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Original Research Article

Evaluation of preliminary qualitative analysis of Clam *Paphia malabarica* extracts from Girgon chowpatty Creek, Mumbai

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ABSTRACT

Marine surroundings could be a very large supply for yet to be discovered enormous bioactive natural bio-products. For the present study, the marine bivalve species *P. malabarica* was taken for the preliminary qualitative analysis of the extraction. Three different extract methods were adapted to identify the secondary metabolites, such as aqueous (A), methanol (M) and acetone & n-hexane (AnH) extracts and each extracts of clam was qualitatively evaluated for the secondary metabolites using general color reactions method and spray reagents on thin layer chromatography. The yields of the extracts were recorded 2.1%, 4.8% and 6.6% respectively. AnH extract showed highest bands followed by other two solvents in Thin Layer Chromatography plates. In addition, the developed TLC plates are illustrated different bio-organic compounds like, alkaloids, phenolics, terpenes and steroids. This is the pioneer evaluation of phenolic compounds from the marine bivalve species. The investigation concluded that *P. malabarica* species can serve as a potential source of active components investigations.

Keyword: Alkaloids; Bio-product; organic compounds; phenolics; TLC

INTRODUCTION

Seventy percentages of the world's earth surface are covered by Marine ecosystems and it is proposed to contain over 80% of the world's flora and fauna in it [1]. Exact marine biodiversity is less certain since between one-third and two-thirds of marine organisms have yet to be described [2]. These marine surroundings might be a great supply for however, to be discovered bioactive natural products. Apart from the food, it has bountiful

diversity of bioactive natural compounds are being isolated and characterized with enormous assure treatment for human diseases [3].

The marine clams, mussels and oysters are leading components of bivalve fishery in coastal area [4] and it is forms exceptionally important source of nutrition for coastal populations [5, 6]. Currently, there is a rising an interest in the bioactive resources of mollusk extracts and albeit a little portion of secondary metabolites only investigated from mollusk species. Some marine gastropods and bivalves are having a great curiosity to produce bio-natural products, yielding an assortment of bioactive compounds and numerous drugs leads are currently in clinical test [7].

In general, there is no more scientific investigations carried out to authenticate the health benefits derivatives from mollusks species and characteristically unidentified bioactive natural constituents are involved in the taxa. Based on the Mollusks evolutionary history and only one of its kinds of life styles, frequent for minor classes of Mollusks species is unjustified because of complete lacks of knowledge is led to novel pathways for secondary metabolism in it. Many arguments were continuing about the secondary metabolites of the phylum Mollusks due to an unclearness of natural bioactive metabolites production is omnipresent contained by the phylum Mollusks. Therefore, in future drug discovery field having excellent scope within this phylum of mollusks and it is exploring novel bioactive natural compounds with newer mode of action in it. [8].

In Indian coastal region, the folks are consuming *Paphia malabarica* (Chemnitz) clam as a marine food in its cooked form [4] and also they are exploited widely both meat and shells for the industrial purpose. The raw and processed clam *P. malabarica* has great market values in some foreign countries like Japan and some other European countries. In addition, this clam species has a very good content of dietary value and percentage edibility [9]. The present works investigates the qualitative evaluation of the major secondary metabolites present in the whole tissue of the clam *P. malabarica*'s in three different extracts.

MATERIALS AND METHODS

Collection Extraction method

P. malabarica 600 gm. was purchased in the Girgoan chowpatty, Mumbai and immediately transferred to laboratory in live condition. Specimens were then washed with distilled water to remove associated debris. For this present study, three different extraction methods were adapted like methanol, acetone & n-hexane (1:3) and aqueous extract have been taken from the investigated species.

For methanol extract, the species (200 g.) were dissected and the whole body tissues were blot dried with tissue paper to remove extraneous water content of the tissue. The whole body tissues (10% w/v) were homogenized and extracted in methanol (90% v/v) by agitation in rotary shaker for 24 hours. The step wise methanol extraction procedure included repeated extractions at every 6 hours intervals. Initially, the whole extract contents were centrifuged (8000 × g for 10 minutes at 4 °C) and supernatant was collected in separate vial. The tissue pellet obtained in consequent steps was further treated similarly with methanol to achieve maximum extraction and recovery of the bioactive compounds. All the fractions were finally pooled together, filtered through Whatman No.1 filter paper and concentrated through Rota evaporator. The yield estimation was carried out by evaporating 1 mL extract in pre-weighted aluminum dish at room temperature (27 ºC) until complete dryness and was expressed as mg (crude dry weight extract)/mL. The condensed methanol extracts were adjusted to 10 mg/ mL either by diluting or by concentrating with the same solvent. Sample extracts were then preserved at-20ºC for further investigations. [10].

