IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138
INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ANTIMICROBIAL ACTIVITY OF EGGS FROM SNAIL, POMACEA DIFFUSA

[1]Nightingale Sheeba, S, [2]Vinoliya Josephine Mary, J and [3]Srinvasa Kumar, KP
[1]Research Scholar, [2]Assistant Professor
[1,2]Department of Zoology, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India.
Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.
[3]Professor, Department of Health Science, Lincoln University, Malaysia.

Abstract

Because of bacterial resistance to a wide spectrum of antibiotics, finding alternatives to antibiotics has become a very fascinating topic in recent years, and studies have begun to look into alternate bactericidal materials to remedy this problem. In contrast to most studies of plant extracts, this study demonstrated the use of *Pomacea diffusa* egg extract as an antimicrobial agent. The agar well diffusion method was used to assess each sample's antibacterial activity *in vitro*. Various extracts of *Pomacea diffusa* eggs, including ethanol, methanol, acetone, ethyl acetate, petroleum ether, Niobium pentoxide, chloroform, and Saline, were tested against bacteria, including *Staphylococcus aureus, Streptococcus mutans, Proteus vulgaris, Klebsiella pneumonia* and *E. coli* and fungi *Aspergillus flavus, Aspergillus niger* and *Rhizopus*. Petroleum ether extracts had the efficacy against the bacteria *S. mutans*. Niobium pentoxide extracts had the inhibitory potency against practically all of the microorganisms tested. Except for ethyl acetate extract, none of the extracts had inhibitory efficacy against fungal strains. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method (MBC). In the broth dilution assay, petroleum ether extract of *Pomacea diffusa* eggs was most effective against *S. mutans* (MIC 12.5 µg/ml; MBC >50 µg/ml), while Niobium pentoxide was most effective against *S. aureus* (MIC 25 µg/ml; MBC >50 µg/ml). While the eggs from *Pomacea diffusa* were somewhat effective against these pathogens, further fractionation to isolate the active components and determination of their optimal concentrations, as well as whether or not they act synergistically, may be useful for human health-related applications in the future.

Index Terms: Antibacterial, Antibiotics, MBC, MIC and *Pomacea diffusa*

1. Introduction

Antibiotics were discovered in the mid-twentieth century, and they greatly reduced the morbidity and death associated with infectious infections. Since the initial revelation of *Streptococcus pneumonia's* diminished susceptibility to penicillin in 1965, the propensity of bacteria to acquire resistance to antimicrobial medicines has been a source of concern. Since many of the early antibiotics were naturally occurring chemicals, researchers have continued to look for novel antimicrobial drugs based on anecdotal evidence and folklore (Davies et al., 2013; Shallcross and Davies, 2014).

Antimicrobial peptides (AMPs) in invertebrates are a hot topic right now. These are tiny molecules (5–15 kDa) that are part of the animal's natural defense system (Soderhall, 2011). Defensins, mytilins, myticins, and mytimacins are examples of molluscan proteins that have been identified (Li et al., 2011). Gastropods have a variety of internal AMPs, but it's also likely that their external secretions contain antimicrobial chemicals that protect them from diseases, similar to the antimicrobial substances found in fish and amphibian mucus (Gerdol et al., 2012).

Snails (Gastropoda) have a mechanism that allows them to produce a large number of chemicals that they employ to protect themselves from bacterial or fungal infections. These active chemicals are involved in the gastropods' humoral and cellular immunological responses. Snails humoral immune component has antibacterial and antifungal properties due to its cytotoxic and haemagglutination action against possible microbial infections (Chmelar et al., 2019).

