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Der Pharmacia Lettre, 2020, 12 (7): 16-26
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Antibacterial Activity of Marine Gastropods *Paphia malabarica*, Dillwyn 1817 and *Crassostrea gryphoides*, Schlotheim 1820 Collected From Ratnagiri, Southwest Part of Maharashtra, India

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ABSTRACT

Objective: The aim of the present study is to evaluate the antibacterial activity of the crude extracts of *Paphia malabarica* and *Crassostrea gryphoides*

Methods: Crude extract of two Bivalvia was tested for inhibition of bacterial and fungal growth. Antimicrobial activity of the whole body of *Paphia malabarica* and *Crassostrea gryphoides* were tested against six bacterial strains and two fungal strains by disc diffusion method. The antimicrobial activity was measured accordingly based on the inhibition zone around the disc impregnated with Crude extract of gastropods.

Results: The inhibition zone of (8 mm) was observed against *Corynebacterium diphtheria* and for fungi inhibition zone of (4 mm) was observed against *Aspergillus niger* in the crude methanol extract of *Crassostrea gryphoides*. In the case of Methanolic extracts of *Paphia malabarica* inhibition zone of (7 mm) was observed against *Proteus vulgaris* and for fungi inhibition zone of (4 mm) was observed against *Malassezia furfur*.

Conclusion: The Methanol crude extracts of *Paphia malabarica* and *Crassostrea gryphoides* show activity against both bacterial and fungal strains.

Keywords: *Paphia malabarica*, *Crassostrea gryphoides*, Antibacterial activity.

INTRODUCTION

Mollusca constitute an imperative component of marine biodiversity of the territory and island coasts of India. Marine molluscs happen in assorted environments: rough coasts, sandy shorelines, ocean grass beds, coral reef biological systems, mangroves additionally at abyssal profundities within the ocean. In any case, in terms of molluscan species differences and plenitude, rough intertidal zones and coral reef ranges of India are wealthy. Mollusca are classified into seven classes, of which five are spoken to in India. Of the 586 worldwide families, 279 are spoken to within the Indian locale, counting approximately 3600 species, of which roughly 2300 (65%) are marine. As there are no appropriate gauges of the number of molluscan species occurring within the marine and coastal biological systems of India, this can be an endeavor towards evaluate ment of marine molluscan differences in India [1].

The emphasis herein is on their potential use in the food and nutraceutical industry. In expansion, mollusks utilization as portion of ordinary eats less can moreover be supportive in maintaining a strategic distance from numerous sicknesses as they are wealthy in crucial supplements and dynamic auxiliary metabolites, as well as have the capacity to improve safe response. Moreover, the available literature recommends that ordinary cooking hones have no outstanding unfavorable impacts on their dietary esteem and they hold certain bioactivities indeed after the activity of stomach related proteins. In spite of the fact that mollusks have been broadly examined in connection to the health-promoting impacts checked on here, there's still more scope for advance investigate in this heading in arrange to completely utilize this colossal source of nourishment and nutraceuticals [2].

Oysters in the oceans are a common sight and are virtually untapped resource of novel compounds. Oysters are bivalve soft bodied molluscs and shellfish under class Bivalve, mostly marine or estuarine in habit. As a sea food, oysters have been introduced and established in food permanently in at least 24 countries. Common species of oyster like *Saccostrea cucullata*, *Crassostrea madrasensis*, *Crassostrea gryphoides*, *Crassostrea rivularis* and *Crassostrea discoidea* found in India. Numerous ponders reveled that molluscs has antitumor, anti-leukemic an anti-viral exercises. These wealthy differences to marine life forms accept a more prominent opportunity for the disclosure of modern bioactive compound. Six oysters used as a supplement of iron and protein for daily diet. So, doctors recommended the oyster as a diet for patients with anemia [3].

These edible oysters are very popular as raw and processed food in South Indian States, Goa and in Europe, USA etc. Therefore, the aim of the present study was to evaluate the antimicrobial activity of the soft body and shell powder of *Crassostrea gryphoides* against different pathogenic bacterial and fungal strains.

