Int J Pharm Bio Sci 2016 April; 7(2): (B) 395 - 405

Original Research Article Microbiology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

SCREENING OF MARINE BROWN ALGAE ASSOCIATED POTENTIAL BACTERIA PRODUCING ANTAGONISTIC BIOACTIVE COMPOUNDS AGAINST UTI PATHOGENS

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ABSTRACT

The discovery, development and clinical use of antibiotics during the 20th century decreased substantially the morbidity and mortality from bacterial infections. Marine bacteria and fungi are of considerable importance as new promising sources for a huge number of biologically active products. For the present study multi antibiotic resistant pathogens from UTI patients were chosen. The brown alga *Turbinaria conoides* associated endophytic and epiphytic bacterial strains were isolated and screened for potential antagonistic activity against UTI pathogens using the Cross streak assay. Three potent antagonistic marine bacteria inhibiting growth of all tested pathogens were mass cultured for ethyl acetate extraction and concentration of extracellular bioactive products in broth medium. The S6-EP marine isolate inhibited the Gram positive and negative UTI pathogens of this study with minimum inhibitory concentration in the range of 250 µg – 500 µg/ml. The bioactive secondary metabolites of concentrated extract of marine strain S6-EP were characterized by Gas chromatography and Mass spectrometry (GC - MS).

KEYWORDS: **Brown sea algea, *Turbinaria conoides,* GC-MS.**

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INTRODUCTION

The life threatening microbial infections are a menace today in spite of the vast many antimicrobial agents available for the treatment and management of infectious diseases. Urinary tract infection (UTI) is the second most common infectious diseases ranking next to upper respiratory tract infection. There are estimated 150 million UTIs per year worldwide. UTIs are the most common bacterial infections affecting humans throughout their lifespan. UTIs encompass a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis. More than 95% of UTI are caused by single bacterial species *E. coli* which is the most frequently infecting organisms. However, many other bacteria can also to cause the infection for example *Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia* and *Neisseria spp.* The treatment of urinary tract infections (UTI) with antibiotics is commonly used, but recurrence and antibiotic resistance have been growing and concerning clinicians[1]. Traditionally, the treatment of UTIs consists of antimicrobial therapy administered in a regimen appropriate to the clinical situation, frequently administered temporally either as a prophylactic to reduce the risk of UTI or as a therapeutic approach. Several antibiotics such as penicillins, sulfanilamide, nitrofurantoin and cephalexin have been used in therapy. This initial therapy is based on knowledge of the predominant pathogens and their antimicrobial susceptibility. Misuse of the antibiotics is leading to the development of antibiotic resistance[2]. According to World Health Report of Infectious Diseases, overcoming antibiotic resistance is the major issue for the next millennium. To prevent antibiotic resistance, new compounds which are not based on existing synthetic antimicrobial compounds have to be used. The severe side effects associated with the use of commercially available antibiotics, especially in immune compromised patients and children, strongly recommend the use of natural products for the treatment of infections. Biologically active compounds from natural resources have always been of great interest to scientists working on different diseases[3]. The production of microbial inhibitory substances from marine species was noted as early as in 1917. Since then, several studies have been carried out to identify novel antimicrobial compounds from marine sources. The ability to produce antimicrobial substances may be significant not only as a defensive instrument for the aquatic plants but also as a good source of the new bioactive compounds from a pharmaceutical point of view[4]. In recent years, many bioactive compounds have been extracted from various marine living beings like tunicates, sponges, soft corals, bryozoans, sea slugs and marine organisms. A number of compounds originating from marine organisms have been reported to possess *in vitro* and *in vivo* antimicrobial activity. Competition among microbes for space and nutrients in the marine environment is a driving force behind the production of such precious antibiotics and other useful pharmaceuticals. Researches in marine organism can provide a very good source for drug discovery program[5]. It was surprising to find that many bioactive compounds, reported from marine living forms are produced by their microbial symbionts. It has been proven that seaweed-associated bacteria are involved in metabolite production originally attributed to the host[6]. Microbes can exist as symbionts living in association with many marine algae as epiphytes or endophytes. Various microorganisms have been found in brown algae too. The symbiotic microbial community is highly novel and diverse[7,8]. Endophytes were shown to produce compounds that target pathogens of the host plant. Many bacterial epiphytes of algal family represent a rich source of toxins, signaling compound, and metabolites with an array of biological activities[9]. Because seaweed mariculture for chemical compound production is technically challenging, the epiphytic or endophytic bacteria may represent a more promising and manageable source of bioactive metabolites. In addition, bacterial bioactive compounds may represent a more promising and easier to handle source of natural products with biotechnological applications in comparison with seaweed-derived compounds[10,11,12]. These associated bacteria of marine algae can be possibly utilized as resources for antimicrobial agent against the newly emerging problematic multidrug resistant pathogens responsible for human infections.The increasing prevalence of antibiotic resistant bacteria, escalating costs of antibiotic therapy and unsatisfactory therapeutic alternatives in recurrent UTIs have stimulated an interest in novel, non-antibiotic based methods for preventing and controlling of the infection[13]. Since many common UTI pathogens have now developed resistance to a number of prevailing antibiotics, the present study was undertaken to evaluate antimicrobial efficacy of the epiphytic and endophytic organisms isolated from *Turbinaria conoides* against the multidrug resistant UTI pathogens isolated from patients in Salem hospitals.