Pomacea freshwater snails (Caenogastropoda, Ampullariidae), especially invasive canaliculata species, lay colourful and deadly egg masses on emergent substrates above the water level, exposing eggs to environmental stressors and terrestrial predators. With the exception of the fire ant *Solenopsis geminata*, these defenses pay off, as their eggs have almost no known predators. Cadierno et al. (2018) discovered that these defenses are provided by proteins (perivitellins) that are already active in the albumen gland of females, where the egg components are synthesized, which may explain why the brown rat (*Rattus norvegicus*) and the snail kite (*Rostramus sociabilis*), which prey on adult snails, systematically discard the albumen gland. The apple snail is found along the surface of rivers, and its eggs are rich in polysaccharides, proteins, and carotenoids. The goal of this study is to look at the antibacterial capabilities of snail eggs, *Pomacea diffusa*, based on their properties.

2. Materials and Methods

2.1. Collection of Animal

Freshwater snail, *Pomacea diffusa* were collected from local aquarium, Kanyakumari District, Tamil Nadu, India.

2.2. Preparation of the extract of snail eggs

The eggs were suspended in different solvents for overnight. The tissues were homogenized using sterile mortar and pestle. The homogenate of egg extracts was centrifuged at 5000 rpm for 15 min and the supernatants were collected and then filtered through Whatman No.1 filter paper. The solvent from combined filtrates was evaporated at reduced pressure and temperatures. The obtained residues were stored in refrigerator for further use (Ramya et al., 2018).

2.3. Antimicrobial activity

The Antibacterial activities of various extracts of eggs, *Pomacea diffusa* were investigated against Gram (-) and Gram (+) bacteria. The pathogenic bacteria are *Staphylococcus aureus*, *Streptococcus mutans, Proteus vulgaris, Klebsiella pneumonia* and *Escherichia coli*. The Antifungal activities of various extracts of eggs, *Pomacea diffusa* were performed under three fungal strains known as *Rhizopus, Aspergillus flavus*, and *Aspergillus niger*. The antimicrobial activity of eight different solvents (like Ethanol, Methanol, Acetone, Ethyl acetate, Petroleum ether, Niobium pentoxide, Chloroform and Saline) of eggs, *Pomacea diffusa* extract was determined using an Agar disc diffusion method followed by the protocol of (Jan Hudzicki et al., 2009).

Each one of the microbial plates was prepared under sterile conditions. The sterile disc was soaked in the respective solvents and kept in a vortex mixer for 15 minutes. The sterile swab is used to equally spread the microbes in the plates. The sample-loaded discs were placed in the plates at labeled positions. At 37°C, all the bacterial plates were incubated for 24 hours. The fungal plates were observed for 48 hours at room temperature (approx. 31°C). The inhibition clear zone against microbes is measured in the millimeter (mm) range.

2.4. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Triplication in the experiment was done. A 96-well microtiter plate was used to perform the Minimum Inhibitory Concentration (MIC) assay. Each well-containing nutrient broth (90 μL) inoculated with bacterial culture 10 μL. In each well, the extract of eggs, *Pomacea diffusa* was added separately with the concentration ranging from 900 μL to 6.25 mg/mL. After 12 hours of incubation of the plate at 37°C, in each plate p-iodonitrotetrazolium, Violet was added (0.4 mg/mL) and again incubated for 6 hours. After incubation, in viable bacteria, the color change from yellow to red to purple was observed and no color change in a lower concentration. The result was noted as MIC. The cultures with no or very little bacterial growth from this assay were then streaked on MHA plates and incubated at 37 °C for 24 h to ascertain the colony count and to determine the MBC, the minimum concentration required to produce no viable bacteria. The procedure was determined and described by (Bag and Chattopadhyay, 2015).

3. Results and Discussion

3.1. Antibacterial activity

The apple snail eggs were collected from an aquarium and cleaned with distilled water before extracting. Table 1 summarizes the antibacterial activity of snail eggs as determined by the agar well diffusion assay (Fig. 1). The petroleum ether extracts inhibited *S. mutans* and *P. vulgaris*. *S. aureus, P. vulgaris*, and *K. pneumonia* were all inhibited by the Niobium pentoxide extract. The antibiotic streptomycin, on the other hand, exhibited much stronger inhibitory activity as measured by the diameter of inhibition zones. The various egg extracts have no inhibitory action against the *E. coli* bacteria.