The marine clams, mussels and oysters are driving components of bivalve fishery in coastal region and it is shapes especially imperative source of sustenance for coastal populations [4]. The bivalve mollusks *Paphia malabarica* and *Villorita cyprinoides* shows Nutritional Qualities collected from Estuarine Waters of the Southwestern Coast of India [5]. The yellow-foot bivalve clam, *Paphia malabarica* Chemnitz (Veneridae) is distributed in the southwest coastal regions of India. The ethyl acetate-methanol extract of this species exhibited significant antioxidant and anti-inflammatory activities [6]. The investigation concluded that *P. malabarica* species can serve as a potential source of active components investigations [7].

Anti-bacterial development was surveyed in various unpleasant extricates of five commercially crucial consumable marine bivalves, specifically *Meretrix casta* (Chemnitz), *Polymesoda* (Geloina) *P. aeruginosa* (Solander), *Perna viridis* (Linnaeus), *Crassostrea gryphoides* (Schlothim) and *Villorita cyprinoides* (Dim), collected from the coast of Goa (India). The antimicrobial movement of the bivalve extricates arranged was assessed against a set of 9 pathogenic micro-organisms (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas* sp., *Streptococcus pyrogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella*

pneumoneae, *Serrratia marganii* and *Candida albicans*) utilizing standard circle dissemination test (subjective) and fluid development restraint assay [8]. The two Bivalve *Perna viridis* and *Crassostrea madrasensis* was utilized for the antibacterial Exercises [9]. Two species of clam *M. meretrix* and *M. casta* Assayed for Antimicrobial Activities against 10 pathogenic micro-organisms and 6 Fungal Pathogens [10]. Extraction of important biologically-active compounds from marine resources will certainly be helpful in protecting and treating various viral diseases in human beings [11]. Molluscs that antimicrobial peptides and proteins have been subject to distinct patterns of diversification and we evidence the existence of different evolutionary routes leading to such sequence variability [12].

MATERIALS AND METHODS

Description & Sampling location

Description: Ratnagiri is a coastal district of Maharashtra state, situated in the western coast of India. It has north-south length of about 180 km and average east-west extension of about 64 km. This district comes between 16.30 to 18.04 north latitudes and 73.02 to 73.53 east longitude. In Ratnagiri coast of Maharashtra with its precious ecosystems such as coral reefs, mangroves, and sea grasses is one of the highly productive coastal areas in India. For collection of specimen survey done among the Fishery market and village near the sea shore level, taking their opinion for the collection of marine Gastropods.

Sampling location: Marine molluscs *Paphia malabarica* and *Crassostrea gryphoides* were collected from different locations in Ratnagiri coast. The sampling locations are shown in Figure 1. The gastropod *Paphia malabarica* and *Crassostrea gryphoides* was collected from juve mangrove ecosystem (16.9650°N, 73.3101°E) and Shirgaon (17.4536°N, 73.6201°E) situated in Ratnagir district of Maharashtra state (India) shown in Figure 2, that was covering a distance of 5-8 km from the Government college of Pharmacy, Ratnagiri, Maharashtra (India).



Figure 1: The Gastropod *Paphia malabarica* was collected from juve mangrove ecosystem.



Figure 2: The gastropod *Crassostrea gryphoides* was collected from Shirgaon.

Preparation of crude extracts of whole selected animal

The soft bodies of the *Paphia malabarica* and *Crassostrea gryphoides* were removed from outer shells and the soft bodies were chopped into small pieces weighed (200 g) and macerated with methanol (500 ml) for 7 days in mechanical shaker, respectively. The extracts were cooled centrifuged at 5000 rpm for 15 min. Samples were centrifuged at 5000 rpm for 15 min and the supernatant were collected. Supernatant liquid concentrated to dryness using a rotary evaporator and the extracts were further stored at 4°C until analysis [13].

Bacterial cultures

six bacterial strains and two fungal strains, viz., *Pseudomonas aeruginosa*, *Corynebacterium diphtheria*, *Salmonella Paratyphi B*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Malassezia furfur*, *Aspergillus niger* were obtained from the Indira Institute of Pharmacy, Sadavli, Ratnagiri.