MATERIALS AND METHODS

(i) *Collection of Brown Algae*

Turbinaria conoides- B:265 (CSMCRI) species of brown algae were collected from Mandadapam area situated in the Palk Strait region of Tamil Nadu. The Latitude and Longitude of Rameswaram mandapam is 9.274897 and 79.123696 respectively. The sea weed sample was used for the isolation of endophytic & epiphytic bacterial strains.

(ii) *Isolation of Epiphytic and Endophytic Organisms*

For isolation of epiphytes, 50 gms of sea weed as single piece was taken in a 100 ml flask containing 50 ml sterile Zoble marine broth, placed in rotary shaker at 200 rpm for 5 minutes and one ml of the wash water was inoculated onto sterile Zobell marine agar medium in triplicates. Thoroughly washed seaweed sample was homogenized using 10 ml sterile sea water, centrifuged at 6000 rpm for 15 minutes. One ml supernatant was

serially diluted upto 10^6 dilution and each dilution was plated on sterile Zobell marine agar medium. The plates were maintained in triplicates. Uninoculated control plates were also maintained. Incubation were done similar to endophyte isolation procedure. The colonies were observed after 24 hrs of incubation at 37°C[14].

(iii) *Isolation of Urinary Tract Pathogens*

For the present study, the urine samples from **30** UTI patients were collected of which 18 were outpatients and 12 were inpatients**.** The patients were suspected of acute cystitis or pyelonephritis as of the prevailing symptoms. Samples were collected before the start of empirical therapy with antibiotics at SKS hospital, one of the busy hospitals in Salem. Patients were provided with sterile bottles for mid-stream urine collection with appropriate instruction to avoid contaminations. Five of the inpatients had indwelling catheters. Urine specimens from catheterized patients were obtained by fresh catheterization. The appropriately labeled samples were brought to the lab at the earliest and immediately processed for culturing and staining tests. Urine culture was used as the reference method for definitive diagnosis of UTIs. A 10 µl aliquot of each urine sample was cultured on blood agar and Mac Conkey agar plates. Plates were incubated in an aerobic atmosphere at 37 °C for 18 h. Plates showing negative results were discarded if they remained negative following a further 24 h incubation. When bacterial growth was present, colonies on blood agar were counted. A specimen was considered positive for a UTI in view of the number of yielded colonies (≥ 10^5cfu/mL with single type of colony morphology). If more than two colony morphologies were present, the urine sample was rejected. Pure culture techniques were done for further bacterial identification process. The genus and species level identifications were done using standard staining, culturing and biochemical test procedures.

