

Bioactive Metabolite Profiling of *Brotia costula*: Linking Traditional Knowledge with Modern Analytical Evidence

LAISHRAM LENIN SINGH, LAKSHMIKANTA KHUNDRAKPAM, BIJAYALAKSHMI DEVI NONGMAITHEM, LAIPHRAKPAM PINKY CHANU, KHANGEMBAMBRAJAMANI MEETEI¹ AND AJIT KUMAR NGANGBAM*

School of Biological Sciences, Manipur International University, Imphal-795 140 (Manipur), India
*(e-mail: ajikumarng83@gmail.com; Mobile: 94852 27590)

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ABSTRACT

Traditional medicinal practices in North-east India particularly Manipur have long utilized several species of freshwater snails including *Brotia costula* for treating skin ailments, wound healing and bone-related disorders. This study aimed at chemically profile the bioactive compounds present in *B. costula* using Liquid Chromatography Mass Spectrometry (LC-MS) and Gas Chromatography Mass Spectrometry (GC-MS) to support its ethno-medicinal relevance. Solvent extracts using chloroform and methanol were prepared from the edible tissue including muscular foot and visceral tissues of *B. costula*. LC-MS analysis revealed the presence of biomedical important compounds such as hesperidin, linoleic acid, chitin, perlolyrine, capsidiol and maslinic acid known for their anti-inflammatory, antioxidant, anticancer and wound healing properties. GC-MS analysis further revealed the presence of octacosanol, tetracosane, 1-hexacosanol, pentacosane, eicosane and hexadecanoic acid methyl ester, many of which have antimicrobial, antioxidant and anticancer activities. Several other bioactive compounds with unknown bioactivity were also detected during this study. This study showed the rich chemical diversity and bioactive potential of *B. costula*, hence supporting its use in traditional medicine and highlighting its importance as a sustainable source for therapeutic and functional food development.

Key words: Freshwater gastropods, functional food, natural product profiling, nutraceuticals, traditional medicine

INTRODUCTION

Traditional knowledge systems provide valuable insights into the health-promoting potential of underutilized freshwater gastropods, particularly in the regions facing nutritional and healthcare issues. Freshwater gastropods mainly the snails, though diverse and widely distributed, remain significantly less studied in natural product research, especially when compared to marine gastropods (Mahmoud *et al.*, 2023). Freshwater snail offers dual benefits as food with nutrient-rich and as a reservoir of medicinally important bioactive compounds which are valuable in the search for sustainable and culturally important therapeutic alternatives (Ngangbam *et al.*, 2024a; Nongmaithem *et al.*, 2024). Gastropods particularly the snails demonstrate high ecological adaptability, enabling them to survive across diverse environments and

potentially produce a wide range of bioactive secondary metabolites of medicinal importance (Ngangbam *et al.*, 2019, 2024b; Nongmaithem *et al.*, 2017, 2018). *Brotia costula*, a freshwater gastropod of the family Pachychilidae, is commonly consumed in the north-eastern part of India particularly Manipur. Community-based study on *B. costula* in Manipur highlighted its traditional use for wound healing, skin infections and bone-related ailments. However, limited awareness of its functional food potential was observed among the local populations of Manipur (Singh *et al.*, 2025). Scientific studies specifically focusing on the bioactive compounds and therapeutic potential of *B. costula* remain limited and under explored despite its nutritional benefits with rich in protein, polyunsaturated fatty acids (PUFAs) and essential micronutrients. Recently, few studies were conducted on the bioactive

¹Krishi Vigyan Kendra, Bishnupur, Utlou-795 134 (Manipur), India.

compound profiling of *B. costula*. Imsong and Murali (2022) demonstrated the presence of bioactive compounds with antioxidant and anti-diabetic activity in *B. costula*, further supported by DNA barcoding and phytochemical analysis. Another preliminary biochemical screening revealed the presence of antimicrobial, antioxidant and nutritional properties in *B. costula*, thereby reaffirming its ethno medicinal relevance (Rout *et al.*, 2022). These studies highlighted the need for more in-depth chemical profiling to preliminary validate its traditional use and pharmacological potential.