Antibacterial assay

In vitro antibacterial activity was assayed by the Standard disc diffusion method [14]. A known amount of crude extract (5 mg) was dissolved in 0.5 ml of methanol and applied to 6 mm sterile disc. The discs were allowed to dry at room temperature. Pathogenic bacterial strains were inoculated in sterile broth and incubated at 37°C for 24 hrs. Pathogens were swabbed on the surface of sterile petri dishes in 20 ml of solidified nutrient agar. The experimental discs were placed in the sterile solidified nutrient agar petri plates to assess the effect of solvent and extracts on pathogens. These agar plates were incubated at 37°C for 24 hrs and the antibacterial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Antibacterial activity was expressed in diameter zone of inhibition which was measured with the outer side of the disc to inner side of the inhibition zone.

Fourier- transformed infrared spectroscopy (FTIR)

Infrared spectrum was measured with Attenuated total reflection (ATR). The sticky extract (1-2 mg) was placed in ATR which has standard technique for the measurement of FT-IR (FTIR-ALPHA, BRUKER model, INDIA). The frequencies of different components present in the sample were analyzed CD technique.

Phytochemical screening

Phytochemical analysis was done using the protocols by Harbone and Sofowora [15,16] for testing the presence of tannins, saponins, steroids, terpenoids, glycosides, alkaloids, anthroquinones and flavonoids in ethanolic extract of *Crassostrea gryphoides*.

RESULTS AND DISCUSSION**Antibacterial screening**

Primary screening for evaluating the antimicrobial potential of the *Paphia malabarica* and *Crassostrea gryphoides* against six bacterial strains and two fungal strains, viz., *Pseudomonas aeruginosa*, *Corynebacterium diphtheria*, *Salmonella Paratyphi B*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Malassezia furfur*, *Aspergillus niger*. The microbial interactions were analyzed by determining the distance of inhibition measured in mm. Microbial strains showing good inhibition activity were selected for secondary screening. All the experiments were performed in triplicate and the average values were considered for analysis [17]. Methanol extract of *Paphia malabarica* and *Crassostrea gryphoides* was described in Table 1, Figure 3 (Antimicrobial activity of methanol extracts of *Paphia malabarica* and *Crassostrea gryphoides*).

Table 1: Zone of inhibition in mm of methanol extracts of *Paphia malabarica* and *Crassostrea gryphoides*

S. No	Name of the Organism	Compound Number along with zone of Inhibition in mm	
		Methanol extract of <i>Paphia malabarica</i>	Methanol extract of <i>Crassostrea gryphoides</i>
1	<i>P. aeruginosa</i>	--	--
2	<i>C. diphtheria</i>	--	4
3	<i>S. paratyphi B</i>	--	--
4	<i>S. aureus</i>	--	--
5	<i>P. vulgaris</i>	6	--
6	<i>E. coli</i>	--	--
7	<i>M. furfur</i>	4	--
8	<i>A. niger</i>	--	4