(iv) Antibiotic Disc Diffusion Method

The panel of antibiotics routinely used for empirical treatment of UTI as per CLSI guidelines taking into consideration the local sensitivity pattern were selected for the study. Also some of the antibiotics were decided based on the type of pathogens isolated from the patients. The isolated UTI pathogens were subjected to antibiotic susceptibility test using Kirby Bauer disc diffusion method on Mueller Hinton agar as described by the Clinical and Standard Laboratory Method**.** The test inoculums in broth cultures were adjusted to McFarland 0.5 standards. The inoculum size was 0.1ml per plate. The plates were aerobically incubated at 35±1ºC, 18±2h. Zone edges were read as the point showing no growth by viewing from the back of the plate against a dark background illuminated with reflected light. The pathogen specific MAR index (Multiple antibiotic resistance value), the resistivity pattern and the sensitivity percentage of antibiotics were calculated[15]. The MAR value is a ratio of the number of effective antibiotics to that of all effective antibiotics tested against the pathogens. The resistivity pattern was obtained by multiplying the MAR index with 100. The pathogenic strains showing resistance to most of the tested antibiotics were selected for further experiment. The sensitivity percentage of antibiotics were calculated by finding the ratio of number of isolates showing sensitivity to the total number of isolates tested against an antibiotic.

(v) Cross Streak Assay

The antagonistic activity of brown algae associated bacterial strains was tested by using cross streak assay against the isolated UTI pathogens. Single streak (4-6mm in diameter) of the pure culture marine strains were streaked on the surface of Muller Hinton Agar plates. On obtaining a ribbon-like growth, the overnight culture of antibiotic resistant pathogens were streaked with equal gaps perpendicularly to the original streak of marine isolates and incubated at 37±2°C. The inhibition was measured after 24 hours in the case of bacteria. A control plate was also maintained without inoculating isolates to assess the normal growth of bacteria[16].

(vi) Mass Cultivation of selected strain and isolation of bioactive compound

Bacterial strains that showed maximum antagonistic effect against tested pathogens were subjected for mass cultivation in Zobell marine broth medium. After an incubation period of 48 hrs, the culture broth was filtered using Whatman filter paper No 1. Filtrate was mixed with equal volume of ethyl acetate (v/v) in a separating funnel and shaken well and allowed to stand undisturbed for 15 minutes. The lower aqueous phase was discarded and upper solvent phase was concentrated in a vacuum evaporator at room temperature for 24 hrs to obtain the powder form of crude extract that was stored in refrigerator for further analysis[17].

(vii) MIC and MBC

For MIC determination 0.5 ml of various concentrations of extracts was prepared with Dimethyl sulphoxide (DMSO) and mixed with 0.5 ml of nutrient broth to give a final concentration of 63, 125, 250,500, 1000, 1500, 2000µg crude extract powder per ml. An aliquot of 50µl of overnight broth cultures of selected UTI pathogens were inoculated into each of the extract dilutions separately. Un-inoculated 1 ml nutrient broth served as negative control. The whole setup in duplicates was incubated at 37°C. Growth was examined on the basis of turbidity. The MIC was the lowest concentration of the extract without any visible growth after 24 hours of incubation. To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds and to confirm whether the MIC concentration is Bactericidal or Bacteriostatic, the minimum bactericidal concentration (MBC) was determined. The cultures from all MIC test tubes were sub cultured on to the sterile Mueller – Hinton agar plates**.** The MBC dilution was noted[18,19].

(viii) GC–MS Analysis

The crude extract was quantified using a gas chromatograph (Shimadzu QP2010) equipped with a VF-5 ms column (diameter 0.25 mm, length 30.0 m, film

thickness 0.25 µm) mass spectrometer (ion source 2000C; Electron Ionization mode operated at 70 eV), programmed at temperature 40–65°C with a rate of 4°C/min; Injector flow rate was 200°C; carrier gas was Helium (He) 99.9995% purity, column flow rate 1.51 ml/min, injection mode-split.

