Bioactivities of freshwater sponges and their associated bacteria: a review

Original Article

LIMNOLOGY FRESHWATER BIOLOGY

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ABSTRACT. This review aims to provide an overview of the biological activities of freshwater sponges and their microbes associated with them. Freshwater sponges, despite their ecological significance and potential as reservoirs of bioactive compounds, remain underexplored compared to their marine counterparts. This review highlights the bioactivities of eight freshwater sponge extracts, among them being *Ochridaspongia rotunda* (Arndt,1937), *Ephydatia fluviatilis* (Linnaeus,1759), *Oncosclera asiatica* (Manconi & Ruengsawan,2012), *Metania reticulata* (Bowerbank,1863), *Drulia browni* (Bowerbank,1863), *Drulia cristata* (Weltner,1895), *Drulia uruguayensis* (Bonetto & Ezcurra de Drago,1969), and *Eunapius carteri* (Bowerbank,1863).Various extracts, including those obtained using methanol, acetone, aqueous, ethyl acetate, and methylene chloride, were employed in previous studies to evaluate a range of bioactivities. Observed bioactivities include antibacterial, antifungal, anti-quorum sensing, antiplasmodial, acetylcholinesterase inhibition, and anticancer properties, which are determined either from the crude extracts of the sponges themselves or by their associated microbes.

Keywords: freshwater sponges, biological activities, bioprospecting, microbial symbionts, anticancer activity, antiplasmodial activity

For citation: Adkar U.S., Chhabda P.J., Harkal A.D., Rao P.R., Devdhe G.R. Bioactivities of freshwater sponges and their associated bacteria: a review // Limnology and Freshwater Biology. 2026. - № 1. - P. 57-62. DOI: 10.31951/2658-3518-2026-A-1-57

1. Introduction

The urgent need to find new drugs to combat existing resistant strains is driven by the increasing prevalence of antibiotic-resistant bacteria, which significantly limits the effectiveness of current treatments. This resistance leads to higher medical costs, prolonged hospital stays, and increased mortality rates, emphasizing the critical necessity for novel antibiotics and alternative therapies to address this global health threat. The development of anti-cancer drugs is crucial due to the limitations and drawbacks of current treatments, such as severe side effects, resistance to chemotherapy, and the inability to effectively target all cancer types.

Extensive research has been conducted to identify effective bioactive molecules from natural sources. Marine sponges have particularly emerged as a prolific source of such compounds (Anbarasu, 2024). However, studies of freshwater sponges remain limited due to various challenges and constraints. Freshwater sponges, belonging to the phylum Porifera, are classified into 45 genera across six families, encompassing a total of 219 species that represent a largely untapped resource of bioactive compounds with significant potential in pharmaceutical and biotechnological applications (Manconi and Pronzato, 2008). They are less diverse compared to their marine counterparts, reducing the likelihood of discovering unique bioactive compounds. These searches for new drugs from freshwater sponges are more challenging.

Sponges are abundant in bioactive molecules due to their high exposure to biotic and abiotic stress. This environmental pressure forces them to develop a wide array of chemical defenses to survive. The need to deter predators has driven sponges to produce various bioactive compounds, ensuring their survival by making

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Received: September 21, 2025;
Accepted after revised: January 16, 2026;
Available online: February 19, 2026

them less palatable or even toxic to potential threats (Thoms et al., 2007). The symbiotic lifestyle of sponges with numerous microorganisms leads to the production of unique bioactive molecules, resulting from complex interactions and mutualistic relationships with their microbial partners (Taylor et al., 2007). Their filter-feeding behavior increases sponge exposure to hazardous particles, prompting the evolution of a diverse arsenal of bioactive compounds to neutralize potential threats and maintain their health (Webster and Thomas, 2016).

2. Reported bioactivities of freshwater sponges

Freshwater sponges were identified as potential sources of bioactive compounds, exhibiting a range of pharmacological and ecological activities. These bioactivities include antimicrobial, antiquorum sensing, cytotoxic, mutagenic, antiplasmodial, and ecological defense mechanisms such as immobility induction and antipredation effects. Table 1 summarizes these bioactivities from different freshwater sponges. The following subsections provide an overview of these activities, highlighting their significance and potential applications.