Ethnic communities across north-eastern states of India and other parts of Asia have long utilized molluscs in traditional medicine for various health conditions (Jadhav *et al.*, 2023; Ngangbam *et al.*, 2024 a,b; Nongmaithem *et al.*, 2024). However, the integration of these traditional practices into evidence-based pharmacology remains limited. This study, therefore, aimed at bridge this gap by conducting chemical profiling of bioactive metabolites through Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) in *B. costula* to support its ethno-medicinal relevance.

MATERIALS AND METHODS

Mature specimens of *B. costula* (n-6) were procured from Waithou, Manipur, India, in 2023 and 2024 for solvent extraction of lipophilic and polar bioactive compounds using methanol and chloroform solvents (Fig. 1). The collected snail's specimens were temporarily kept in a well-aerated aquarium tank for 24 h in the laboratory. A localized mechanical pressure was applied, fracturing the shell at the spire-body whorl to facilitate easy extraction of the internal soft tissue and the opercula were detached from the flesh using a sharp, sterile scalpel. The pool flesh tissues (Three snails were pooled each for chloroform and methanol solvent extraction) were co-homogenized and subjected to solvent extraction.

Ten g of co-homogenized flesh extracts of *B. costula* were prepared using 50 ml each of HPLC grade (Sigma-Aldrich, USA) chloroform and methanol solvents and then transferred to clean glass vials. The solvents were decanted and replaced with fresh solvents every 2 h,

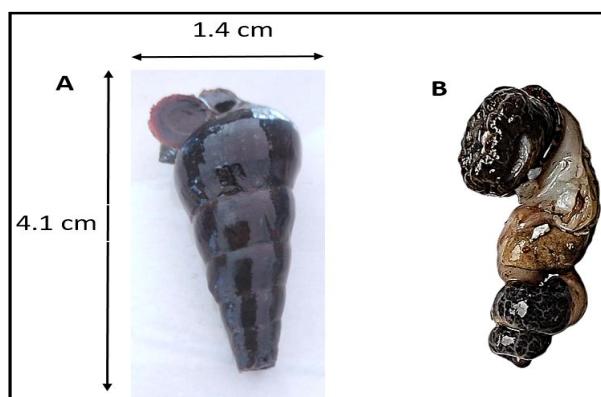


Fig. 1. A. Adult *B. costula* and B. Internal soft edible tissue of *B. costula*.

repeating the process four times, until the solvent colour was stabilized, followed by a final overnight soak in a refrigerator (Videocon 190 L, Videocon, Mumbai, India) at 4°C. The solvent extracts were then filtered through a Whatman No. 1 filter paper. The resulting filtrates were concentrated under reduced pressure using a rotary evaporator (Buchi, Flawil, Switzerland) at 40°C and 150 mb pressure to remove residual solvents. 0.5 g of the dried extract was re-dissolved in 5 ml of the respective solvent and transferred into pre-weight glass vials and stored at -20°C in a freezer (Blue Star, India). The extracts were subjected to LC-MS analysis with an Agilent 1260 Infinity II/LC-MSD iQ system. The volatile metabolites were identified via GC-MS with an Agilent 7890A GC system coupled with MS 5977B VL MSD (Agilent Technologies, Palo Alto, CA, USA).

The bioactive compounds from the methanol and chloroform extracts of *B. costula* were screened via LC-MS analysis on an Agilent 1260 Infinity II/LC-MSD iQ system, following the protocols prescribed by Nongmaithem *et al.* (2017). The chromatographic separation was carried out on a 2.1 × 50 mm C18 column, with a linear gradient over 7 min from 5:95 to 95:5 acetonitrile/water containing 0.1% formic acid. Mass spectrometry data were carried out in both ESI⁺ and ESI⁻ ionization modes within a mass range of 100-800 m/z. The injection volume for all samples was maintained at 10 µl. The chromatogram spectral data were analyzed using the Agilent ChemStation software and the identification of the resulting compounds was tentatively matched against the predicted chemical formulae in the National Library of Medicine, USA.

