49.39	295.3; 282.1	4.88 $\pm$ 0.43	Unknown	ND
Dihydrodactysterol <sup>ME</sup>	C <sub>28</sub> H <sub>46</sub> O	50.86	275.2; 282.0	12.84 $\pm$ 0.12	Synthetic analogue of vitamin D, bone mineralization	Cordy (1977)
Nonadecyl heptafluorobutyrate <sup>ME</sup>	C <sub>23</sub> H <sub>39</sub> F <sub>7</sub> O <sub>2</sub>	52.40	294.3; 279.3	3.89 $\pm$ 0.13	Unknown	ND

<sup>1)</sup>The total Area % for the compounds presented here does not add up to 100% due to the presence of other unidentified compounds in the extracts.

CE, chloroform extract; ME, methanol extract; ND, not detected.



**Fig. 3. Gas chromatography-mass spectrometry (GC-MS) chromatograms of *Cipangopaludina lecythis* shell and opercula extracts. A, chloroform extract; B, methanol extract.**

wound-healing and anti-apoptotic properties due to it. Anti-cancer, anti-inflammatory, and anti-oxidant properties of 6-gingerol have earlier been reported by Wang et al. (2014). The fact that *C. lecythis* contains 6-gingerol demonstrates how this snail is used in traditional medicine.

The shell and opercula of *C. lecythis* extract contained major bioactive compounds that were likely to be allantoin as well as several other minor bioactive compounds of medical significance (Table 3 and Fig. 5). Allantoin was the most prevalent compound in the shell and opercula extract based on the peak area obtained (Fig. 5). Allantoin was known to possess cytoprotective, gastroprotective, antioxidant, and anti-inflammatory properties (Dinica et al., 2021). Allantoin, an imidazolidine-2,4-dione, is a safe and efficient active ingredient for skin protection that is primarily used in modern medicine and cosmetics. It is utilized in over-the-counter cosmetics as well as other commercial goods like shampoos, conditioners, sun protection products, and oral hygiene products (Dinica et al., 2021).

Allantoin was used as an ingredient in cosmetic skin creams, was previously identified as coming from the garden snail, *Helix aspersa*. El Mubarak et al. (2013) established a protocol for the simultaneous detection of allantoin and showed that there was a notable variation in the amounts of these compounds amongst various cosmetic creams, which may indicate the product's quality or the effectiveness of the snail mucus content. The positive bioactive effect of the compound was largely driven by the quantity/volume used for specific efficacy (El Mubarak et al. 2013). Given the toxicity of certain natural products, one should never assume that using traditional or natural medicine is safe. Therefore, quantification of the specific dose is a must needed step of any drug discovery protocol. This may be the reason that the traditional preparation techniques employed the method which may be able to lessen or eliminate the harmful elements in some traditional medical systems. The pharmaceutical industry uses only the shells of the mollusc species with toxic venoms such as neogastropods and cone snails for har-



**Table 3. Chemical constituents of *Cipangopaludina lecythis* flesh and shell-opercula extracts using liquid chromatography-mass spectrometry (LC-MS)**

Sample details	Compounds	Molecular formula	Retention time (min)	Major ion	Area % compound $\pm$ SD <sup>1)</sup>	Bioactive properties or uses	References
Flesh	Chitin <sup>CE</sup>	(C <sub>8</sub> H <sub>13</sub> O <sub>3</sub> N) <sub>n</sub>	1.283	203.1; 202.0	29.21 $\pm$ 0.41	Tissue engineering, wound dressings, antimicrobial agents, antiaging cosmetics, and vaccine adjuvants	Ali-Korri & Hamblin (2016)
Flesh	6-gingerol <sup>CE</sup>	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	1.729	294.7; 236.0	29.03 $\pm$ 0.17	Anticancer activities, anti-inflammation, and anti-oxidation	Wang et al. (2014)
Flesh	Chamazulene <sup>ME</sup>	C <sub>14</sub> H <sub>16</sub>	1.176	188.9; 185.0	16.45 $\pm$ 1.01	Antioxidant properties and reverses Osteoarthritis inflammation	Capuzzo et al. (2014)
Flesh	Carotene-Zeta <sup>ME</sup>	C <sub>40</sub> H <sub>60</sub>	1.488	541.7; 540.8	7.07 $\pm$ 0.78	Unknown	ND
Flesh	Hesperidin <sup>ME</sup>	C <sub>28</sub> H <sub>34</sub> O <sub>15</sub>	1.719	742.6; 637.0	5.45 $\pm$ 0.47	Antioxidant, antibacterial, antimicrobial, anti-inflammatory and anti-carcinogenic	Pyrzyńska (2022)
Flesh	Linoleic acid <sup>ME</sup>	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	2.374	279.7; 201.6	7.12 $\pm$ 0.33	Anti-inflammatory properties	Özççek et al. (2023)
Shell/opercula	Allantoin <sup>CE</sup>	C <sub>4</sub> H <sub>6</sub> N <sub>4</sub> O <sub>3</sub>	1.822	159.7; 116.9	20.31 $\pm$ 0.38	Antioxidant, anti-inflammatory Cytoprotective	Dinica et al. (2021)
Shell/opercula	Lupanine <sup>ME</sup>	C <sub>13</sub> H <sub>24</sub> N <sub>2</sub> O	1.117	249.0; 232.9	8.26 $\pm$ 0.09	Antioxidant, anti-cholinergic and antidiabetic activity	Kolak et al. (2011)
Shell/opercula	Syringic acid <sup>ME</sup>	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	1.228	197.5; 195.8	12.22 $\pm$ 0.25	Anti-oxidant, antimicrobial, anti-inflammatory, antiendotoxic	Srinivasulu et al. (2018)