2.1. Antimicrobial activity

1. The freshwater sponge, *Ephydatia fluviatilis*, from Vinkeveense Plassen lake in the Netherlands was found to harbor Pseudomonas species. In the study, 90 isolates of Pseudomonas were screened for biofilm production, antimicrobial activity, and protozoan grazing resistance. The *E. fluviatilis* sponge revealed 15 fingerprint clusters (BOX I–XV), containing 2 to 17 isolates after BOX-PCR genotyping. Each cluster was checked against *R. solani*, *F. moniliforme*, *P. ultimum*, or *B. subtilis*. Box 1 represents the *P. protegens*-like isolates, while

Table 1. Summary of reported bioactivities of freshwater sponges, their associated species, mechanisms, and references

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Antimicrobial	****Ephydatia fluviatilis*** (Linnaeus,1759)	1. The sponges harbor *Pseudomonas* species that exhibit antibacterial, antifungal, and anti-oomycetal activities. 2. In-vitro antagonistic activity was observed against *Rhizoctonia solani, Fusarium moniliforme*, *Pythium ultimum*, and *Bacillus subtilis* in 68 out of 90 Pseudomonas isolates. 3. A positive relationship exists between biofilm production capacity and the detection of antagonistic activities in these species.	*Pseudomonas* species 1. *P. protegens* as isolates 2. *P. jessenii* as isolates 3. *P. oryzihabitans* as isolates	(Keller-Costa et al., 2014)
	Ochridaspongia rotunda (Arndt,1937)	1. Five crude extracts showed antimicrobial activity against eight bacterial and eight fungal strains. 2. The methanolic extract exhibited the strongest antibacterial activity with MIC values of 7.5–15 μg/mL and MBC values of 15–30 μg/mL. 3. The acetone extract exhibited maximum antifungal activity with MIC of 7.5–45 μg/mL and MFC of 15–60 μg/mL.		(Pejin et al., 2014b)
	****Oncosclera asiatica*** (Manconi & Ruengsawan,2012)	1. The sponge symbiont bacterium, *Pseudomonas moraviensis*, exhibited antibacterial activity. 2. It was effective against *Escherichia coli* (*E. Coli*) and *Staphylococcus aureus* (*S. Aureus*).	*Pseudomonas moraviensis*	(Wulandari et al., 2023)
	****Metania reticulata*** (Bowerbank,1863)	1. Two bacterial strains, MERETb.761 and MERETb.762, and one fungal strain, MERETf.010, were associated with sponge antimicrobial activity. 2. Both bacterial strains inhibited *Aspergillus* sp. 3. Fractions from fungus MERETb.762 and MERETf.010 inhibited *S. Aureus* 4. HPLC fractions of MERETb.762 inhibited the release of beta-hexosaminidase in the RBL-2H3 degranulation assay.	MERETb.761 (Genbank accession number KF305316) MERETb.762 (Genbank accession number KF305317) MERETf.010 (Genbank accession number KF305318)	(Rozas et al., 2016)