The volatile constituents were analyzed via GC-MS on an Agilent 7890A GC coupled with an MS (5877B VL MSD, Agilent Technologies) as per the protocols outlined by Nongmaithem *et al.* (2017). The initial column temperature was programmed at 40°C for 5 min, then increased to 120°C at a rate of 3°C /min for 2 min, and finally increased at a ramp rate of 250°C (8°C/m) for 10 min. The total analytical run was 60 min. The resulting compounds were identified based on the library databases updated by the National Institute of Standards and Technology (NIST).

RESULTS AND DISCUSSION

The chemical profiling of *B. costula* chloroform and methanol extracts using LC-MS and GC-MS revealed the presence of several medicinally important bioactive compounds. These preliminary studies validated the traditional medicinal uses of *B. costula* and its potential as a valuable source for nutraceutical development and drug discovery.

The LC-MS analysis of the chloroform extract of *B. costula* revealed the presence of three major compounds, namely, hesperidin, linoleic acid and chitin (Table 1) and several other minor bioactive compounds. Hesperidin was also reported from freshwater snails, *Cipangopaludina lecythis* and known to have anticancer, neuroprotective, antioxidant and anti-inflammatory activities (Aalikhani *et al.*, 2021; Ngangbam *et al.*, 2024 a, b). The presence of this bioactive compound supports the potential of *B. costula* in inflammatory disease interventions. Linoleic acid, which is known for its anti-inflammatory and immunomodulatory properties, was also earlier reported from freshwater snails (Özçicek *et al.*, 2023;

Ngangbam *et al.*, 2024 a,b). Linoleic acid role in immune response modulation supports the nutritional and therapeutic importance of *B. costula*. Chitin, a structural polysaccharide, has widespread applications in tissue engineering, wound healing and vaccine delivery systems. It is also known to have antimicrobial and anti-aging effects and earlier reported its presence in *C. lecythis* (Ali-Komi and Hamblin, 2016; Ngangbam *et al.*, 2024 a, b). The presence of these bioactive compounds in the edible tissues of *B. costula* supported its utility as a functional food.

The methanol extracts revealed the presence of several bioactive compounds such as perlolyrine, cucurbitine, maslinic acid and capsidiol (Table 2). Perlolyrine has been reported to have chemopreventive and antiproliferative properties (Lee *et al.*, 2016). Cucurbitine is known to have antihelmintic activity and used in skincare products for its anti-allergenic properties (Gawe³-Bében *et al.*, 2022). Maslinic acid is also reported to have a wide range of bioactivities such as anti-tumor, anti-parasitic, hypoglycemic and anti-inflammatory effects (Yan *et al.*, 2024). Capsidiol exhibits strong antifungal activity (Mal *et al.*, 2025), suggesting potential use in fungal infections. The identification of these bioactive compounds in *B. costula* highlights the importance of this freshwater snail in pharmaceutical industry.

The GC-MS analysis of the chloroform extracts showed the presence of several bioactive compounds such as heneicosane, tetracosane, octacosanol, eicosane, 2,4-dimethyl, 1-hexacosanol, nonadecylheptafluorobutyrate, pentacosane, dotriacontane, 2-methylhexacosane nonadecane, eicosane, nonyltetracosyl ether, 2-methyl-cis-7,8-

Table 1. Major compounds identified from *B. costula* chloroform extracts using LC-MS

Compound	Chemical formula	Structure	Retention time (min)	Major ion (m/z)	Bioactivity	References
Hesperidin	C ₂₈ H ₃₄ O ₁₅		1.702	441.6	Anticancer, neuroprotective, antioxidant, anti-inflammatory	Aalikhani <i>et al.</i> (2021)
Linoleic acid	C ₁₈ H ₃₂ O ₂		1.178	281.6	Anti-inflammatory properties	Özçicek <i>et al.</i> (2023)
Chitin	(C ₈ H ₁₃ O ₅ N) _n		1.344	203.1	Tissue engineering, wound healing, antimicrobial, anti-aging and vaccine adjuvants	Ali-Komi and Hamblin (2016); Ngangbam <i>et al.</i> (2024a, b)

Table 2. Major compounds identified from *B. costula* methanol extracts using LC-MS