<sup>1)</sup> The total Area % for the compounds presented here does not add up to 100% due to the presence of other unidentified compounds in the extracts.  
CE, chloroform extract; ME, methanol extract; ND, not detected.

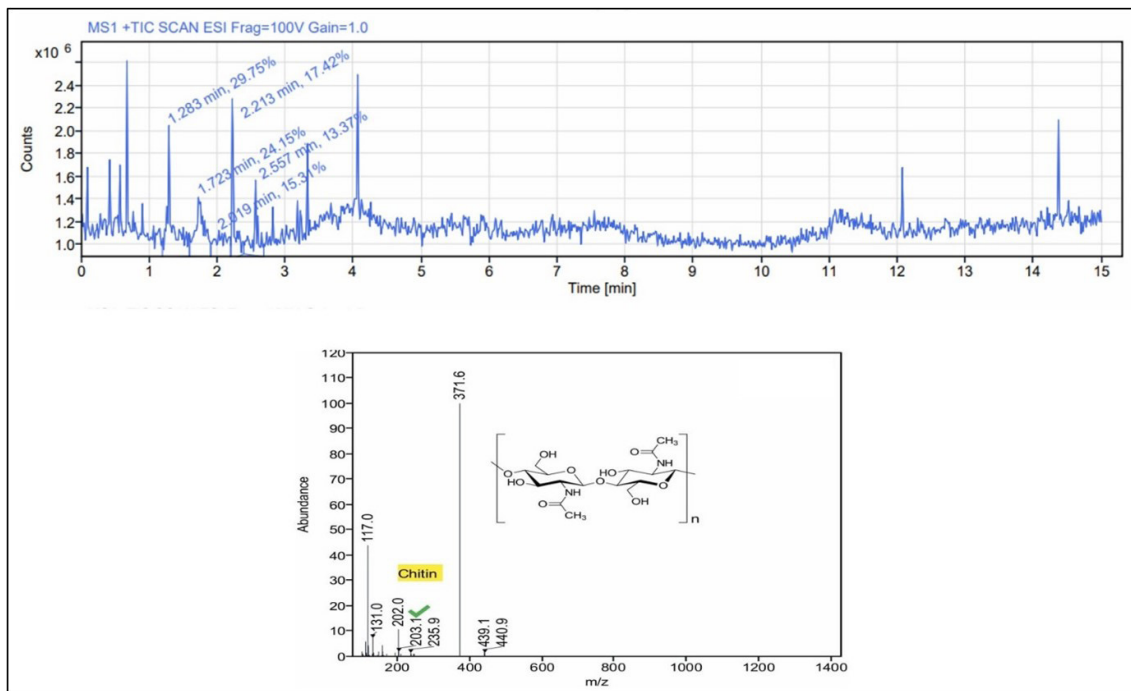


Fig. 4. Liquid chromatography-mass spectrometry (LC-MS) flesh extracts of *Cipangopaludina lecythis*, showing identification of chitin.