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Immobility and anti-predation (anti protozoan)	****Ephydatia fluviatilis*** (Linnaeus,1759)	The sponge harbors Pseudomonas spp., which shows the immotility effect on the *C. steinii* ciliate. 1. Between 5 and 15 minutes, cells became immotile. After 2 hours, cell lysis of *C. steinii* was initiated. After 24 hours, nearly all *C. steinii* were a broken structure. 2. 35% of isolates resisted to the *C. steinii* ciliate predation.	*Pseudomonas* species 1. *P. oryzihabitans* as isolates 2. *P. protegens* as isolates	(Keller-Costa et al., 2014)
Anti-quorum sensing activity	***Ochridaspongia rotunda*** (Arndt,1937)	1. The methanolic extract of the sponge showed more pyocyanin inhibitory activity than its acetone extract. 2. The acetone extract reduced more biofilm production than the methanolic extract towards *P. aeruginosa*. 3. Both methanolic and acetone extracts reduced the flagellar motility and twitching ability of *P. aeruginosa*.		(Pejin et al., 2014a)
Acetylcholinesterase inhibitory activity	***Ochridaspongia rotunda*** (Arndt,1937)	The acetone extract of sponge inhibited acetylcholinesterase under both liquid and solid conditions.		(Talevska et al., 2017)
	Drulia browni (Bowerbank,1863)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Drulia cristata (Weltner,1895)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Drulia uruguayensis (Bonetto & Ezcurra de Drago,1969)	The methanolic extract strongly inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
	Metania reticulata (Bowerbank,1863)	The methanolic extract inhibited acetylcholinesterase.		(Manço da Costa Bolson et al., 2019)
Anti plasmodial activity	***Metania reticulata*** (Bowerbank,1863)	The methanolic extract of the sponge inhibited the *P. falciparum* (FCR3) parasite with IC50 of 2.7 μg/mL.		(Manço da Costa Bolson et al., 2019)
	Drulia browni (Bowerbank,1863)	The methanolic extract of the sponge inhibited the *P. falciparum* (FCR3) parasite with IC50 of 9.9 μg/mL.		(Manço da Costa Bolson et al., 2019)
Cytotoxicity	***Ochridaspongia rotunda*** (Arndt,1937)	1. The methanolic extract exhibited cytotoxicity towards the malignant brain U-251 MG tumor cell line with IC 50 of 1.87 ± 0.09 μg/mL at 96h. 2. The micronucleus test revealed that the genotoxicity of the methanolic extract at the chromosomal level was over 4.5 times lower than that of doxorubicin.		(Pejin et al., 2021)
	****Drulia cristata*** (Weltner,1895)	1. Crude extract showed no cytotoxic activity against the colorectal cell line (HCT-116). 2. Ethyl acetate extracts of bacterial strains DTR1 and DTR2 isolated from sponges exhibited the MTT assay with >50% inhibition.	DTR1 and DTR2 bacterial strains	(da Costa et al., 2019)

Bioactivity	Freshwater sponge	Properties/mechanism	Microbes associated with sponge activity	References
Free radical generation	**Eunapius carteri* (Bowerbank,1863)	1. Some fractions (F4) of the sponge isolated by density gradient centrifugation and flow cytometry generated a significant amount of superoxide anions than other fractions (F1, F2, and F3). 2. Fractions P2 and P3 generated nitric oxide ions.		(Mukherjee et al., 2015)

Note: * Indicates crude extract of the sponge used.

** Indicates extracts of bacterial/fungal isolates from the sponge used.

Box 6 represents the *P. jessenii*-like isolate species of Pseudomonas. Of the seven isolates antagonistic to *F. moniliforme*, six belonged to BOX PCR cluster VI (P. jessenii), and all *P. protegens*-like isolates showed strong inhibition towards *B. subtilis*. *P. umsongensis* type isolates, biofilm formers, showed moderate antagonism and resistance against *B. subtilis*. *P. oryzihabitans* showed antagonistic activity towards *B. subtilis*. Biofilm production was observed in 58% of the isolates, dominated by mucilaginous biofilms. Antagonism against phytopathogenic fungi (*Rhizoctonia solani*, *Fusarium moniliforme*, and *Pythium ultimum*) and the *Bacillus subtilis* bacterium was observed in 75% of isolates, with maximum inhibition against *P. ultimum* and *B. subtilis*. Additionally, 35% of the isolates were also resistant to predation by the *Colpoda steinii* protozoan. A principal component analysis (PCA) revealed a co-variation between biofilm production and antagonism, with *Pseudomonas* protegens-like isolates demonstrating high antagonism, particularly against *B. subtilis* and *C. steinii*. (Keller-Costa et al., 2014).

2. Five *O. rotunda* crude extracts were screened for their antibacterial and antifungal activities against eight bacterial strains and eight fungal species by the microdilution method. The methanolic extract of *O. rotunda* displayed the strongest antibacterial activity, with a MIC (Minimum Inhibitory Concentration) of 7.5 to 15 μg/mL and an MBC (Minimum Bactericidal Concentration) of 15 to 30 μg/mL, which was greater than that of the positive control antibiotics streptomycin and ampicillin. *Bacillus cereus* was the most susceptible organism, and *Listeria monocytogenes* was the most resistant to the antibacterial activity of the extracts. The acetone extract of *O. rotunda* was the most active antifungal, with MIC of 7.5 to 45 μg/mL and MFC of 15 to 60 μg/ml. *Trichoderma viride* was the most sensitive fungus, whereas *Candida albicans* was the most resistant to the antifungal action of the extracts (Pejin et al., 2014b).

