Research Article

PRELIMINARY QUALITATIVE CHEMICAL EVALUATION OF THE EXTRACTS FROM MUSSEL *PERNA VIRIDIS*

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ABSTRACT

The Indian green mussel *Perna viridis* is an edible mytilid bivalve seen all along both east and west coasts of India. Even though it is well documented as a commercial cultivable species and as a sentinel organism in pollution research, the species is rarely been studied for its pharmacological properties. Nevertheless, the active ingredients involved are typically unknown. The present work is carried out to qualitatively evaluate the secondary metabolites present in the extract taken from the tissue of mussel *Perna viridis*. The whole mussel tissue was successively extracted with ethylacetate, methanol & water: ethanol; 7:3. Each of the extracts was qualitatively analyzed for the secondary metabolites using general colour reactions and spray reagents on thin layer chromatography. The developed plates were analyzed under UV and exposed to iodine vapour. In the present work, preliminary qualitative chemical test for different extract showed the presence and absence of alkloids, phenolics, sterols, terpenes & saponins.

Keywords: Mollusc; *Perna viridis*; secondary metabolites; TLC; spray reagents.

INTRODUCTION

Currently there is increasing interest in the bioactivity of molluscan extracts and secondary metabolites[1] even though the overall secondary metabolites investigated from molluscan species form a tiny proportion (<1%). Some marine gastropods and bivalves have been of great interest to natural products chemists, yielding a diversity of chemical classes and several drug leads currently in clinical trials. In most cases, there has been no scientific research undertaken to substantiate the health benefits derived from molluscs and the active ingredients in the taxa involved are typically unknown. The complete disregard for several minor classes of molluscs is unjustified based on their evolutionary history and unique life styles, which may have led to novel pathways for secondary metabolism. Nevertheless, it is presently unclear whether the production of bioactive secondary metabolites is ubiquitous within the phylum Mollusca and several disputes were ongoing about the topic. Therefore, there is much scope for future drug discovery within this phylum, exploring novel compounds with newer mode of action.

The present work investigates the qualitative evaluation of the major secondary metabolites present in the whole tissue of the mussel *Perna viridis* in view of its importance as an edible item and a commercially important cultivable species. The species *P. viridis*, commonly known as the Indian green mussel, is a widely distributed edible mytilid bivalve seen all along both east and west coast of India. Even though many works has been undertaken with regard to pollution studies as a sentinel organism, *P. viridis* is been sparsely included in pharmacological studies. Therefore, the present work is first of its kind in attempting to find out the chemical constituents present in the above organism.

MATERIALS AND METHODS

Collection of specimen

The mussel *Perna viridis* was collected from its natural bed at Anthakaranazhi, Alappuzha District (Kerala, India). They were brought to the lab in aerated plastic containers filled with sea water of ambient salinity. Mussels of both sexes were selected and washed in a jet of water and cleaned thoroughly to get rid of the attached algae and debris.

Extraction

The shells were separated in the lab and whole mussel tissue weighing 300g was macerated first with ethyl acetate (EtOAc) in a blender. The mixture was subjected to mechanical stirring overnight at room temperature. Subsequently the suspension was centrifuged at 8000 rpm for 20 min, and the supernatant solvent was collected and stored. The residue was again treated with more solvent and the whole process was repeated two or more times. The residue after centrifugation was serially extracted with methanol and then with water: ethanol (7:3) as described above. Each supernatant were concentrated using vacuum evaporator (35-55^0 C) under reduced pressure. The resultant viscous mass were weighed and kept in clean glass vials at -80 ^{0}C until use.

Preliminary Identification of the chemical components using general detection reagents

The identification of the chemical constituents (alkaloids, flavonoids, phenolics, saponins & sterols) present in the three extracts of *P. viridis* were carried out using various general detection reagents as described by Cannell (1998) in the year 1998[2].

Thin layer chromatography (TLC) of the extracts

The slurry was prepared by mixing 60 gm of silica gel G with required amount of distilled water and applied on a glass plate (20 x 20cm and 5x20 cm) at a uniform thickness of 0.5 mm using a spreader. The plates were allowed to air dry and further activated in oven at 110^0 C for 1 hr. Concentrated extracts dissolved in appropriate solvents were spotted and allowed to develop with Individual solvent systems for each extracts. A few drops of ammonium solution was added to the solvent system for methanol and water : ethanol (7:3) extracts for better resolution. The developed TLC plates were visualized under UV lamp fixed in UV chamber; afterward they were exposed to iodine vapour to visualize the components which were UV invisible. The solvent systems used for each extracts were given below.