For acetone & n-hexane extract, the clam (200 g.) was homogenized and extracted with acetone & n-hexane (1:3 ratios) and then agitated for 15 minutes by using a magnetic agitator. The extract was filtered through cellulose under vacuum, the residue was repeatedly extracted and final extracts were made up to 3 mL and stored in deep freezer at -20 °C for further analysis [11].

For aqueous extract, the clam tissue (200 g.) sample was weighed and ground in 300 ml of distilled water using sterile mortar and pestle. After a systematic grinding, the crude extract was filtered using Whatman No. 1 (42 µm) filter paper and the residue was again ground with 200 ml of water and filtered [12]. The filtrate (500 ml) the crude filtered extract of clam sample was evaporated through Rotary evaporator and stored in deep freezer at -20 °C for further analysis.

Preliminary Identification of the chemical components test

The chemical elements of alkaloids, flavonoids, phenolics, saponins and sterols are present in the three extracts of *P. malabarica* were carried out using various general detection reagents as described by [13].

Thin layer chromatography (TLC) of the extracts

The Concentrated extracts dissolved in suitable solvents and each extracts were spotted on readymade TLC plate (10 x 10cm) and allowed to develop with individual solvent systems for each extracts. A few drops of ammonium solution were added to the solvent system for methanol and aqueous extracts for better resolution. The developed TLC plates were visualized under UV lamp fixed in UV chamber; afterward they were exposed to iodine vapor to visualize the components which were UV invisible. The solvent systems used for each extracts were given below.

Acetone & n-Hexane (1:3) extract:

Hexane : ethyl acetate (70:30)

Methanol extract:

Methanol : Dichloromethane: chloroform (30:35:35)

Aqueous extract:

Ethyl acetate: acetic acid (50: 30: 20)

Spray reagent detection method on TLC

The developed TLC plates are following to visualization under UV light were sprayed with various spray reagents to find out the presence of secondary metabolites like alkaloids, phenolics, steroids and terpenes according to standard protocols illustrated by [13].

RESULT

In this present investigation, the yield of extracts from *P. malabarica* in acetone & n-hexane extract has given good quantity then other two extract methods. The extract of acetone & n-hexane gave 6.6%, methanol delivered 4.8 % and aqueous extract gave lower amount of 2.1% yield (Table1). The presence and absence of convinced chemical elements of the investigated extracts are showed by preliminary chemical analysis (Table 2).

Thin layer chromatography of three extracts of *P. malabarica* exposed the occurrence of different chemical elements in the chromatogram. The developed TLC plate of methanol extract exhibited a colored chromatogram with the universal solvent system of hexane: ethyl acetate at 7:3 ratios. On observation under UV 254 nm and 365 nm, the chromatogram reflected different colored spots like pink, white, blue, yellow, brown, violet etc., seven, five and four separate spots were noticed on the developed TLC plates of acetone & n-hexane, aqueous and methanol extracts respectively. The R_f vales were shown in Table 3. Some spots are colorful and some are not visible in the day light period that spots can see under UV radiation only. The Spray reagent detection of the developed TLC plates

of the three different solvent extracts illustrated different bio-organic compounds like alkaloids, phenolics, terpenes and steroids (Table 4).

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

S.No.	Name of the extract	Color	Consistency	% yield
1	Methanol	Brown	Sticky	4.8
2	Acetone& n-hexane	Green	Sticky	6.6
3	Aqueous	White	Sticky	2.1

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

S.No.	Test	Extracts of *P. malabarica*		
		Acetone & n-hexane	Methanol	Aqueous
1	**Alkaloids**			
	Mayer's test	+V	-V	-V
	Dragendorff's reagent	-V	-V	-V
	Wagner's reagent	+V	-V	+V
2	**Flavonoids**			
	Shinoda's test	-V	-V	-V
	Poly phenols	+V	+V	-V
3	**Sesquiterpene lactones /Cardiac glycosides**			
	Baljet reagent	-V	-V	-V
	Legal reagent	-V	-V	-V
4	**Sterols**			
	Liebermann-Buchard test	+V	+V	-V
	Salkowski reaction	+V	+V	-V
	Saponins	-V	+V	+V
5	**Tannins**	-V	-V	-V

Table 3: R_f value of different extract of *P. malabarica*

Acetone & n-hexane	Methanol	Aqueous extract
AnH1- 0.07	M1- 5.7	A1- 0.21
AnH2- 0.15	M2- 5.5	A2- 0.22
AnH3- 0.21	M3- 4.5	A3- 0.65
AnH4- 0.26	M4- 4.8	A4- 0.91
AnH5- 0.36	-	-
AnH6- 0.66	-	-
AnH7- 0.83	-	-

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

S.No.	Compounds	Identified compounds of the three extracts by R_f value		
		Acetone & n-hexane	Methanol	Aqueous extract
1	Alkaloids	AnH1, AnH2 & AnH3	-	A1 & A2
2	Phenolics	AnH7	-	-
3	Terpenes	AnH4 & AnH7	M3& M4	A3
4	Steroids	-	M1 & M2	A4

DISSCUSSION

A number of Marine Mollusks are used as traditional Indian, Chinese, South African and Middle Eastern Countries as folk medicines [14, 15, 16, 17, 18, 19, 20 and 21] as well as in homeopathic remedies [22].