3. Seventeen bacterial isolates were recovered from *Oncosclera asiatica* from Kali Porong, East Java. Paper disc inhibition assay exhibited isolate 2 with effective antibacterial against *E. coli* and isolate 14–against *S. aureus*. Sequence relationships revealed that isolate 2 had the highest relationship with *Pseudomonas moraviensis* strain 3N. Isolate 2 also had the NRPS gene that encodes antibacterial compounds (Wulandari et al., 2023).

4. Sometimes, symbionts together with sponges produce immunomodulators to interact with the host immune system. Such studies were performed on bacterial and fungal strains that were cultured from the freshwater sponge, *Metania reticulata*, from the Negro River of the Amazon central basin region. Two bacillus strains, MERETb.761(Genbank accession number KF305316) and MERETb.762 (Genbank accession number KF305317), and one fungal strain MERETf.010 (Genbank accession number KF305318) were selected of these MERETb.762 and MERETf.010 exhibited potent antibacterial activity against Staphylococcus aureus, while both bacillus strains inhibited *Aspergillus sp*. Two of its extracts from MERETb.762, containing nitroaromatic compounds, exhibited inhibition to degranulation of RBL-2H3 cells (Rozas et al., 2016).

2.2. Anticancer activity

1. The methanolic extract of *O. rotunda* sponge collected from Lake Ohrid was evaluated for its anticancer activity against brain tumor cell lines (Neuro-2A, U- 251MG, and U-87 MG). MTT assay revealed that the extract was most effective against U-251 MG (IC50 1.87 ± 0.09 μg/mL) and was five times more selective to the U-251 MG than the U-87 MG cell line. At the same time, it was less cytotoxic to normal cells when doxorubicin was used as a control. For assessing its genotoxicity, the micronucleus test was performed, which indicated that the methanolic extract lacks genotoxicity at the chromosomal level (Pejin et al., 2021).

2. The freshwater sponge, *Drulia cristata*, collected from Maracana beach, on the banks of the Tapajos River, the State of Para, was evaluated for its cytotoxic activity. Bacterial strains associated with the sponge were cultured and named DTR-1 and DTR-2. The ethyl acetate extracts of both strains were assessed with the MTT assay against the HCT-116 cell line. Crude extract of *Drulia* did not exhibit any cytotoxic activity, while extracts of bacterial strains showed moderate cytotoxic activity. DTR2 strains showed more potent cytotoxic activity than DTR1 (da Costa et al., 2019).

2.3. Acetylcholinesterase inhibitor activity

1. The Ellman method showed that *O. rotunda* acetone extract in liquid exhibited potent acetylcholinesterase activity with an IC_{50} of 23.07 μg/mL. The Marston method revealed stronger activity in solid form with an IC_{50} of 1.5 μg/mL. (Talevska et al., 2017)
2. *Metania reticulata*, *Drulia uruguayensis*, *Drulia browni*, and *Drulia cristata* collected from the Amazonian water were assessed for their acetylcholinesterase inhibitor activity by the Ellman method. Methanolic extracts of all sponges inhibited the acetylcholinesterase enzyme (AChE from electric eel), while none of them inhibited the butyrylcholinesterase enzyme (BChE from Equine serum). *Drulia uruguavensis* showed the most potent acetylcholinesterase inhibitory activity with 52 percent of enzyme inhibition. The percent inhibition was found as follows in increasing order: *Drulia cristata* < *Drulia browni* < *Metania reticulata* < *Drulia uruguayensis*. IC_{50} of *Drulia uruguayensis* was 1.04 ± 0.01 mg/mL (Manço da Costa Bolson et al., 2019).

2.4. Anti-quorum sensing activity

The anti-quorum sensing activities of the freshwater sponge, *Ochridospongia rotunda*, were assessed towards *Pseudomonas aeruginosa* (PA01). Since twitching and flagella motility are critical for the formation of biofilms, and the ability to form a biofilm is a major Quorum Sensing-controlled virulence trait, the ability of an extract to effectively reduce these motilities is considered a mechanism of anti-QS activity. Methanolic and acetone extracts were used to study pyocynin production, biofilm production, and twitching and motility of the bacterium. Pyocynin is essential for up-regulation of QS genes in stationary phase growth of *Pseudomonas*. Both extracts inhibited pyocyanin production by 49.9% and 42.44%, respectively. Methanolic and acetone extracts exhibited anti-biofilm activity by 48.29 and 53.99, respectively, compared to that of ampicillin (30.84). *Pseudomonas* colonies that were grown in the control group had larger diameters than those grown in methanolic and acetone extracts, suggesting that flagellar motility was reduced, and the twitching ability of *Pseudomonas* was compromised (Pejin et al., 2014a).

