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Research Article

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Antimicrobial substances of potential biomedical importance from *Babylonia zeylanica*

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ABSTRACT

The present study has been aimed to ascertain the antimicrobial activity of extracts from B. zeylanica, against various pathogenic bacterial and fungal strains using the agar disc diffusion method and the most probable antimicrobial compound by GC-MS study. The crude methanolic extract of B. zeylanica the range varied from 6mm to 15mm. Of the five column chromatographic fractions, maximum number of pathogens was inhibited by F4 and F5 fractions and the highest activity was exhibited against A. hydrophila (9mm) and the least against V.cholerae 0139 (3mm) in F4 fraction and in F5 against E.coli and S.typhi (9mm) and V.cholerae classical (4mm) respectively. MIC of F4 fraction showed maximum inhibitory zone against A. hydrophila (8mm) at 100mg concentration and at the same concentration in F5 fraction S. typhi and E.coli (8mm) respectively. Effects of crude extracts of B. zeylanica on fungi range varied from to 3mm (P. oxallcium, A. terreus, Trichoderma sp. and Rhizopus sp.) to 6mm (A. fumigatus). Among the fractions, F4 and F5 showed little activity against the tested fungi. The GC/MS study of B.zeylanica reveals the probable antimicrobial compounds such as 2-piperidinone, undecanal, 2-methyl-, 1,2-benzenedicarboxilic acid, diisooctyl ester, 3-hexadecyloxycarbonyl -5- (2-hydroxyethyl) -4- methylimidazolium ion, a-D-mannofuranoside, farnesyl-, trans-a-bergamotene, diethyl phthalate,phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-(S), and 2,2-dimethyl-6- methylene-1-(3,5-dihydroxy- 1-pentanyl) cyclohexane-1-perhydrol. Of the five fractions, the number of fractions active was F4 and F5 and A. hydrophila, E.coli and S. typhi were the most susceptible pathogens in concern with the Babylonia extract and the antimicrobial compounds identified by GC-MS were responsible for the inhibition of tested pathogens.

Key words: *Babylonia zeylanica*, antimicrobial activity, inhibitory zone, GC-MS

INTRODUCTION

Most of the pathogens are increasingly resistant to the major classes of the routinely used anti-biotic. Many diseases were initially controlled exclusively by the use of anti-microbial drugs. The massive use of anti microbial 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 anti microbial 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. Natural products isolated from marine organism's increases rapidly and hundreds of new compounds being discovered every year [2]. Marine invertebrates offer good source of potential anti microbial drugs [3, 4]. Among the invertebrates, the

molluscs are very good source for biomedical important products [5]. Many classes of molluscs exhibits bioactive compounds like antitumour, antileukemic, antibacterial, cytotoxic, anti-inflammatory and antiviral properties have been reported [6,7]. Discovered bioactive compounds in molluscs were identified and they are presented specific types of activities [8]. The presence of antimicrobial activity in molluscs has been reported from the egg mass and purple fluid of the seahare *Dobella auricularia* [9]. These reports suggest that molluscs are a 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 ascertain the antimicrobial activity of extracts from *B. zeylanica,* against various pathogenic bacteria and fungal strains.

EXPERIMENTAL SECTION

Collection and Preparation of samples

The molluscs *B.zeylanica* was collected from muddy bottom of deep waters of Gulf of Mannar, near by Theraspuram Tuticorin, situated in the South east coast of India, during April 2010 to December 2010. 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 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 10 different bacterial pathogens, viz.,*Aeromonas hydrophila, Bacillus cereus, Escherichia coli, Pseudomonas aerogenosa, , Salomnella typhi, Shigella flexneri, Vibrio cholera 0139, Vibrio cholera classical, Vibrio cholerae 01790 and Vibrio cholerae EITOR*. These clinical strains were obtained from Basic Biomedical sciences, Bharathidasan University, Trichy. The fungi pathogens *Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Aspergillus fumigatus, Fusarium moniliforme, Trichoderma sp. Penicillium citrinum, Penicillium oxallicum and Rhizopus sp.* were obtained from TNAU, Coimbatore. Pathogenic fungal strains were inoculated in potato dextrose agar medium and incubated at 48 hrs.