Table 1. Antibacterial activity of eggs from snail *Pomacea diffusa*

Extracts	Bacterial Strains				
	S. aureus	***S. mutans***	***P. vulgaris***	***K. pneumonia***	***E. coli***
Ethanol	NZ	9	NZ	NZ	NZ
Methanol	NZ	NZ	NZ	NZ	NZ
Acetone	NZ	NZ	NZ	NZ	NZ
Ethyl acetate	NZ	NZ	NZ	NZ	NZ
Positive	28	28	28	25	27
Negative	NZ	NZ	NZ	NZ	NZ
Petroleum Ether	NZ	15	7	NZ	NZ
Niobium pentoxide	12	NZ	9	11	NZ
Chloroform	7	NZ	NZ	NZ	NZ
Saline	NZ	NZ	NZ	NZ	NZ
Positive	25	35	25	20	25
Negative	NZ	NZ	NZ	NZ	NZ

NZ- No Zone

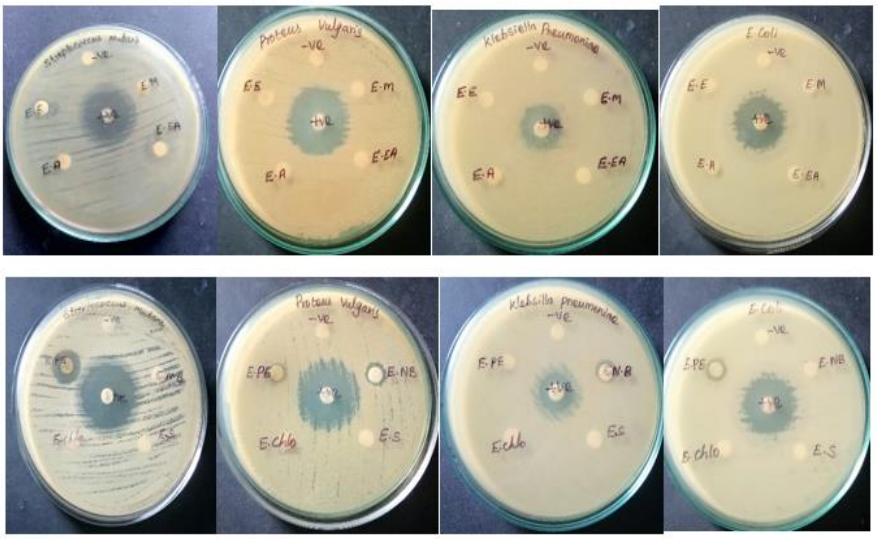

Figure 1. Antibacterial activity of eggs from snail *Pomacea diffusa*

3.2. Antifungal activity

Except ethyl acetate extracts, there was no effect of egg extract on the growth of pathogenic fungi (Table 2; Fig. 2).

Antifungal activity of eggs from snail *Pomacea diffusa*

Extracts	Fungal Strains		
	A. flavus	***A. niger***	***Rhizopus***
Ethanol	NZ	NZ	NZ
Methanol	NZ	NZ	NZ
Acetone	NZ	NZ	NZ
Ethyl acetate	9	NZ	NZ
Positive	27	12	10
Negative	NZ	NZ	NZ
Petroleum Ether	NZ	NZ	NZ
Niobium pentoxide	NZ	NZ	NZ
Chloroform	NZ	NZ	NZ
Saline	NZ	NZ	NZ
Positive	20	13	13
Negative	NZ	NZ	NZ