2.5. Anti-protozoan activity

Pseudomonas sp. were isolated from *Ephydatia fluviatilis* that showed predation resistance and toxicity towards *Colpoda steinii* strain Sp1, which was assessed by microtiter plate assay adapted from predation resistance strains in Jousset et al. They were defined as bacteria with <40 reductions of initial optical density after 22h of exposure to protozoa. These strains were assessed further for toxicity. Dried bacterial extracts were introduced to the *C. steinii* strain. Extracts of *Pseudomonas proteogens* as isolates, *P. jessenii* as isolates SF-2-02 and SF-3-03 (BOX VI), and isolates SG-1-02, SH-1-17, and SH-2-01 demonstrated toxicity towards protozoa. Within 5-15 minutes, all protozoan motility stopped, and after two hours of exposure, protozoans started to lyse. After 24 hours, all protozoan cells became broken (deformed with broken structures) (Keller-Costa et al., 2014).

2.6. Antiplasmodial activity

FCR3, a chloroquine-resistant strain of *P. falciparum*, was used to assess anti-plasmodial activity by extracts of *M. reticulata* and *Drulia browni*. The evaluation of antiplasmodial activity was assessed according to Rabelo et al. Among all extracts, the methanolic extract of *Metania reticulata* showed IC50 of 2.7μg/mL, while *Drulia browni* showed IC50 of 9.9μg/mL (Manço da Costa Bolson et al., 2019).

2.7. Free radical generation

E. carteri cells were separated into four fractions (F1–F4, top to bottom) using a discontinuous Ficoll gradient (5–25%) as outlined by De Sutter and Van de Vyver (1977) and Zhang et al. (2003) for subsequent analyses. Freshly dissociated sponge cells were filtered through a 35 μm nylon mesh and sorted with a BD FACS Aria III flow cytometer. Cell morphotypes were identified based on size (forward scatter, FSC) and granularity (side scatter, SSC) at 140V and 406V, respectively. Dot plots captured 50,000 events per sample, with three fractions (P1, P2, and P3) gated to exclude debris and cell doublets. Intracellular superoxide anion production in fractions from density gradient centrifugation (F1–F4) and flow cytometry (P1–P3) was quantified spectrophotometrically via the Nitroblue tetrazolium (NBT) reduction method. Fraction F4 produced significantly more superoxide anions than fractions F1, F2, and F3. Nitric oxide production was measured as nitrite release using Griess reagent (Green et al., 1982; Aktas et al., 2013), with fractions P2 and P3 showing nitric oxide generation (Mukherjee et al., 2015).

3. Conclusion

This review emphasizes the diverse bioactivities of freshwater sponges and their associated bacteria, underlining their potential as sources of novel therapeutic agents. The antimicrobial, anticancer, and anti-quorum sensing, as well as anti-plasmodial and acetylcholinesterase inhibitory activities, highlight the medicinal value of these organisms.

Notably, *Ephydatia fluviatilis* and *Ochridaspongia rotunda* with *Pseudomonas* species have very good antimicrobial and anti-quorum sensing activities, while others, such as *Metania reticulata* and *Drulia browni*, have good anti-plasmodial and acetylcholinesterase inhibitory activities. *Ochridaspongia rotunda* and *Eunapius carteri* also exhibit good cytotoxic and nitric oxide production activities, which can be used for cancer and inflammatory disease research. Furthermore, mutagenicity and cytotoxicity assays indicate that

some extracts, such as those of *Ochridaspongia rotunda*, have low genotoxic activity, making them promising candidates for drug development research.

Further exploration of the chemical and microbial diversity within freshwater sponges could uncover new bioactive compounds to combat antimicrobial resistance and neurodegenerative diseases, underscoring the importance of conserving freshwater ecosystems for future bioprospecting efforts.

Acknowledgements

The author expresses his gratitude to B.P.H.E. Society's Ahmednagar College, Ahilyanagar (formerly Ahmednagar) affiliated to Savitribai Phule Pune University, Pune for providing necessary facility for the research and to the Council of Scientific and Industrial Research (CSIR), New Delhi for providing a research fellowship.

Conflict of interest

All authors declare no conflict of interests.

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