RESULTS

1.Epiphytic and Endophytic Organisms isolated from brown seaweed

From the brown alga *Turbinaria conoides,* about 36 bacterial species were identified using Zobell marine agar medium. Among them 22 epiphytes (S1-EP to S22- EP) were isolated with wash water as inoculum and 14 endophytes (S23-ED to S36- ED) were isolated from the tissue homogenate of brown alga. The bacterial strains were isolated based on the differences in the colonial morphological appearance and appropriately labeled. Twenty three percent of the colonies were pigment producers. The gram positive colonies constituted 58% and of them were Gram negative organisms. The genus and species level identification of the marine bacterial strains was not done in this study. The study was aimed to focus on the biological property namely the antagonistic activity of the marine isolates against the human UTI pathogens. Pure cultures of morphologically distinct colonies were maintained under appropriate labelling using which their antagonistic activity for selected pathogens was checked further.

2. UTI Test Pathogens

In blood agar the total plate count values of above 10^4 cfu / ml in all the processed urine samples indicated significant bacteriuria in patients. From the urine culture results the single pathogenic non-fastidious bacterial strains per sample were identified using staining, motility and biochemical test results (Table-1). They belonged to the genus *Escherichia, Klebsiella, Streptococci* and *Pseudomonas*. The most predominantly isolated organism was *Escherichia coli 60%* (18 nos) followed by *Klebsiella* 23% (7 nos), *Enterococci* 13% (4 nos) and *Pseudomonas* 4% (1 no.).

Table 1
Biochemical test results for urine sample associated pathogens

Organisms	Gram Staining	Motility	I	MR	VP	C	TSI	CA	OX
Escherichia coli	'-'ve short rod	Actively Motile	+	+	-	-	+	+	-
Pseudomonas aeruginosa	'-'ve slender rod	Swarming Motile	-	-	-	+	+	-	+
Klebsiella pneumoniae	Short thick '-'ve rod	Non Motile	-	-	+	+	+	+	-
Streptococcus faecalis (Enterococci)	'+'ve rod	Non Motile	-	-	+	-	-	-	-

I - Indole, MR - Methyl Red, VP - Voges Proskauer, C - Citrate, TSI -Triple Sugar Iron , CA – Catalase, X – Oxidase, Positive, [-] Negative

3. Antibiotics selected for Disc diffusion assay

The testing was designed such that the total number of antibiotics tested against each bacterial genus was 10 numbers each. A battery of antibiotics was selected for Disc diffusion assay for the isolated Enterobacteriaceae organisms, *Enterococci* and Non enterobacterial *Pseudomonas* sp. The broadspectrum fluoroquinolones antibiotics like Ciprofloxacin, Levofloxacin, and Norfloxacin commonly used for therapeutics of isolated pathogens were included in the sensitivity test. The antibiotics specific for Enterobacteriaceae or *Enterococci* - Ampicillin, Fosfomycin, Nitrofurantoin and the antibiotics specific for Enterobacteriaceae and *Pseudomonas* - Piperacillin, Amikacin, Aztreonam, Imipenem were included. The antibiotics like Chloramphenicol, Streptomycin, Penicillin-G, Vancomycin for Enterococci and the antibiotics like Ceftazidime, Cefepime, and Gentamycin (HLG) for *Pseudomonas* were also used in this study. The detail of antibiotic types and concentrations used against each pathogen was given in Table-2.

