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Bioactive potential of *Purpura persica*

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ABSTRACT

The present study has been aimed to ascertain the antimicrobial activity of extracts from Purpura persica against various pathogenic bacterial and fungal strains using the agar disc diffusion method and the most probable antimicrobial compound by GC-MS study. Crude and eluted fractions were assayed for anti microbial activity against six human bacterial pathogens viz Vibrio cholerae, Salmonella typhi, Shigella flexneri, Escherichia coli, Mycobacterium tuberculosis and Pseudomonas fluorescens and three fungal pathogens viz. Candida albicans, Aspergilus flavus and Actinomyces sp. Maximum inhibition zone were obtained against S. typhi , S.flexneri V.cholerae and E.coli. Of the five column chromatographic fractions, maximum number of pathogens was inhibited by F3 followed by F2 and F1 respectively. Maximum effects of crude extracts of P. persica on fungi were exhibited by chloroform extract. Among the fractions, F2 and F3 showed very good activity against the tested fungi (C. albicans (19mm) and Actinomyces sp.). The GC/MS study of P.persica revealed the probable antimicrobial compounds such as a steroid compound with chloridate cholest-5-en-3-01(3a) - carbano chloridate and a chloride compound 9, 12-octadecadienoyn chloride (z, z), in F2 fraction and in F3 fraction six antimicrobial compounds were identified such as a phenolic compound eugenol, three Plasticizer compounds, dibutyl phthalate, 1,2-Benzenedicarboxylic acid, diisooctyl ester and Phthalic acid, bis (7-methyloctyl) ester, and a steroid cholest-5-ene, 3-bromo-(3a).

Keywords: Antibacterial activity, solvents, inhibitory zone, GC-MS analysis, test pathogens.

INTRODUCTION

Most of the pathogens are increasingly resistant to the major classes of the routinely used antibiotic. Many diseases were initially controlled exclusively by the use of antimicrobial drugs. The massive use of antimicrobial for diseases control and growth promotion in animals increases the selective pressure exerted on the natural emergence of bacterial resistance [1]. So there is an urgent need for the discovery of the new and novel antimicrobial drugs to effectively combat not only the drugs resistance but also the new disease producers, hence the search for active drugs from alternative sources including marine environment, obviously becomes imperative. The rich diversity of marine organism assumes a great diversity of the discovery of new bioactive substances. The ocean remains as an untapped source for many drugs and contemporary experimental studies which indicate that, pharmacologically active substances could be isolated from marine organism [2].

Natural products isolated from marine organisms have increased rapidly and hundreds of new compounds being discovered every year [3, 4]. Marine invertebrates offer good source of potential antimicrobial drugs [5, 6, 7].

Studies on antimicrobial mechanisms and compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in molluscs.

Among the invertebrates, the molluscs are very good source for biomedical important products [8]. Many classes of molluscs with bioactive compounds like antitumour, antileukemic, antibacterial, cytotoxic, anti inflammatory and antiviral properties have been reported [9, 10]. These reports suggest that molluscs are the rich source for discovering novel lead compounds for the possible development of new types of antibiotics for pharmaceutical use. Keeping the importance of gastropods in terms of bioactive compounds with antibacterial properties, the present study has been undertaken to determine the antibacterial activity of extracts from *P.persica* against various human pathogenic microorganisms.

EXPERIMENTAL SECTION

Collection and Preparation of Samples

The mollusc *P.persica* was collected from intertidal rocky shore of harbour area of Gulf of Mannar, near by Theraspuram Tuticorin, situated in the south east coast of India, between April 2015 and December 2015. The collected samples were rinsed with sterile sea water to remove the associated debris and salt. Test animals were first carefully removed from their shells. The flesh was cut into small pieces and air-dried. The air-dried flesh was immersed in 100% A.R. Grade ethyl acetate, chloroform and methanol for 10 days at room temperature. The extract from the solvents was filtered by using Whatman no.1 filter paper and evaporated to dryness in rotary evaporator and the dried extract was stored at 0°C for further use.