NZ- No Zone

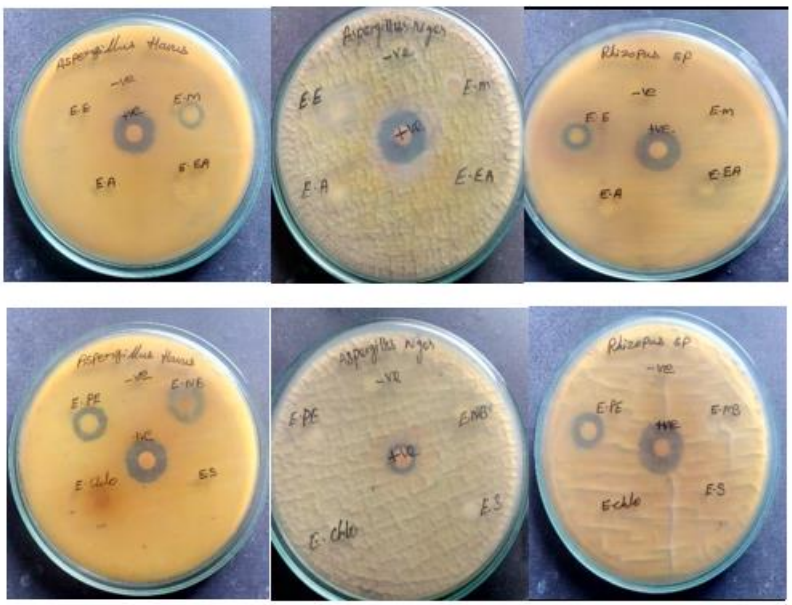

Figure 2. Antifungal activity of eggs from snail *Pomacea diffusa*

3.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of petroleum ether extract from eggs *Pomacea diffusa* were evaluated against the two bacterial strains using the broth dilution method. The extract showed a weak antibacterial activity (>50 µg/ml) against all the pathogenic bacteria, while Niobium pentoxide from eggs *Pomacea diffusa* had too low an antibacterial activity to determine MIC and MBC values (Table 2).

MIC and MBC activity of Petroleum Ether extracts of eggs from snail *Pomacea diffusa*

Bacteria	Petroleum Ether Extract (µg/ml)	
	MIC	MBC
S. mutans	12.5	>50
P. vulgaris	25	>50

MIC and MBC activity of Niobium pentoxide extracts of eggs from snail *Pomacea diffusa*

Bacteria	Niobium pentoxide Extract (µg/ml)	
	MIC	MBC
S. aureus	25	>50
P. vulgaris	>50	>50
K. pneumonia	>50	>50

Many invertebrate species lay fertilized eggs in nutritious egg masses that are ideal for microorganism development (Mukai et al., 2004). Despite the fact that parental protection of eggs appears to be critical for species survival, there are little investigations on the immunological protection of invertebrate eggs. Eggs from the mollusk *Aplysia kurodai* included an antibacterial protein called aplysianin-A, while eggs from the pulmonate snail *Helix pomatia* contained a N-acetyl-galactosamine-binding lectin that agglutinates bacteria (Sanchez et al., 2006). In a proteomic analysis of *Biomphalaria glabrata* egg masses, peptides of LBP/BPI proteins were discovered alongside aplysianin peptides (Hathaway et al., 2010).

Janthima et al., (2018) used a biological source derived from golden snail egg extract to produce silver nanoparticles (AgNPs). Both Gram-positive Bacterium *Staphylococcus aureus* and Gram-negative bacterium *Escherichia coli* were resistant to the antibacterial action of the produced AgNPs. The results of this study clearly demonstrated the possible application of apple snail egg extract for the green synthesis of tiny AgNPs with antibacterial activity.

Ghada et al., (2019) synthesized silver nanoparticles (AgNPs) using a biological source from the golden snail egg extract. It also showed antibacterial activity against Gram positive (*Staphylococcus aureus, Staphylococcus epidemidis, Streptococcus pyogens*) and Gram negative (*Salmonella, Escherichia coli, Klebsiella, Pseudomonas aeruginosa* and *Enterobacter*) bacteria by well diffusion assay and tube dilution method. Antibiofilm activity was detected by crystal violate staining assay through the use of AgNPs synthesized in this study and results showed very good inhibition in biofilm production at low concentrations of AgNPs. So, additional identification will be needed for the possibility of antibacterial impact.