Compound	Chemical formula	Structure	Retention time (min)	Major ion (m/z)	Bioactivity	References
Perolyrine	C ₁₆ H ₁₂ N ₂ O ₂		0.957	264.8	Chemopreventive and antiproliferative	Lee <i>et al.</i> (2016)
Cucurbitine	C ₅ H ₁₀ N ₂ O ₂		0.957	131.1	Anthelmintic, anti-allergen in skin care products	Gawel-Beben <i>et al.</i> (2022)
Maslinic acid	C ₃₀ H ₄₈ O ₄		1.583	473.9	Anti-tumor, hypoglycemic, anti-inflammatory and anti-parasitic	Yan <i>et al.</i> (2024)
Capsidiol	C ₁₅ H ₂₄ O ₂		2.034	236.9	Bacteriostatic against <i>Helicobacter pylori</i>	Mal <i>et al.</i> (2025)

epoxynonadecane, nonahexacontanoic acid, heneicosylpentafluoropropionate and eneicosylheptafluorobutyrate (Table 3). Octacosanol, which is a long-chain fatty alcohol, is known to have cholesterol lowering effect and also used as a nutritional supplement and functional food (Singh *et al.*, 2020). 1-hexacosanol exhibits antioxidant activity (Andrew *et al.*, 2024), while 1-octadecanol was also reported from freshwater snails (Ngangbam *et al.*, 2024 a, b) and known to have antibacterial, antifungal and anti-larval activities (Gnanashree and Mohamed, 2018).

Tetracosane was reported to have apoptotic effects in cancer cell studies (Parimalachelvam *et al.*, 2023), and pentacosane are known to have anti-inflammatory and antioxidant properties (Hagaggi *et al.*, 2025). Bioactive compounds such as dotriacontane and 2-methylhexacosane were reported for their antimicrobial properties (Bordoloi *et al.*, 2017; Chakraborty *et al.*, 2022). Nonadecane and eicosane are also known to have anti-fungal properties (Ahsan *et al.*, 2017). Several other bioactive compounds such as nonadecyl heptafluorobutyrate, heneicosyl heptafluorobutyrate and nonahexacontanoic acid were also detected with unknown and uncharacterized bioactivity.

The methanol-based GC-MS analysis further revealed the presence of bioactive compounds such as sulfurous acid, 2-ethylhexyl hexyl ester, nonane, 5-(1-methylpropyl), hexadecanoic acid, methyl ester, 1,2-Bis(trimethylsilyl) benzene, 11-Eicosenoic acid, methyl ester, 1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy) and 2,6-Difluorobenzoic acid, 4-nitrophenyl ester (Table 4). Hexadecanoic acid, methyl ester was

reported to have antimicrobial properties (Daben *et al.*, 2017). 11-eicosenoic acid, methyl ester is associated with antioxidant and nematicidal activities (Ayoola *et al.*, 2020). Sulfurous acid, 2-ethylhexyl hexyl ester is known to function as a free radical scavenger (Viraj *et al.*, 2021). Bioactive compound such as 1,2-bis(trimethylsilyl)benzene was reported to have anticancer and antibacterial properties (Lingfa and Ankanagari, 2023), thereby supporting the therapeutic importance of *B. costula*.

The presence of several bioactive compounds of medicinal importance in *B. costula* supports the use of this snail in traditional medicine. The presence of bioactive compounds with anticancer, antioxidant, anti-inflammatory and antimicrobial properties further provides preliminary scientific validation for its ethno-medicinal relevance. This study suggests that *B. costula* has the potential to be a sustainable source for the development of therapeutics and functional food.