Microbial strains used

Antimicrobial activity of tissue extracts were determined against 6 different bacterial pathogens, viz., *Shigella flexneri, Vibrio cholerae, Salmonella typhi, Mycobacterium tuberculosis, Pseudomonas fluorescens and Escherichia coli* and three fungal stains viz., *Candida albicans, Aspergilus flavus* and *Actinomyces* sp.

Antibacterial susceptibility assay

In vitro anti bacterial activity was assayed by the disc diffusion method [11]. A known amount of crude whole body gastropod extract was dissolved in 0.6ml of solvent (methanol) and applied to 6mm sterile disc. In the same way for control 0.6 ml of methanol was soaked in sterile disc. Both 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. In vitro antifungal activity was determined using the techniques of Kelman *et al.,* 2001. Pathogenic fungal strains were inoculated in potato dextrose agar medium and incubated at 48 hrs. Pathogens were swabbed on the surface of sterile petri dishes in 20ml of solidified nutrient agar. The control and 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 for antibacterial activity and 48hrs for fungal was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Antimicrobial 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. Each active extract was tested thrice for confirmation of activity.

Crude extract was fractionated and elusions were made with ethyl acetate (F1), ethyl acetate: chloroform (1:1) (F2), chloroform (F3), ethyl acetate: methanol (1:1) (F4) and methanol (F5). Eluted fractions were assayed for anti microbial activity following the above mentioned disc diffusion method.

Identification of compounds

The most potent crude extract of the test animal was subjected to GC-MS study which was carried out on a GC Clarus 500 Perkin Elmer system for the identification of probable antimicrobial different compounds.

RESULTS AND DISCUSSION

Antibacterial activity of extracts from *P. persica*

The crude ethyl acetate extract of *P. persica* the range of activity varied from 2mm (*P. florescens and S. flexneri*) to 6mm (*S. typhi*) (Fig. 1), in crude chloroform extract from 2mm (*M. tuberculae*) to 7mm (*S. typhi*) (Fig.2) and in methanol extract from 2mm (*P. fluorescens* &*M. tuberculae*) to 7mm (*S. typhi*) (Fig. 3). Of the five columns

chromatographic fractions, maximum numbers of pathogens were inhibited by F3 fractions followed by F1 and F2 (Fig.6, 4 &5).

The highest activity of F3 fraction was exhibited against *V. cholerae* (19mm) and the least against *M. tuberculae and S. flexneri* (2mm) (Fig.6). F1 fraction showed maximum activity against S. typhi (17mm) (Fig. 4) and in F2 fraction the highest was against *V. cholerae* (11mm) (Fig.5). In concern with *P. persica's* extract the most sensitive pathogens were *V. cholerae, S. typhi* and *E. coli*.Santhana Ramasamy and Murugan experimentally analyzed the methanolic extract of *C.virgineus* and *C.ramosus* and they also observed the broad spectrum antibacterial activity of body tissue extract [12]. Highest activity was observed against *Klebsiella pneumoniae* and *Staphylococcus epidermis* by the extract of acetone and against *Salmonella paratyphi* by the extract of chloroform column purified whole body extract of the winged Oyster *Pteria chinensis* were reported by Chellaram [13].

Apart from methanol extract in the present investigation chloroform fraction of *P.persica*, inhibited *S.flexeri, S.typhi and V.cholerae* . Similar result was reported by Chellaram [13] in chloroform extract of *Pterai chinensis* which inhibited eight fish pathogens. Anbuselvi [14] found that acetone column purified fractions of *Trochus tentorium* shown highest antibacterial activity. *T*he present study with test animal *P.persica* corroborates the earlier findings of suppressing the activity of *S.typhi*. In the present investigation higher degree of inhibition was confined to F3, F1 andF2 fractions of *P.persica* indicating the substances involved in producing the antibacterial effect could be a medium polar compound, *Cypraea erronus* was reported to have antibacterial activity at the non polar end of the step gradient by the column-purified fractions [15]. Difference in antibacterial activity found with prosobranchs extracts may depend on extracting capacity of the solvents and the compounds extracted [16]. Many antimicrobial screening studies have shown that Gram-negative bacteria are more sensitive than Gram- positive bacteria [17].