4. Conclusion

The eggs from *Pomacea diffusa* had a moderate effect against bacterial strains, according to this study. However, after fractionation to identify the active compounds and optimal doses, as well as whether or not they act synergistically, snail eggs may still have potential as alternative therapeutic agents against pathogenic bacteria that cause skin disease, and may be useful in human health-related applications in the future. Furthermore, it is obvious from the review that the synthesis of metallic nanoparticles from snail eggs has a larger potential for antibacterial action, which could lead to the development of a drug delivery system.

5. References

1. Bag, A. and Chattopadhyay, RR. 2015. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. PLoS ONE, 10(7).
2. Cadierno, MP, Dreon, MS. and Heras, H. 2018. Validation by qPCR of reference genes for reproductive studies in the invasive apple snail *Pomacea canaliculata.* Malacologia, 62(1):163–170.
3. Chmelar, D, Hinstova, R, Kuzma, J. and Hajek, M. 2019. Determination of antimicrobial activity of *Achatina reticulata* slime. Epidemiol. Mikrobiol. Immunol., 68(2):65-70.
4. Davies, SC, Fowler, T, Watson, J, Livermore, DM. and Walker, D. 2013. Annual Report of the Chief Medical Officer: infection and the rise of antimicrobial resistance. Lancet, 381(9878):1606–1609.
5. Gerdol, M, De Moro, G, Manfrin, C, Venier, P. and Pallavicini, A. 2012. Big defensins and mytimacins, new AMP families of the Mediterranean mussel Mytilus gallo-provincialis. Dev Comp Immunol, 36(2):390–399.
6. Ghada, MS, Shaymaa, SN. And Abass, M. 2019. Golden apple snail Eggs Extract: Biosynthesis of Nanoparticles and Its Antibacterial effect. J. Pharm. And Tech., 12(7):3444-3450.
7. Hathaway, JJ, Adema, CM, Stout, BA, Mobarak, CD. and Loker, ES. 2010. Identification of protein components of egg masses indicates parental investment in immunoprotection of offspring by *Biomphalaria glabrata* (gastropoda, mollusca). Dev Comp Immunol, 34:425-435.
8. Jan Hudzicki. 2009. Kirby-Bauer disk diffusion susceptibility test protocol.
9. Janthima, R, Khamhaengpol, A. and Siri, S. 2018. Egg extract of apple snail for eco-friendly synthesis of silver nanoparticles and their antibacterial activity. African Cells, Nanomedicine, and Biotechnology, 46(2):361-367.
10. Li, H, Parisi, MG, Parrinello, N, Cammarata, M. and Roch, P. 2011. Molluscan antimicrobial peptides, a review from activity-based evidences to computer-assisted sequences. Invertebrate Surviv J, 2011; 8:85-97.
11. Mukai, ST, Hoque, T, Morishita, F. and Saleuddin, ASM. 2004. Cloning and Characterization of a Candidate Nutritive Glycoprotein from the Albumen Gland of the Freshwater Snail, *Helisoma duryi* (Mollusca: Pulmonata). Invertebrate Biology, 123:83-92.

12. Ramya, S, Ravichandran, S. and Mohan, K. 2018. Antimicrobial potential of crude extracts from Sea slug, *Euselenops luniceps* (Cuvier, 1817). Drug Discovery, 12:40-53.
13. Sanchez, JF, Lescar, J, Chazalet, V, Audfray, A. and Gagnon J. 2006. Biochemical and structural analysis of *Helix pomatia* agglutinin. A hexameric lectin with a novel fold. J Biol Chem, 281:20171-20180.
14. Shallcross, LJ. and Davies, SC. 2014. The World Health Assembly resolution on antimicrobial resistance. J Antimicrob Chemother, 69(11):2883–2885.
15. Soderhall, K. 2011. Invertebrate immunity. New York: Springer Science & Business Media.