Ethyl acetate - hexane: ethyl acetate (70:30)

Methanol - methanol : dicholoromethane : chloroform (30:35:35)

Water : ethanol (7:3) - ethanol : ethyl acetate : acetic acid (50: 30: 20)

Spray reagent detection of the components of extracts on TLC

The developed plates subsequent to visualization under UV were sparyed with various spray reagents to detect the presence of secondary metabolites like alkaloids, phenolics, steroids and terpenes according to standard protocols described by Cannell (1998)[2].

RESULTS

The percentage yield of the three extracts of *P. viridis* viz; ethyl acetate, methanol and water : ethanol (7:3) were 1.8%, 6.4% & 3.2 % respectively (table1). Methanol extract showed higher percentage yield compared to other two. The yield of extracts ranked in the order Methanol < water : ethanol (7:3) < ethyl acetate. Preliminary chemical analysis showed the presence and absence of certain chemical constituents in these extracts. The results were shown in table 2.

Table 1: Percentage yield & physical properties of three extracts of *P. viridis*

Name of the extracts	Colour	Consistency	% yield
Ethyl acetate	Dark brown	Non sticky	1.8
Methanol	Yellowish brown	sticky	6.4
Water : ethanol (7:3)	Yellowish brown	sticky	3.2

Table 2: Components of *P. viridis* extracts identified by general detection reagents

Test	Extracts of *P. viridis*		
	Ethyl acetate	Methanol	Water:ethanol; 7:3
Alkaloids			
Mayer's test	-ve	-ve	+ve
Dragendorff's reagent	+ve	+ve	+ve
Wagner's reagent	-ve	-ve	-ve
Flavonoids			
Shinoda's test	-ve	-ve	-ve
Poly phenols	+ve	+ve	-ve
Sesquiterpene lactones/Cardiac glycosides			
Baljet reagent	-ve	-ve	-ve
Legal reagent	-ve	-ve	-ve
Sterols			
Liebermann-Buchard test	+ve	+ve	-ve
Salkowski reaction	+ve	+ve	-ve
Saponins	-ve	+ve	+ve

Table 3: R_f values of extracts of *P. viridis*

Ethyl acetate	Methanol	Water: ethanol (7:3)
E1- 0.17	M1- 0.03	W1- 0.12
E2- 0.28	M2- 0.07	W2- 0.21
E3- 0.50	M3- 0.12	W3- 0.45
E4- 0.60	M4- 0.22	W4- 0.56
E5- 0.71	M5- 0.33	W5- 0.66
E6- 0.83	M6- 0.77	W6- 0.79
E7- 0.93	M7- 0.89	W7- 0.90

Table 4: Compounds detected in the extracts of *P. viridis* using different spray reagents on TLC

Compounds	Separated Components of the three extracts		
	Ethyl acetate	Methanol	Water: ethanol (7:3)
Alkaloids	E1 & E2	M1 & M2	W2
Phenolics	E4	M4	—
Terpenes	E5	M6	W5
Steroids	E7	M7	W7

Thin layer chromatography of three extracts revealed the presence of different chemical constituents in the chromatogram. The developed TLC plate of ethyl acetate extract displayed a coloured chromatogram with the universal solvent system, hexane : ethyl acetate; 7:3, which made the detection easier than other two. On examination under UV, the chromatogram reflected different coloured bands like, lilac, white, blue, violet etc. Seven separate bands were observed on the developed TLC plate of methanol & water: ethanol (7:3) extracts. The R_f vales were shown in table 3. All the bands were colourless/ not visible in the day light, but the bands M1, M2, M3 & W7 were visible under UV. On subsequent exposure to iodine vapour all the separated bands of three extracts rendered visible and showed yellow to dark brown colouration.

Spray reagent detection of the developed plates of the three extracts showed different organic constituents like, alkaloids, phenolics, terpenes and steroids (Table 4).

DISCUSSION

The yield of extraction depends upon the solvent, time and temperature of extraction as well as the chemical nature of the sample. Several criteria have been used in evaluating the effectiveness of the extraction method and the suitability of a solvent for a particular extraction procedure. The most commonly encountered criterion is extraction yield, i.e. the total yield or the yield of a certain target compound or compounds[3-5]. The yield from the whole tissue of *P. viridis* (300g) was higher for methanol compared to the other two solvents. The better yield of methanol extract may be due to the presence of more of polar ingredients. Similar results were also observed in the case of Lotus rhizome extracts[6].