Anti microbial susceptibility Assay:

In vitro anti bacterial activity was assayed by the disc diffusion method [10]. Invitro antifungal activity was determined using the techniques of Kelman *et al.,* [11].After initial screening the extracts showing broad spectrum were fractionated using normal phase silica gel 160-120 mesh(Glaxo, Bombay) column chromatography with low polar to high polar solvent. Hexane: Chloroform (F1); Chloroform (F2); Benzene (F3); Benzene: Methanol (F4), and Methanol F5. Eluted fractions were assayed for antimicrobial activity following the above mentioned disc diffusion method. To estimate the minimum inhibitory concentration of extract three different concentrations such as 1, 10 and 100 mg/ml were prepared for all five fractions and they were tested against the pathogens. After 24hsr for bacterial and 48 hrs for fungal inhibition, the plates were removed and observations were made for inhibition zone against the pathogens respectively. The most potent fractions of test molluscs were subjected to GC-MS to characterize the possible compounds responsible for antimicrobial activity. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC 20i auto sampler and gas chromatography interfaced to a mass spectrometer(GC-MS) instrument.

RESULTS

The crude methanol extract of *B. zeylanica* the range varied from 6mm (*P. aerogenosa* and *V. cholerae classical*) to 15mm (*A. hydrophila and S. typhi*) (plates 1-4). Of the five column chromatographic fractions, maximum number of pathogens was inhibited by F4 and F5 fractions (plates5-7). The highest activity of F4 fraction was exhibited against *A. hydrophila* (9mm) (plate 5) and the least against *V.cholerae* 0139 (3mm). F5 fraction showed maximum activity against *E.coli* and *S.typhi* (9mm) (plate 6, 7) and the lowest against *V.cholerae* classical (4mm). MIC of various fractions revealed that *A. hydrophila* showed maximum inhibitory zone of 8mm at 100mg (plates 8- 10) concentration when treated with F4 and at the same concentration in F5 fraction *S. typhi and E.coli* growths were arrested with the formation of 8 mm (plates 11- 14).

Antibacterial activities of crude and various fractions of *Babylonia zeylanica* against pathogens

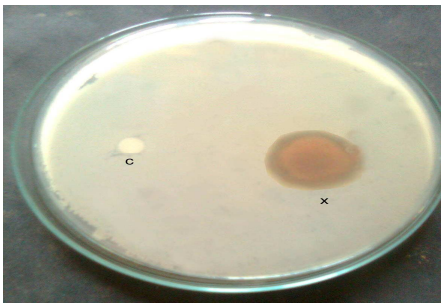


Plate 1: Crude *Bacillus cereus*

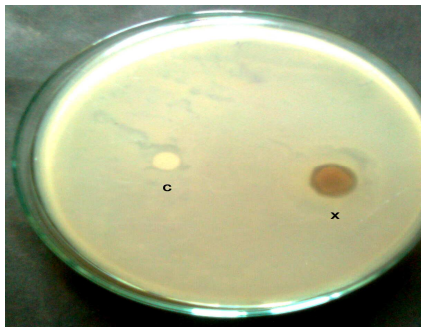


Plate 2: *Aeromonas hydrophila*

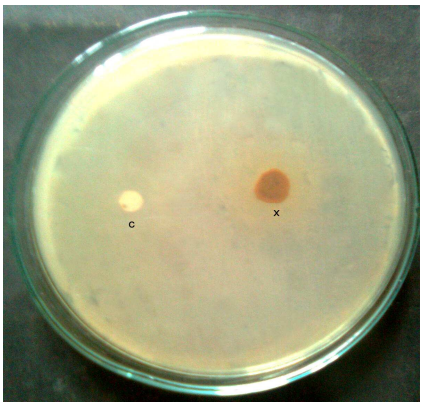


Plate 3: *Salmonella typhi*

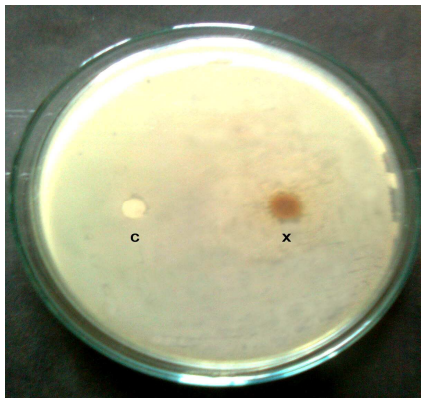


Plate 4: *Pseudomonas aerugenosa*

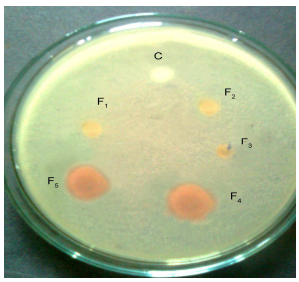


Plate 5
F4 *Aeromonas hydrophila*

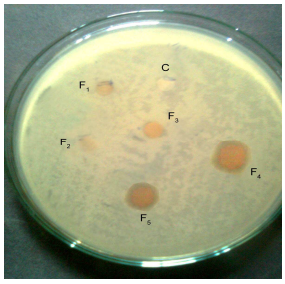


Plate 6
F5 *Escherichia coli*

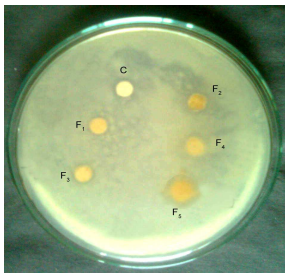


Plate 7
Salmonella typhi

Plate 8: Antibacterial activities of minimum inhibitory concentration of *Babylonia zeylanica* (F4) against pathogens *Aeromonas hydrophila*