Table 2
Antibiotics selected against UTI pathogens

S.No	Antibiotic Class	Antibiotic Name & Conc. Used	Tested Pathogens				CLSI Std: Zone Size Interpretation
			E.coli	***Kleb.***	***Ent.***	***Pseu.***	
1	Fluoroquinolones	Ciprofloxacin (CIP-5mcg)	+	+	+	+	R ≤ 28 mm, S ≥ 29 mm I= -
2	Fluoroquinolones	Levofloxacin (LE-5mcg)	+	+	+	+	R ≤ 19 mm, S ≥ 20 mm I= -
3	Fluoroquinolones	Norfloxacin (NX-10mcg)	+	+	+	+	R ≤ 28 mm, S ≥ 29 mm I= -
4	Penicillin	Ampicillin (AMP-10mcg)	+	+	+	-	R ≤ 15 mm, S ≥ 19 mm I= 18-20 mm
5	Fosmidomycin	Fosfomycin (FO-200mcg)	+	+	+	-	R ≤ 15 mm, S ≥ 21 mm I=16-20 mm
6	Miscellaneous	Nitrofurantoin (NIT-300mcg)	+	+	+	-	R ≤ 12 mm, S ≥ 16 mm I= 13-15 mm
7	Miscellaneous	Chloramphenicol(C-30mcg)	-	-	+	-	R ≤ 12 mm, S ≥ 18 mm I= 13-17
8	Aminoglycoside	Streptomycin (HLS-300mcg)	-	-	+	-	R ≤ 11 mm, S ≥ 15 mm I= 12-14
9	Penicillin	Penicilln-G (P-10 units)	-	-	+	-	R ≤ 28 mm, S ≥29 mm I= -
10	Glycopeptide	Vancomycin (VA-30mcg)	-	-	+	-	R ≤ 14 mm, S ≥ 17 mm I= 15-16
11	Penicillin	Piperacillin (PI-100mcg)	+	+	-	+	R ≤ 14 mm, S ≥ 18 mm I= 15-17 mm
12	Aminoglycoside	Amikacin (AK-30mcg)	+	+	-	+	R ≤ 14 mm, S ≥ 17 mm I= 15-16 mm
13	Monobactum	Aztreonam (AT-50mcg)	+	+	-	+	R ≤ 13 mm, S ≥ 16 mm I= -
14	Carbapenem	Imipenem (IPM-10mcg)	+	+	-	+	R ≤ 13 mm, S ≥ 16 mm I= 14-15 mm
15	3rd - Generation Cephalosporin	Ceftazidim (CZX-30mcg)	-	-	-	+	R ≤ 17 mm, S ≥ 21 mm I=18-20
16	4th - Generation Cephalosporin	Cefepime (CPM-30mcg)	-	-	-	+	R ≤ 14 mm, S ≥ 18 mm I= 15-17
17	Aminoglycoside	Gentamycin (HLC-120mcg)	-	-		-(HLG)	R ≤ 6 mm, S ≥ 10 mm I= 7-9
		Total	10	10	10	10	

Note: '+' – Antibiotic selected. E.coli - Escherichia coli, Ent. –Streptococcus faecalis, Kleb - Klebsiella pneumoniae, Pseu –Pseudomonas aeruginosa

4. Results of Antibiotic susceptibility test

The zones of inhibition to different antibiotics exhibited by the pathogens on Mueller Hinton agar plates were measured. The zone size (zone diameter in mm) interpretations were made based on the CLSI Guidelines. The resistivity pattern of pathogenic strains to tested antibiotics was tabulated in Table 3. The *E. coli* strain EC-6 showed maximum resistance % or MAR index against the tested antibiotics. The resistivity pattern for *Klebsiella, Enterococci* and *Pseudomonas* were also provided in the Table 3. The percentage sensitivity of the antibiotics to the tested pathogenic strains was given in Table 4. The resistivity pattern ranged from 20-70%. None of the antibiotics in this study exhibited 100% sensitivity against the tested pathogens on the whole and the sensitivity percentage ranged from 3 – 86%, the antibiotic Fosfomycin showing the highest percentage.

Table 3
Antibiotic Resistivity % Pattern for UTI Pathogenic Strains

S.No	Strain.Nos	Resistivity% Pattern of Antibiotics
1	EC-1	40
2	EC-2	50
3	EC-3	40
4	EC-4	30
5	EC-5	20
6	EC-6	70
7	EC-7	30
8	EC-8	50
9	EC-9	40
10	EC-10	50
11	EC-11	60
12	EC-12	50
13	EC-13	30
14	EC-14	50
15	EC-15	60

S.No	Strain.Nos	Resistivity% Pattern of Antibiotics
16	EC-16	50
17	EC-17	50
18	EC-18	40
19	KL-1	40
20	KL-2	70
21	KL-3	40
22	KL-4	40
23	KL-5	20
24	KL-6	60
25	KL-7	30
26	EN-1	40
27	EN-2	30
28	EN-3	30
29	EN-4	50
30	PS-1	50