Visualization of developed plate under UV light showed different coloured zones corresponding to the separated bands for EtOAc extract of *P. viridis*. While that of methanol and aqueous/ethanol extract, only few bands showed fluorescence. This may be due to the fact that many analytes do not absorb visible or UV light, quench fluorescence, or fluoresce when excited by visible or UV light. Chromatographic zones normally appear dark on a lighter background or if fluorescence occurs, a variety of visible spectrum colours are seen. The entire chromatogram of EtOAc extract showed fluorescence in UV, reflecting distinct zones of different colours like blue, violet, purple and white. These colours could indicate the presence of alkaloids (blue/blue green or violet), flavonoids (dark yellow, green or blue fluorescence) and saponins (not detectable).

Separated chromatographic zones on a TLC/HPTLC layer may appear colourless in normal light, but can absorb electromagnetic radiation at shorter wavelengths. Often exposure to UV light at short wave radiation (254 nm) or long wave radiation (366 nm) is all that is necessary for absorbing or fluorescing substances to be observed. The components of methanol and aqueous extracts that are not visible in normal light and under UV were detected by exposing to iodine vapour.

The chromatograms of three extracts on exposure to the iodine vapour produced yellow and brownish bands corresponding to the separated zones. The zones which are not fluorescing under UV could thus be detected on reaction with iodine vapour. The so-called ''iodine reaction'' possibly results in an oxidative product. In most instances the reaction is observed in the regions of separated chromatographic zones, where organic unsaturated compounds are present. However,

electrophilic substitutions, addition reactions, and the formation of charge-transfer complexes do sometimes occur. The use of iodine as vapour enables the detection of separated substances rapidly and economically before final characterization with a group specific reagent. The iodine molecules will concentrate in the lipophilic zones present on a chromatographic layer giving yellow–brown chromatographic zones on a lighter yellow background[7].

The chemical composition of the *P. viridis* extracts were found to be complex as judged by thin layer chromatographic separation and spray reagent detection which revealed the presence of diverse group of secondary metabolites like alkaloids, polyphenols, terpenes, steroids and saponins in these extracts. The secondary metabolites isolated from molluscs fall into a wide range of structural classes, with some compounds being more dominant in certain taxa. They make up a vast repository of compounds with a wide range of biological activities. But it can be clearly stated that dietary sources contribute significantly to the chemical diversity found in molluscs. Nevertheless, evidence for *de novo* biosynthesis has been reported in several molluscan taxa[8-11]. Although most major types of secondary metabolites are represented in both classes of mollusc, in Gastropoda terpenes dominate, whereas terpenes are scarcely reported from bivalves. Sterols are the dominant compounds in bivalves, but are the least frequently reported in gastropods. The relatively large research effort on sterols in bivalves is probably due to their importance in fisheries and aquaculture, with interest focusing on biochemical changes over the reproductive cycle. Alkaloids have been isolated in reasonably large numbers from both classes of molluscs, whereas aliphatic nitrogen containing compounds are relatively uncommon[12].

The secondary metabolites are reported to exhibit variety of pharmacological properties. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as efficient radical scavengers and it is believed to be mainly due to their redox properties[13]. Phenolics are also found to cause substrate deprivation & membrane disruption. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[14] with possible interaction with cell wall and DNA. A wide range of biological activities of alkaloids have been reported: emetic, anti-cholinergic, antitumor, diuretic, sympathomimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antimicrobial and anti-inflammatory[15]. Saponins are freely soluble in both organic solvents and water and have been suggested as possible anti-carcinogens also found inhibitory to fungi & bacteria. They possess surface-active characteristics that are due to the amphiphilic nature of their chemical structure, causing membrane disruption. Terpenes are naturally occurring substances produced by a wide variety of plants and animals. A broad range of the biological properties of terpenoids is described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic activities. The extracts were also positive for steroids, which are very important compounds especially due to their relationship with compounds such as sex hormone[16].

Since most of the works have been done on plant secondary metabolites there is certainly a lack of information about the molluscan secondary metabolites and its biological activities in general. The chemical constituents present in the *P. viridis* have not been studied so far. Therefore, this is the first of its kind using general detection reagents & TLC to evaluate the chemical composition of the extracts from *P. viridis*. However further studies need to be carried out to know the exact source, structure and function of these biomolecules.

CONCLUSION

The present study on the qualitative evaluation of components present in the *P. viridis* extract has shown the presence of various secondary metabolites like alkaloids, polyphenolics, sterols and terpenes. All of these compounds are well known for their biological activities curing different ailments. So we can conclude that *P. viridis* can serve as a potential source for these components and further research is needed to explore the biological activity of these extracts.

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