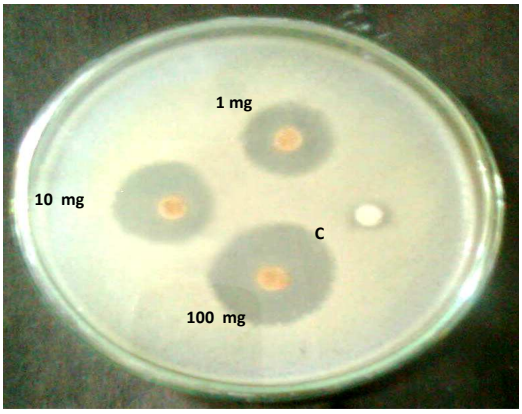


Plate 9: *Pseudomonas aerugenosa*

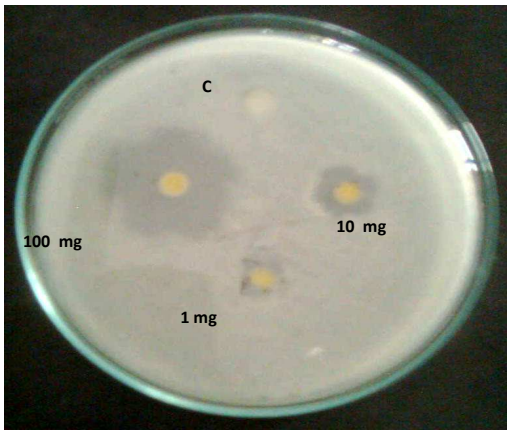


Plate 10: *Salmonella typhi*

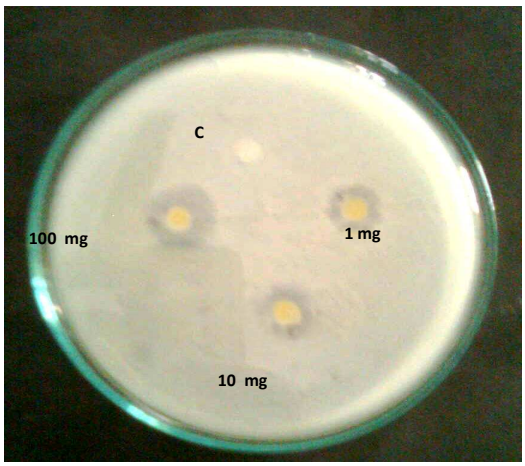


Plate 11: (F5) *Salmonella typhi*

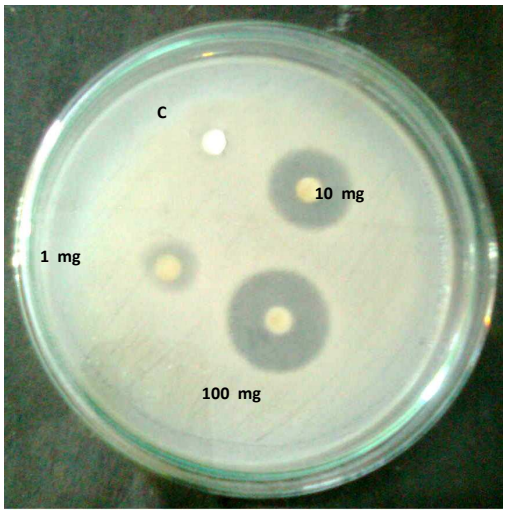


Plate 12: *Escherichia coli*

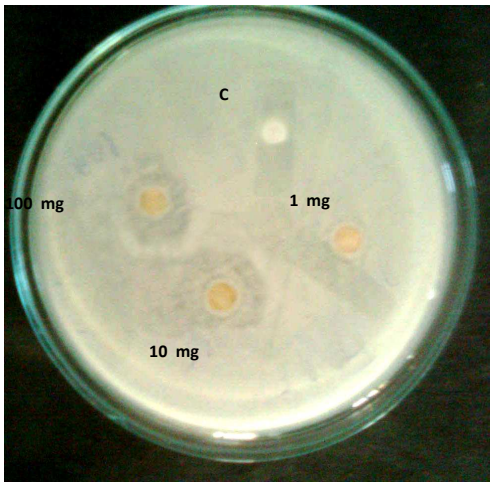


Plate 13: *Pseudomonas aerugenosa*

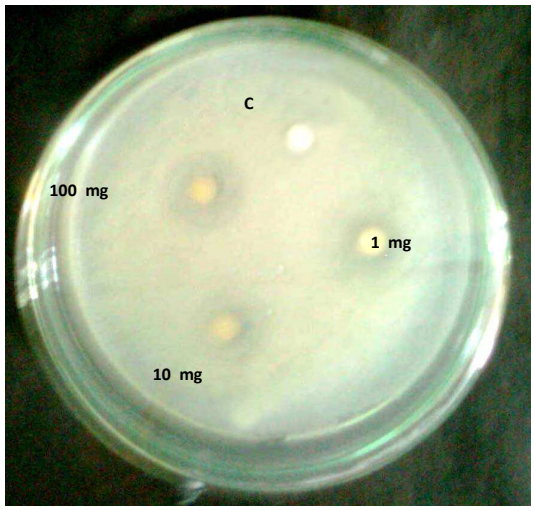


Plate 14: *Aeromonas hydrophila*

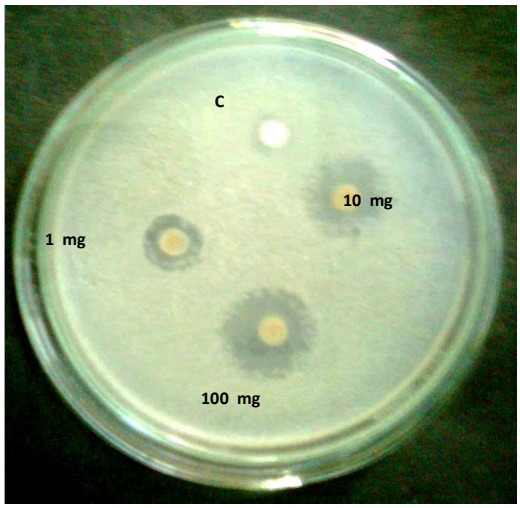
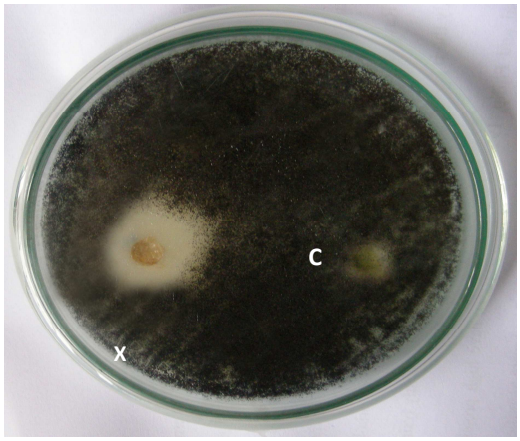


Plate 15: Antifungal activity of crude methanol extract of *B.zeylanica* against *Aspergillus fumigatus*



Effects of crude extracts from *B. zeylanica* range varied from to 3mm (*P. oxallcium, A. terreus, Trichoderma sp.* and *Rhizopus* sp.) to 6mm (*A. fumigatus)* (plate 15*).* Among the fractions, F4 and F5 showed little activity against the tested fungi. Maximum activity was noticed against *A. terreus* (4mm) and minimum in *A. flavus and P. oxallicum* (2mm) in F4 fraction. Highest inhibition zone was developed against *F.moniliforme* (5mm) and the lowest in *Trichoderma sp., P. citrinum and Rhizopus sp.*(2mm) in F5 fraction. Since the activity among fractions was traceable MIC was not warranted.

Marine molluscan extract are usually complex mixtures of bioactive molecules mainly proteins, peptides and sterols. The GC/MS study of *B.zeylanica* reveals (Table 1&2) the probable antimicrobial compounds such as 2-piperidinone, undecanal, 2-methyl-, 1,2-benzenedicarboxilic acid, 3-hexadecyloxycarbonyl -5- (2-hydroxyethyl) -4-methylimidazolium ion, a-D-mannofuranoside, farnesyl-, trans-a-bergamotene, diethyl phthalate,phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-(S), and 2,2-dimethyl-6- methylene-1-(3,5-dihydroxy-1-pentanyl) cyclohexane-1-perhydrol which are responsible for the inhibition of *A.hydrophila, E.coli,* and *S. typhi*.