By the TLC separation method, the secondary metabolites of the *P. malabarica* extracts were known to be complex and spray reagent detection method which exposed the presence of assorted group of secondary metabolites like alkaloids, polyphenols, terpenes, steroids and saponins in the present biochemical evaluation.

The yields of the different extractions were depending upon the solvent, time and temperature of extraction in addition to the chemical nature of the extracted sample. Numerous decisive factors are applied in evaluating the efficiency of the extraction method and the appropriateness of a solvent for a particular extraction method. In general, the majority of an encountered decisive factor is yield extraction, i.e. the total yield [23, 24 and 25]. Similar results were observed on *Perna viridis* species by [7] and *Lotus rhizome* extracts by [16].

Visualization of developed TLC plate under UV light showed different colored zones parallel to the separated spots of n-hexane & acetone (1:3) extract of clam *P. malabarica*. During the methanol and aqueous extracts observation, few spots only exposed as fluorescence. This may happen owing to the fact that various analysts are not absorbing on visible or UV radiation. Chromatographic zones usually emerge in dark background and a multiplicity of visible spectrum colors will appear during the fluorescence. The entire chromatogram of n-hexane & acetone extract showed fluorescence in UV, reflected individual zones of different colors like blue, green, purple and white. These colors could indicate the presence of alkaloids, flavanoids and saponins.

Some alienated chromatographic zones might appear colorless in the normal light absorbance although it can absorb on electromagnetic radiation at shorter wavelengths of 254 nm or in long wave radiation of 366 nm. The components of methanol and aqueous extracts that are not visible in the normal light and under UV were detected by exposing to iodine vapour.

In the present study the *P. malabarica* extracts has showed flavonoids in methanol and aqueous extract. Flavonoids are having a good potent of antioxidants, scavengers of a broad range of reactive oxygen species and lipid peroxidation inhibitors also has a potentially therapeutic agents against an extensive variety of diseases and disorders. In human food diet it

may well decrease the risk of various tumors and preventing menopausal symptoms [26, 27].

Acetone & n-hexane and Aqueous extracts showed the presents of alkaloids in it. From mollusks species the alkaloids have been isolated in sensibly in huge numbers whereas aliphatic nitrogen holding elements are moderately uncommon [13].

In the present study, polyphenolic compounds were noticed in Acetone & n hexane and methanol extract. The polyphenolic compounds presence is representing a very good indication of antioxidant activities in terms of their capability of free radicals scavenging and it is a readymade uses of its phenol rings as an electron traps for free radicals [28, 29]. The physical, chemical and biological characteristics of phenolic compounds are used as drugs and it is responding as an antimicrobial, anti-inflammatory, anti-feedant, anti-viral, anticancer and vasodilator agents [30].

The plants and animals are producing a large assortment of terpenes, which is naturally present in it and an extensive range of biological active properties terpenoids are illustrated some great activities such as cancer chemo preventive effects, antimicrobial, anti-fungal, anti-viral, anti hyperglycemic, anti-inflammatory and anti-parasitic activities. The investigated clam extracts were also given positive results on steroids and it is very vital elements particularly due to their connection with compounds such as sex hormone [31].

In the present study, saponins were noticed in clam extracts and tannins are absents in this sample. In Chinese traditional medicine they are considering Saponin as key ingredients and in many noticed natural biological activities the saponins getting responsible for that active effect. Saponins are identified as an inhibitor of anti-inflammatory activity. There is a wonderful, beneficially ambitious support of saponins as nutritional supplements and nutriceuticals. The physical, chemical and biological characteristics of saponins are having a great useful of producing drugs. These are having some superior activities on antimicrobial, anti inflammatory, anti-feedent and hemolytic effects [32, 33 and 34].

CONCLUSION

The present investigation deals with pioneer estimation of significant active component in marine bivalve species of *P. malabarica.* The qualitative evaluation of components extract has proved the presence of various secondary metabolites like alkaloids, polyphenolics, sterols and terpenes. All indentified compounds are well known for their biological activities curing diverse ailments. The results of the evaluation concluded that *P. malabarica* can serve as a potential source for essential active components and further research can explore the biological activities.

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