Antifungal activity of extracts from *P. persica*

Antifungal activity of *P.persica* were presented in Figures (9 -16). Crude ethyl acetate extract of *P.persica´s* inhibitory range varied in between 3mm (*C. albicans*) and (*A.flavus*) 5mm, in chloroform extract 5mm (A*ctinomyces* sp.) to 8mm (*C. albicans*) and in methanol extract 4mm (*A.flavus*) to 7mm (A*ctinomyces* sp.) (Fig.7,8 & 9). Among the silica gel fractions, maximum activity was observed in fraction F2 and F3. Maximum inhibition zone was obtained against *C. albicans* (19mm) and minimum against A*ctinomyces* sp. (17mm) in fraction F2 (Fig 11). It also showed higher degree of inhibition zone against *A.flavus* (18mm), Fractions F3 also shown inhibition zone against *C. albicans* (Fig 12). As a result, potential antifungal activity was found in *P.persica's extract* against all the tested fungal pathogens. When F2 fraction was subjected to GC-MS analysis a steroid compound with chloridate cholest-5-en-3-01(3a) - carbano chloridate with maximum percentage of 72.31 % and a chloride compound 9, 12-octadecadienoyn chloride (z, z), were identified as antimicrobial compounds (Fig.17 &18) (Table 1). F3 fraction (Fig. 19 - 22) (Table 2) of *P.persica* only six antimicrobial compounds were identified such as a phenolic compound eugenol, three Plasticizer compounds, dibutyl phthalate, 1,2-Benzenedicarboxylic acid, diisooctyl ester and Phthalic acid, bis (7-methyloctyl) ester, and a steroid cholest-5-ene, 3-bromo-(3a)-(Table 5). Among the antimicrobial compounds present, 79.95 % was the 1, 2-Benzenedicarboxylic acid, diisooctyl ester. Several marine natural products showed significant antifungal activity; Callipeltins J and K, MIC at 1 µm [18]; the triterpene glycoside holothurin B, MIC at 1.56µg/ml [19]; the macrolids neopeltolide MIC at 0.62 µg/ml [20]; and pseudoceratins A&B, MIC at6.5-8.0 µg/disc [21]. A polypeptide type AMP (Antimicrobial peptide) isolated from the Chilean scallop *Argopecten purpuratus*, showed antifungal activity against F. *oxysporum* and *Saprolegnia parasitica* [22].

Fig. 1 Antibacterial activity of crude Ethyl acetate extract of *P.persica* against pathogens

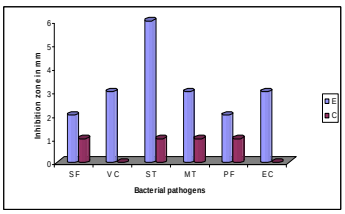

Fig. 2 Antibacterial activity of crude Chloroform extract of *P.persica* against pathogens

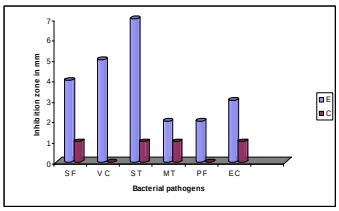

Fig. 3 Antibacterial activity of crude methanol extract of *P.persica* against pathogens

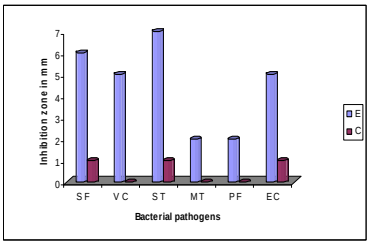

Fig. 4 Antibacterial activity of various fractions of *P. persica* against pathogens F1