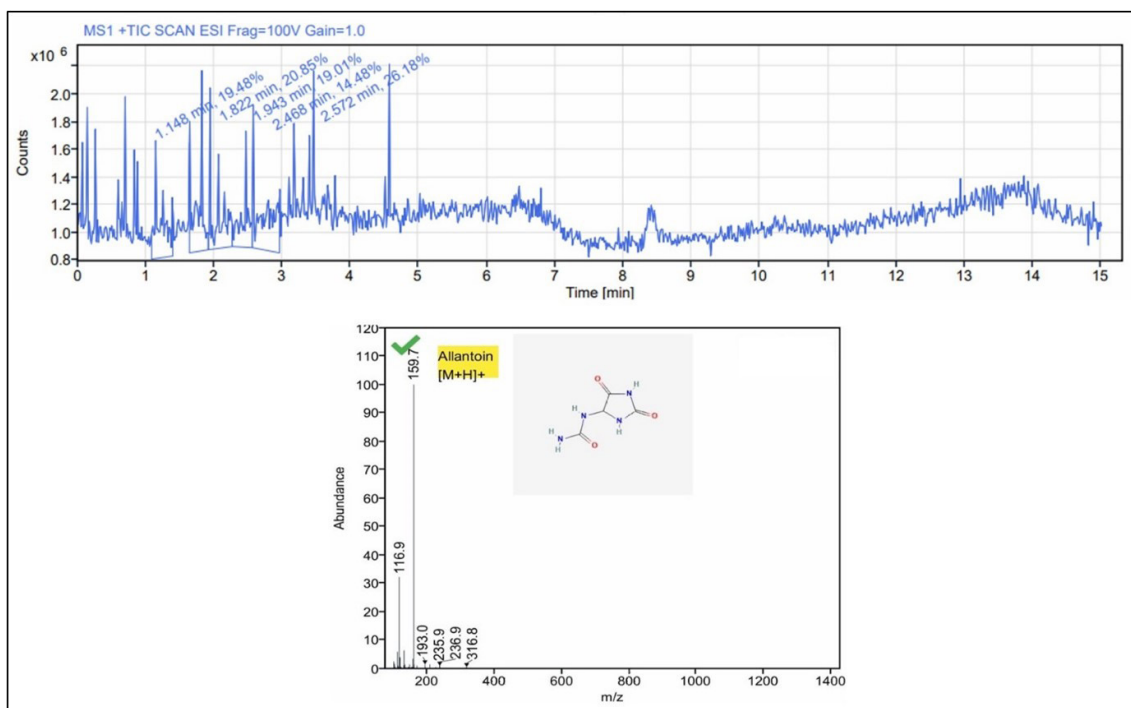


Fig. 5. Liquid chromatography-mass spectrometry (LC-MS) flesh extracts of *Cipangopaludina lecythis*, showing identification of allantoin.

nessing only the good compounds while leaving the toxic ones present in the flesh. Since *C. lecythis* extracts could be made widely available, highly affordable, and utilized in traditional medicine, they may prove to be a promising natural alternative to synthetic drugs in therapeutic products.

The chemical studies on molluscs have led to the isolation of several bioactive primary and secondary metabolites. The molluscs themselves may have synthesized the bioactive metabolites, or they may have obtained them from food sources or the microbial symbionts (Ngangbam et al., 2019). Snails are known to produce slime, a viscous secretion, which is valuable and gaining interest due to its potential applications in medicine. There are numerous studies which have examined and verified the beneficial effects of bioactive compounds found in gastropods, such as their antibacterial, anti-inflammatory, antioxidant, anti-tumoral, anti-aging, tissue regeneration, wound healing, and numerous other attributes (Dinica et al., 2021). The biological activities of snails have garnered a lot of attention lately because of their diverse and possibly beneficial properties.

## Conclusion

This study provides the first evidence of the presence of medicinally significant bioactive compound from *C. lecythis*, a freshwater snail. Bioactive compounds of pharmacological importance such as chitin, 6-gingerol, linoleic, hexamethylcyclotrisiloxane, eicosane, 1-octadecanol, octacosanol, eicosane, 9-cyclohexyl, nonyl tetracosyl ether and octamethyl were among the main compounds found in the flesh; whereas, allantoin, dihydrotachysterol and 2-methylhexacosane were identified in the shell and opercula extract of *C. lecythis*. The presence of these bioactive compounds justifies the use of these snails for treating various human ailments by traditional healers. Further investigation is required in finding the concentration, quantification and methods standardization of the target bioactive compound that may be used for future medical research and comprehending their biosynthetic origin through the use of an omics approach. Overall, *C. lecythis*, an underexplored freshwater snail could be a valuable source of bioactive compounds for application in human medicine and also significant for the pharmaceutical industry in terms of sustainable drug manufacturing.

## Competing interests

No potential conflict of interest relevant to this article was reported.

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## Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

## Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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