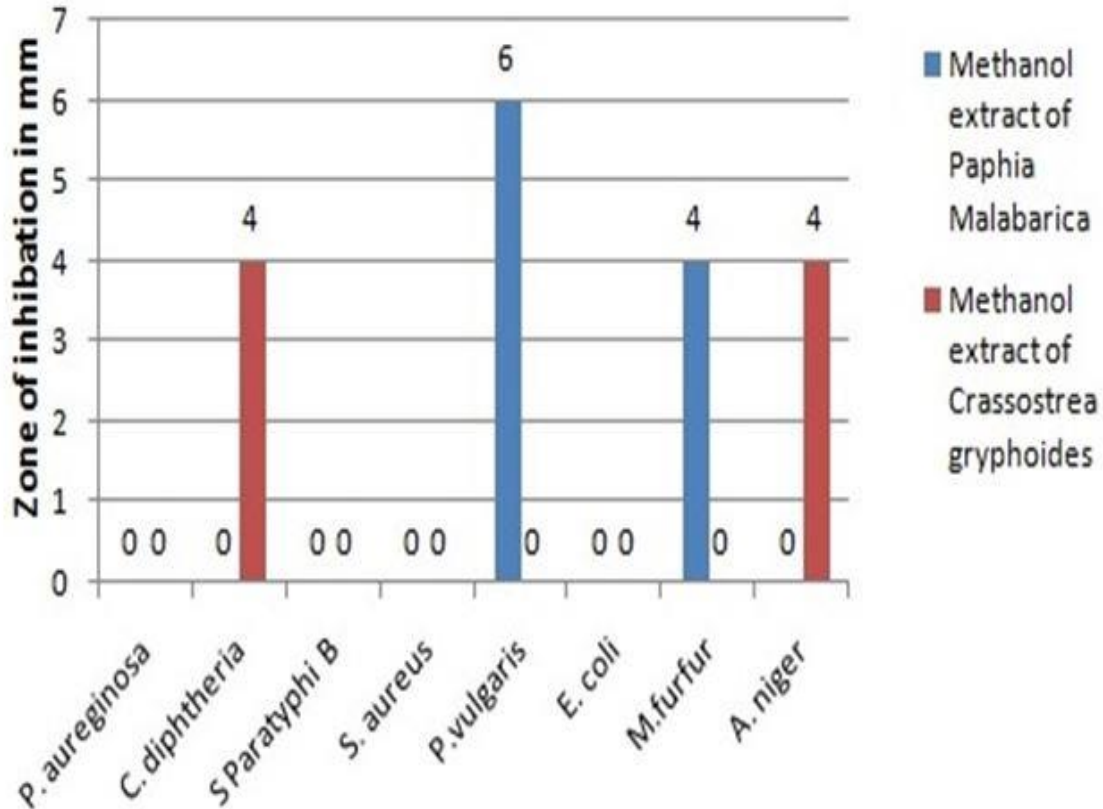


Figure 3: Antimicrobial activity of methanol extracts of Paphia malabarica and Crassostrea gryphoides.

After confirmation of primary screening against the test organisms, secondary screening was done with different concentration (100, 200, 400 µg/ml) and checked against *C. diphtheria*, *P. vulgaris*, *M. furfur*, *A. niger*. The positive control (ciprofloxacin) was active against all the bacterial strains and Nystatin against Fungus tested. Zone of Inhibition in mm of methanol extract of *Crassostrea gryphoides* was described in Table 2, Figure 4 (Antimicrobial activity of methanol extract of *Crassostrea gryphoides*) and methanol extract of *Paphia malabarica* was described in Table 3, Figure 5 (Antimicrobial activity of methanol extract of *Paphia malabarica*).

Table 2: Zone of inhibition in mm of methanol extract of Crassostrea gryphoides

Organism	Crassostrea gryphoides methanolic extract			
	100	200	400	standard
<i>C. diphtheria</i>	2	4	8	12
<i>A. niger</i>	0	5	8	12