EC- E.coli, KL- Klebsiella, EN- Enterococci, PS- Pseudomonas

Table 4
Sensitivity Percentage of Antibiotics for Test Pathogens

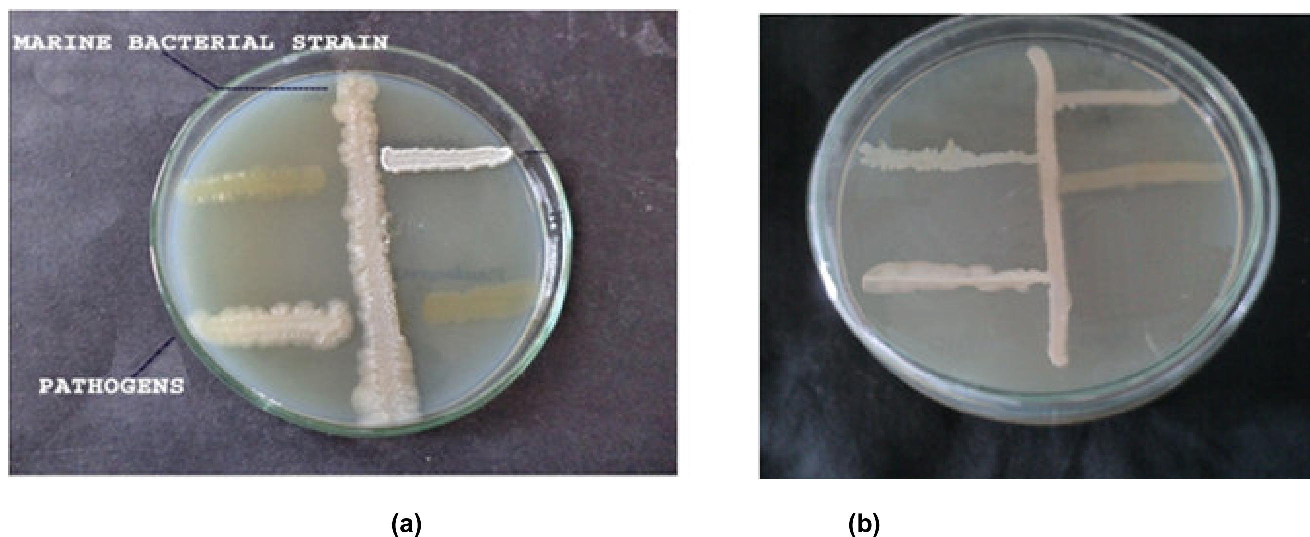
Antibiotics	% Sensitivity to Pathogenic Strains
CIP	30
LE	50
NX	40
AMP	23
FO	86
NIT	56
PI	40
AK	33
AT	43
IMP	60
C	6
HLS	6
P	10
VA	13
CZX	3
CPM	3
HLG	3

Among the many isolates of pathogenic genus the ones with maximum MAR index EC-6 (0.7), KL-2 (0.7), EN-4 (0.5) and the single *Pseudomonas* isolate PS-1 (0.5) were tested for their response to antagonism by marine brown algae associated bacterial strains.

5. Results of Antagonistic activity using Cross streak assay

Cross streak assay was performed on Mueller-Hinton agar plates with the selected UTI pathogens having comparatively higher MAR index. The antagonistic activity of all the thirty six bacterial isolates from *Turbinaria conoides* were tested against the four human UTI pathogens. From the test results it was noted that only twelve of the endo and exo symbiotic strains showed antagonism against one or other tested pathogenic bacteria (Table-6). Antagonism was decided upon the absence of growth of pathogens in the area adjacent to marine isolate. After overnight incubation period in cultured plates, if growth of the pathogens were found in the vicinity of algal associated marine bacterial strain, it indicated absence of antagonism (Fig 1 a & b). Among the isolated marine organisms 32% of epiphytes and 36% of endophytes showed antagonism towards the tested pathogens.