DISCUSSION

Several marine molluscan extracts possessed broad spectrum antibacterial activities affecting the growth of bacteria, fungi and yeasts [6, 7]. Antibacterial activity has previously been described in a wide range of molluscan species [12, 13]. The antibacterial activity of common marine molluscs from Parangipettai coast was studied and reported that the methanolic extract of molluscs exhibited significant activity against *Escherichia coli* [7]. This finding

corroborate the results of the present study since methanol extract of *B. zeylonica* showed pronounced activity against *E. coli.* The inhibitory action of the methanol fractions of *Perna viridis* was reported [14] against bacterial and fungal strains. Similar result was also reported in four bivalves against few pathogens and found that methanol extracts showed significant activity against *Bacillus subtilis*[15]. The crude methanol extracts of *Cypraea errones* exhibited promising results for antibacterial activity [7]. The methanol extract of *Didemnum candidum* maximum antibacterial activity was noted against *Salmonella typhi, Pseudomonas aeroginosa* and *Vibrio cholerae[16]*.

In the present investigation crude and column fractionated extracts of *B. zeylonica* had no distinct antifungal activity against most of the pathogenic fungi tested. Moderate antifungal activity from the extract of various bivalve molluscs was reported [7]. The fungi are more resistant than the bacterial strains to the tested compound this could be leads to the nature of fungal cell wall made up of chitin which is relatively resistant including microbial decomposition [17]. Lesser degree of inhibition by the column fractionated extracts in comparison to the crude in *B. zeylonica* in fungi could be concluded that the active compound may be degraded or modified during the fractionation process as reported by Cannell [18]

Table 1 Activity of components identified in the column fractions of *B.zeylanica* by GC-MS

B.zeylanica **(F_4) Benzene : Methanol**

S.No	RT	Name of the compound	Peak Area %	Compound Nature	*Activity
2	4.30	2-Piperidinone	6.55	Alkaloid	Antimicrobial Anti inflammatory
3	9.19	Diethyl Phthalate	0.73	Plasticizer compound	Anti fouling Antimicrobial
4	12.67	9-Hexadecenoic acid, methyl ester, (Z)-	1.83	Oleic acid ester	Anti inflammatory, Antiandrogenic, Cancer preventive,
6	16.34	9-Octadecenamide, (Z)-	0.94	Amide compound	Antimicrobial
7	17.70	Undecanal, 2-methyl-	0.94	Aldehyde compound	Antimicrobial
9	21.27	1,2-Benzenedicarboxylic acid, diisooctyl ester	2.46	Plasticizer compound	Anti fouling Antimicrobial
10	22.34	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl) -4-methylimidazolium ion	3.88	Nitrogen compound	Antimicrobial
11	26.49	á-D-Mannofuranoside, farnesyl-	18.12	Terpene compound	Antimicrobial Anti inflammatory Anticancer

Table 2

B.zeylanica **(F_5) Methonal**

	RT	Name of the compound	Peak Area %	Compound Nature	*Activity
1	8.22	trans-à-Bergamotene	2.35	Sesquiterpene	Antimicrobial Anti inflammatory
2	9.19	Diethyl Phthalate	4.70	Plasticizer compound	Antimicrobial Antifouling
3	11.02	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	2.98	Phenolic compound	Antimicrobial Anti inflammatory Antioxidant
4	21.68	1,2-Benzenedicarboxylic acid, diisooctyl ester	78.27	Plasticizer compound	Antimicrobial Antifouling
5	30.38	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl] cyclohexan-1-perhydrol	5.78	Alcoholic compound	Antimicrobial

The present findings of compounds identified by GC/MS and HPLC study of *B.zeylanica* are in agreement with Emiliano Manzo 19] who reported that two novel triterpenoids, aplysoils A and B, ßEtzionin a tyrosin derived compound exhibited antibacterial activity against *Bacillus subtilis.* An antimicrobial peptide from the seminal plasma of the mud crab *Scylla serrata* was isolated [20]. A terpenoid caribenol A and B inhibited the growth of *Mycobacterium tuberculosi* [21]. Three antimicrobial linear ß-substituted sesterterpenes were isolated from nudibranch *Hypselodoris capensis* [22]. On account of their broad spectrum antimicrobial activity and the previous

available literature *B.zeylaica* was expected to be a new potential producer of new antibiotics.

CONCLUSION

Of the five fractions, the number of fractions active was F4 and F5 respectively in the column as well as in MCH. *A. hydrophila, E.coli and S. typhi* were the most susceptible pathogens in concern with the *Babylonia* extract and the antimicrobial compounds identified by GC-MS were responsible for the inhibition of tested pathogens.

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