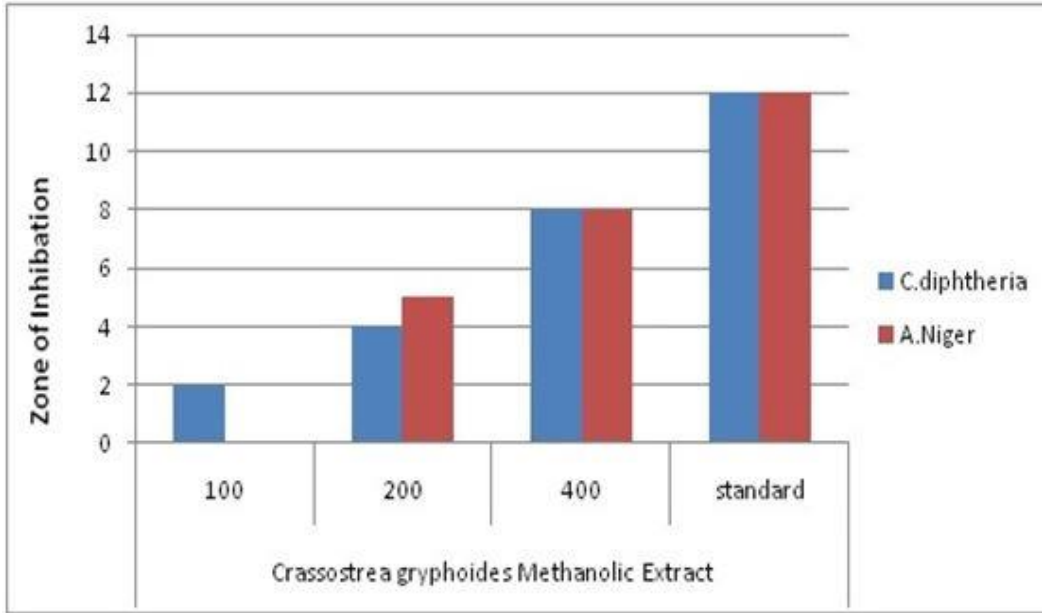


Figure 4: Antimicrobial activity of methanol extract of *Crassostrea gryphoides*.

Table 3: Zone of inhibition in mm of methanol extract of *Paphia malabarica*.

Organism	<i>Paphia malabarica</i> Methanolic Extract			
	100	200	400	standard
<i>P. vulgaris</i>	3	5	7	16
<i>M. furfur</i>	0	4	6	0

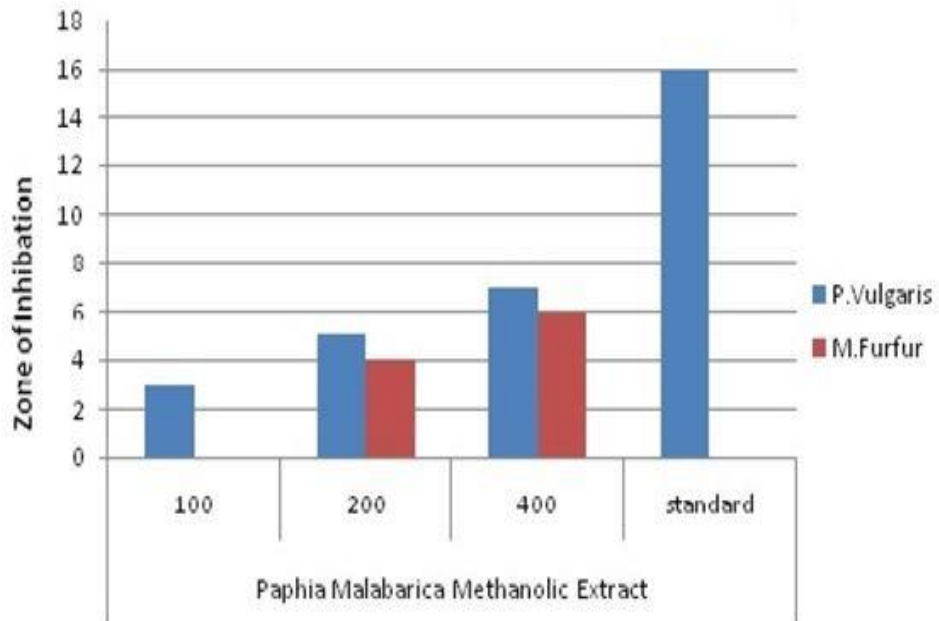


Figure 5: Antimicrobial activity of methanol extract of *Paphia malabarica*.

Fourier transform infrared spectrum of the sample of paphia malabarica

FTIR spectrum of the sample *Paphia malabarica* showed that 7 major peaks were shown at, 3274.3, 2930, 2361, 1456, 1340, 1147, 1075/cm showing the presence of Alcohol, alkenes, carbon dioxide, alkane, aromatic amine, aliphatic ether, and primary alcohol functional groups, whereas all the remaining peaks were very close at 1636, 1456, 1417, 1340, 1147, 1075, 1015, 920, 845 and 507/cm (Figure 6).