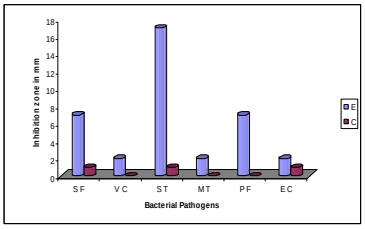

Fig. 5 F2

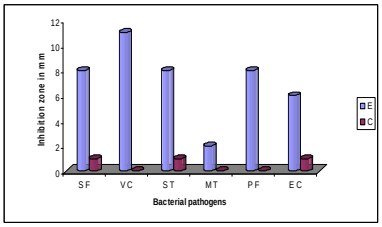

Fig. 6 F3

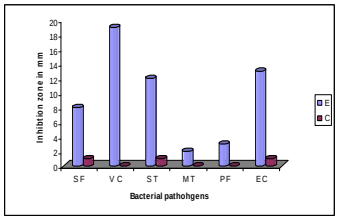

Fig. 7 F4

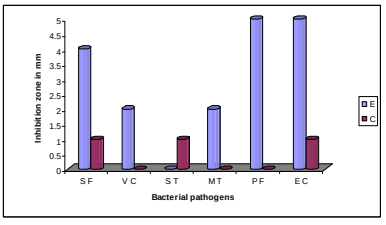

Fig.8 F5

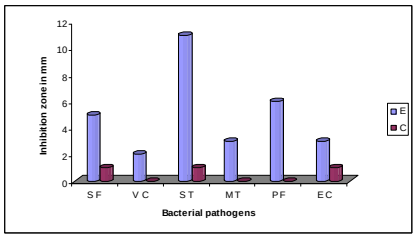

Fig. 9 Antifungal activity of crude Ethyl acetate extract of P. persica

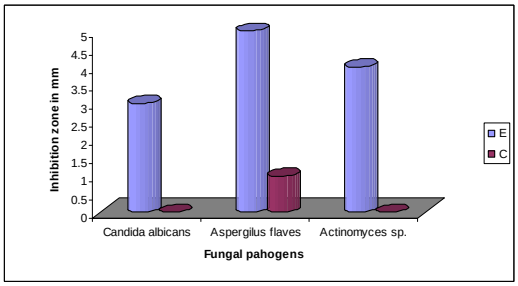

Fig. 10 Antifungal activity of P. persica of crude Chloroform extract

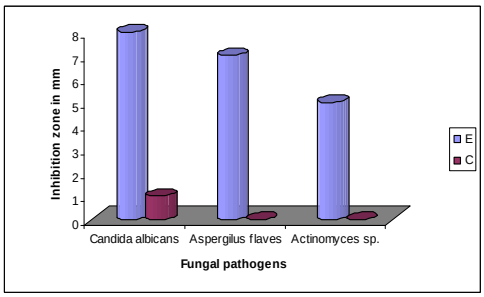

Fig. 11 Antifungal activity of crude methanol extract of *P. persica*

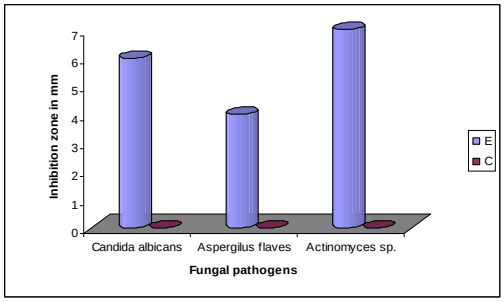

Fig. 12 Antifungal activity of Column fractionated extract of *P.persica* F1

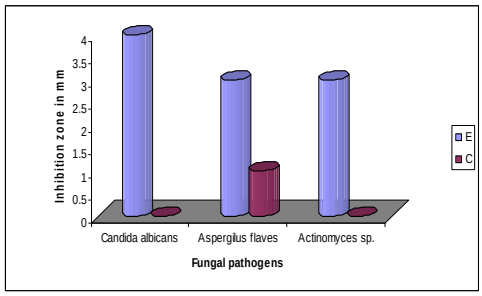

Fig. 13 F2

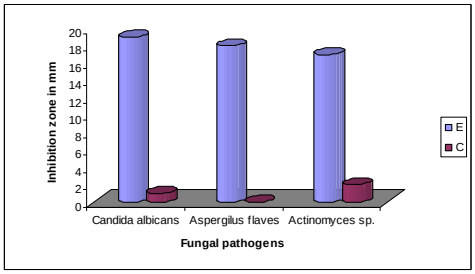

Fig. 14 F3

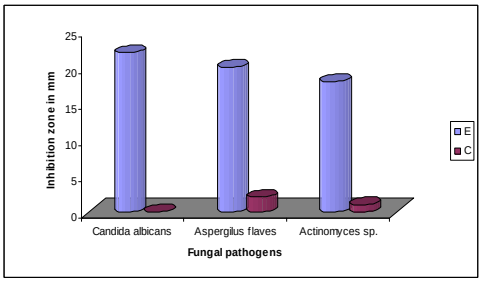

Fig. 15 F4

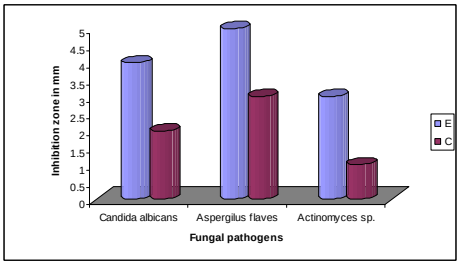

Fig. 16 F5

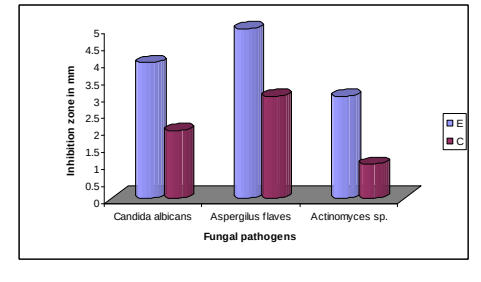

Fig. 17 Chromatogram of column extract of F2 fraction of *P. persica* by GC-MS

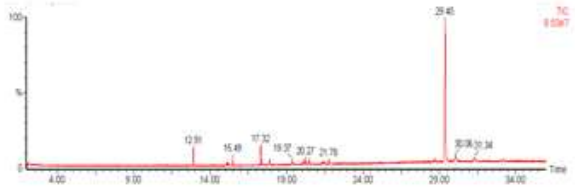