CONCLUSION

The chemical profiling of freshwater snail, *Brotia costula* using LC-MS and GC-MS revealed a wide range of bioactive compounds with proven bioactivities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. This study provided preliminary scientific evidence supporting its traditional use among indigenous communities in Northeast India particularly Manipur. Bioactive compounds such as hesperidin, chitin, linoleic acid, maslinic acid, and octacosanol underscored its importance in nutraceutical and pharmaceutical industry. The integration of traditional knowledge with scientific validation further highlighted the potential of using *B. costula* as a sustainable

Table 3. Major compounds identified from *B. costula* chloroform extracts using GC-MS

Compound	Chemical formula	Structure	Retention time (min)	Major ion (m/z)	Bioactivity	References
Heneicosane	C ₂₁ H ₄₄		39.44	43	Unknown	
Tetracosane	C ₂₄ H ₅₀		43.20	44.03	Apoptotic effect	Parimalachelvam <i>et al.</i> (2023)
Octacosanol	C ₂₈ H ₅₈ O		48.09	55	Anti-fatigue, anti-hypoxia, antioxidant, anti-inflammatory, antitumor	Singh <i>et al.</i> (2020)
Eicosane, 2,4-dimethyl	C ₂₂ H ₄₆		48.29	57.1	Unknown	
1-Hexacosanol	C ₂₆ H ₅₄ O		49.27	82.1	Antioxidant against	Andrew <i>et al.</i> (2024)
Nonadecylhepta-afluorobutyrate	C ₂₃ H ₃₉ F ₇ O ₂		52.40	58.90	Unknown	
Pentacosane	C ₂₅ H ₅₂		53.73	49.66	Anti-inflammatory, antioxidant, antibacterial	Hagaggi <i>et al.</i> (2025)
Dotriacontane	C ₃₂ H ₆₆		51.30	56	Antifungal, antibacterial	Bordoloi <i>et al.</i> (2017)
2-Methylheptacosane	C ₂₇ H ₅₆		49.41	57.64	Antifungal, antibacterial	Chakraborty <i>et al.</i> (2022)
Nonadecane	C ₁₉ H ₄₀		44.68	58.07	Antioxidant	Ahsan <i>et al.</i> (2017)
Eicosane	C ₂₀ H ₄₂		36.09	44.05	Anti-inflammatory, analgesic, antipyretic, antifungal	Ahsan <i>et al.</i> (2017)
1-Octadecanol	C ₁₈ H ₃₈ O		45.86	68	Antibacterial, antifungal, anti-larval	Gnanashree and Mohamed (2018)
Nonyltetraicosyl ether	C ₃₃ H ₆₈ O		46.21	57.77	Unknown	
2-Methyl-cis-7,8-epoxynonadecane	C ₂₀ H ₄₀ O		51.31	56.1	Antioxidant, anti-inflammatory	Nguyen <i>et al.</i> (2020)
Nonahexacontanoic acid	C ₆₉ H ₁₃₈ O ₂		52.41	57.88	Unknown	
Heneicosylpentadfluoropropionate	C ₂₄ H ₄₃ F ₅ O ₂		52.59	71.1	Unknown	
Heneicosylhepta-afluorobutyrate	C ₂₅ H ₄₃ F ₇ O ₂		52.71	57.22	Unknown	

and culturally important source of therapeutic agents. Future studies on *in vivo* and clinical studies are needed to explore its full biomedical potential and its inclusion in evidence-based alternative medicine and health supplements.

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Table 4. Major compounds identified from *B. costula* methanol extracts using GC-MS

Compound	Chemical formula	Structure	Retention time (min)	Major ion (m/z)	Bioactivity	References
Sulfurous acid, 2-ethylhexyl hexyl ester	C ₁₄ H ₃₀ O ₃ S		14.44	31	Antioxidant	Viraj <i>et al.</i> 2021
Nonane, 5-(1-methyl-propyl)	C ₁₃ H ₂₈		26.79	59.06	Unknown	
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂		36.36	74	Antimicrobial	Daben <i>et al.</i> (2017)
1,2-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂		41.95	79.83	Anticancer, antibacterial	Salem <i>et al.</i> (2016)
11-Eicosenoic acid, methyl ester	C ₂₁ H ₄₀ O ₂		42.30	55	Antioxidant, nematicide, antialopellic	Ayoola <i>et al.</i> (2020)
1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)	C ₂₂ H ₃₀ OSi		101	22.18	Unknown	
2,6-Difluorobenzoic acid, 4-nitrophenyl ester	C ₁₃ H ₇ F ₂ NO ₄		27.25	126	Unknown	

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