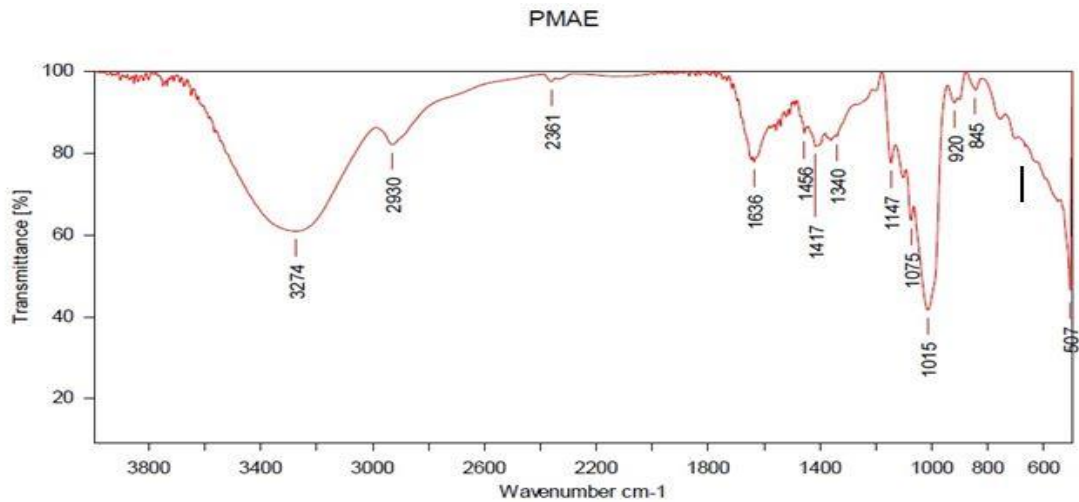


Figure 6: Fourier transform infrared spectrum of the sample of *paphia malabarica*.

Fourier transform infrared spectrum of the sample of Crassostrea gryphoides

FTIR spectrum of the sample *Crassostrea gryphoides* showed that 5 major peaks were shown at, 2975, 2364, 1569, 1119, 1039/cm showing the presence of alkanes, carbon dioxide, alkene, aliphatic ether, , and sulfoxide functional groups, whereas all the remaining peaks were very close at 1569, 1460, 1401, 1119, 1039, 927, 847/cm (Figure 7).

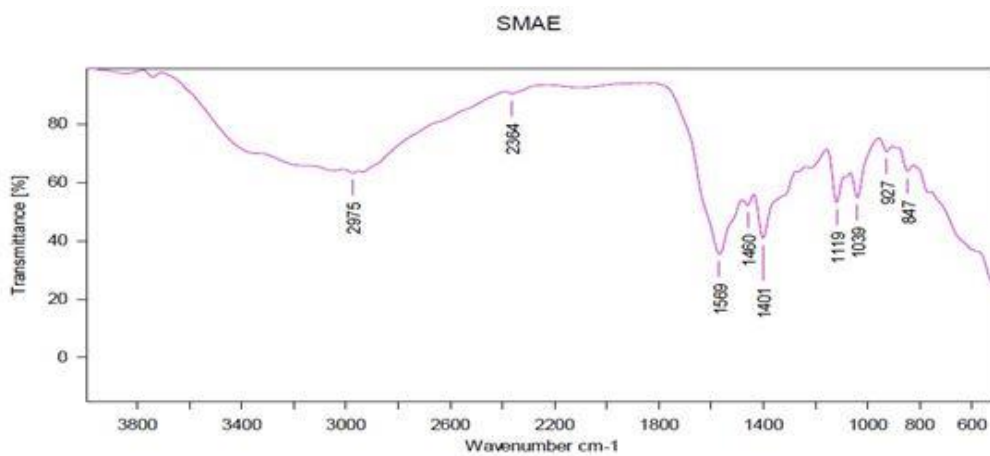


Figure 7: Fourier transform infrared spectrum of the sample of *Crassostrea gryphoides*.