Fig. 18 Chromatogram Components in F2 fraction of *P. persica*

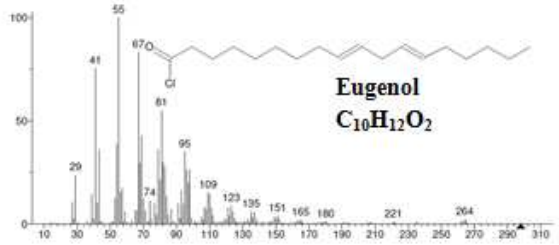

Fig. 19 Chromatogram of column extract (F3) of *P. persica* by GC-MS

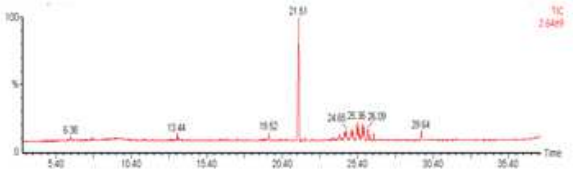

Fig. 20 Chromatogram Components in F3 fraction of *P. persica*

9,12-Octadecadienoyl chloride, (Z,Z)-
$C_{18}H_{31}ClO$

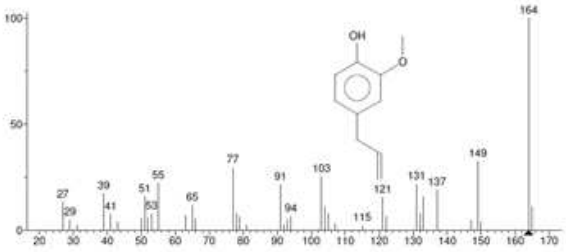

Fig. 21

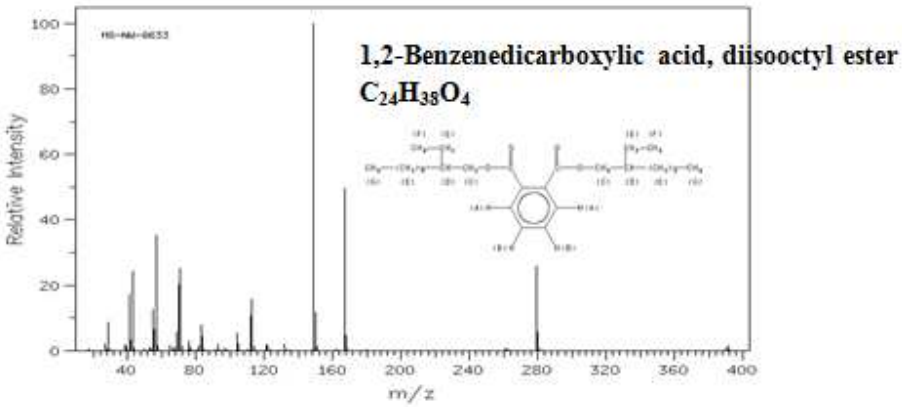

Fig. 22

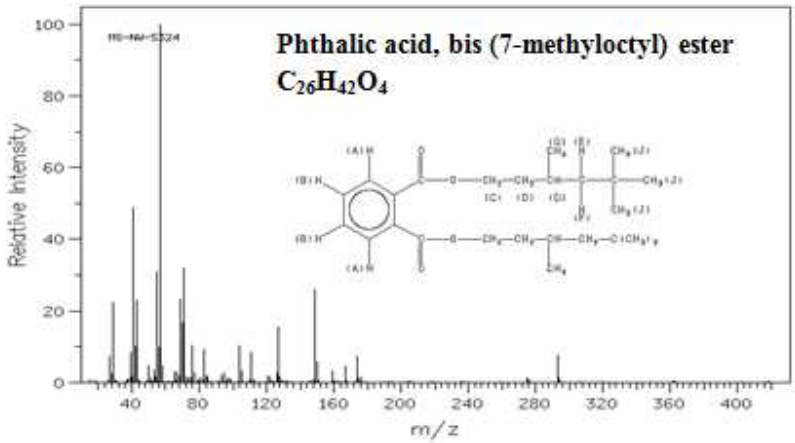

Table 1 Showing various activities of identified compounds by GC-MS in test animal *P.persica*

P.persica **ethyl acetate: chloroform (1:1) (F2)**

S. No.	RT	Name of the compound	Peak Area %	Nature of compound	*Activity
1	17.90	9,12-Octadecadienoyl chloride, (Z,Z)-	2.34	Chloride compound	Antimicrobial
2	20.17	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.52	Linoleic acid ester	Anti-inflammatory, Cancer preventive, Antihistaminic, 5-Alpha reductase inhibitor, Antiarthritic, Anticoronary, Insectifuge
3	29.40	Cholest-5-en-3-ol (3á)-, carbonochloridate	72.31	Steroid	Antimicrobial, Antiasthma, Anti-inflammatory

Table 2 **chloroform (F3)**

S. No.	RT	Name of the compound	Peak Area %	Compound Nature	*Activity
1	6.38	Eugenol	1.35	Phenolic compound	Analgesic, Antibacterial, Anticonvulsant, Anti-inflammatory, Antipyretic, Antisalmonella, Antistaphylococc, Antiseptic
2	13.44	Dibutyl phthalate	1.40	Plasticizer compound	Antimicrobial, Antifouling
3	21.51	1,2-Benzenedicarboxylic acid, diisooctyl ester	79.95	Plasticizer compound	Antimicrobial, Antifouling
4	25.36	Phthalic acid, bis(7-methyloctyl) ester	9.70	Plasticizer compound	Antimicrobial, Antifouling

CONCLUSION

In the present study the activity of the *P. persica* was found to be high from its broad spectral activity which is considered to be a promising source of antifungal source, will definitely expected to be a potential producer of new antibiotics. Extraction of this biologically active compound from marine resources will certainly be helpful in protecting and treating various fungal diseases in human beings.

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