Table 4: Preliminary phytochemical analysis of screened *Paphia malabarica* & *Crassostrea gryphoides*

S. No	Test	<i>Paphia malabarica</i>	<i>Crassostrea gryphoides</i>
1	Carbohydrate	+	+
2	Protein	-	-
3	Amino acid	+	+
4	Alkaloids	+	+
5	Flavonoids	+	+
6	Tannins	+	+
7	Steroids	+	+

Diverse ranges such as marine science, marine environment, natural chemistry, chemistry, pharmacology and biotechnology developing intrigued in marine characteristic items or marine auxiliary metabolites because of their potential pharmacological utilization. A wide variety of bioactive substances are being isolated and characterized from the food that is derived from the marine environment, several with great promise for the treatment of human and fish diseases. For the past two decades, the pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants. However, the advent of multiple resistant mechanism has limited the use of many major classes of antimicrobial compounds [18].

Antibacterial activity from crude methanol extract of *Crassostrea gryphoides* was found to be wide activity against a set of 9 pathogenic micro-organisms (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas* sp., *Streptococcus pyrogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marshallii* and *Candida albicans*) were reported. It was found that acid-enzyme hydrolysis (AEH) extracts showed higher activity against the tested organisms as compared to Methanol (MeOH) and phosphate buffer saline (PBS) extracts [19]. Antimicrobial activity in bivalves appeared to be dependent on the extraction process according to that variation was also observed. However in the present study, *Crassostrea gryphoides* was tested against *Corynebacterium diphtheria* and *Aspergillus niger*. whereas *Paphia malabarica* was tested against *Proteus vulgaris* and *Malassezia furfur*.

In the Primary screening the Methanol crude extracts of *Paphia malabarica* and *Crassostrea gryphoides* show activity against both bacterial and fungal strains. *Paphia malabarica* shows the shown inhibitory zone 6mm to *Proteus vulgaris* and for fungi shown inhibitory zone 4mm to *Malassezia furfur*. Whereas *Crassostrea gryphoides* shows inhibitory zone 4mm to *Corynebacterium diphtheria* and for fungi shows inhibitory zone 4 mm to *Aspergillus niger*.

Antibacterial effects of different concentrations of althaea officinalis root extract versus 0.2% Chlorhexidine and Penicillin on *Streptococcus mutans* and *Lactobacillus* (*In vitro*) [20]. In this study secondary screening was performed against two bacterial and fungal strains viz., *Proteus vulgaris*, *Malassezia furfur*, *Corynebacterium diphtheria* and *Aspergillus niger* with different concentration (100, 200, 400 µg/ml). The results showed a significant antibacterial effect of the Methanolic crude extract of *Paphia malabarica* on the Gram-Negative bacterial species (*Proteus vulgaris* and *Malassezia furfur*) and fungus and Methanol crude extracts of *Crassostrea gryphoides* on the Gram positive bacteria and fungus (*Corynebacterium diphtheria* and *Aspergillus niger*). Therefore, it was concluded that the Methanol crude extracts of *Paphia malabarica* and *Crassostrea gryphoides* is a good choice for treating Gram-positive and gram negative bacterial as well as fungus infections.

The results of FTIR analysis reveal the presence of bioactive compound signals at different range (600.0-3800.0/cm). It was found that all the Tissue extract have considerable proportion of important phytochemicals that are easily detected by

qualitative tests. In our analysis it was cleared that the *Paphia malabarica* and *Crassostrea gryphoides* is rich in Carbohydrate, Amino acid, alkaloids, flavonoids, tannins and sterols (Table 4). The vital thing is that all plant tests contain one common and abundant auxiliary metabolite, flavonoid. From the literature study it was found that flavonoids have wide extend of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic antitumor properties [21]. Alkaloids compounds along with hypoglycemic, antidiabetic properties [22-25] also exhibit anti-inflammatory, antimicrobial and antioxidant effects [26].

CONCLUSION

Considering the significant effect of the Methanol crude extracts of *Paphia malabarica* and *Crassostrea gryphoides* is a good choice for treating Gram-positive and gram negative bacterial as well as fungus infections, further studies are recommended to find medicinal drugs as a sustainable natural source. It also concluded that as the tissue extract studied, found to rich in phytochemicals, and are full of pharmacological and medicinal significance. Further study is required to find their potentials in the mentioned biological properties such as antidiabetic, anti-tumor, etc.

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