Figure 1
a & b: Cross streak assay plates with antagonistic and non-antagonistic activity



(a) **(b)**

Table 6
Cross streak assay: Antagonism of marine isolates to UTI pathogens

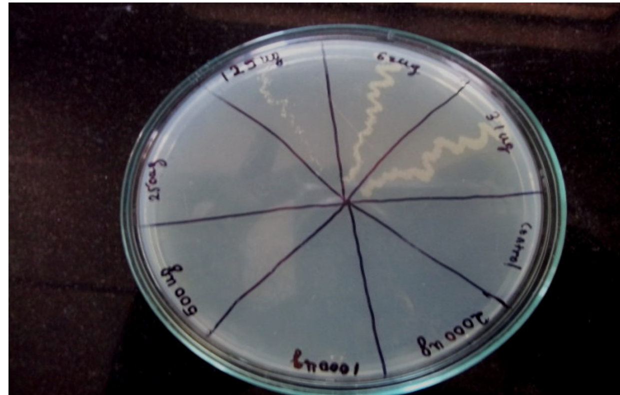
S.No	Strain No	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
1	S 1-EP	+	-	+	-
2	S 6-EP	+	+	+	+
3	S 10-EP	+	+	-	-
4	S 11-EP	+	+	-	-
5	S 15-EP	+	-	+	+
6	S 17-EP	+	-	-	-
7	S 20-EP	-	-	-	+
8	S 25-ED	+	-	-	-
9	S 26-ED	+	-	-	-
10	S 30-ED	+	+	+	+
11	S 35-ED	-	+	-	+
12	S 36-ED	+	+	+	+

[+] Presence of bacterial growth inhibiting activity
[-] Absence of bacterial growth inhibiting activity

6. Results of MIC and MBC

Three bacterial species S 6-EP, S 30-ED and S 36-ED which exhibited prominent antagonism against all the four tested pathogens were mass cultured and proceeded with ethyl acetate extraction experiment. The filtrates of culture broths of the three strains after ethyl acetate extraction and concentration were separately analyzed for MIC and MBC by standard methods. With an increase in extract concentrations a proportionate decrease in the bacterial growth was seen. It was noted that the extract of strain S 6- EP, exhibited an MIC value of 250µg against three tested pathogens *Enterococci, Escherichia coli* and *Klebsiella*. They were bactericidal to *Pseudomonas* at 500 µg concentration. Whereas the extracts of other strains (S 30-ED and S 36- ED) were inhibitory at higher concentration ranges of 500µg to 2000µg. The strain S 30- ED extract showed an MIC value of 1000µg against *Klebsiella* and *Enterococci.* Their MIC value against *E. coli* was 500µg. They were inhibitory to *Pseudomonas* at a concentration of 2000 µg. The strain S 36- ED extract has MIC value of 500 µg against *Enterococci* and 1000µg against *E. coli* or *P. aeruginosa*. They inhibited *Klebsiella* at an MIC value of 2000 µg. When a loopful of culture from all MIC test tubes was streaked on to the specific marked area on Mueller Hinton agar MBC plates, growth was observed in plates inoculated from MIC test experiment inoculums with concentrations below the MIC value determined. This confirmed that the turbidity observed in MIC test experiment tubes is due to bacterial growth only (Fig 2). The inhibitory action of bioactive compound in the MIC tube and other higher concentrations must have caused death and total lysis of pathogens giving clarity in broth tubes.

Figure 2
MBC assay plate of S-6 Ep extract for Klebsiella



In the present study the MBC values corresponded to the MIC values. No growths were observed in the plates corresponding to the tubes with MIC value and other higher dilutions indicating the bactericidal nature of the extracts. The strain S 6- EP had effective antagonistic activity with comparatively lower MIC values towards the tested UTI pathogens. To find out the possible active antibacterial component in the ethyl acetate extract of the strain SD-6 EP, the concentrated extract was subjected to GC-MS analysis with standard specifications which provided a characteristic chromatogram (Fig-3). From the chromatogram results many biological compounds were found to be present which were known for their antibacterial activity. The identified bioactive compounds are listed in Table-7. The known applications of the chromatographed compounds are described in Table-8. Interestingly, some of our resultant spectra compounds exhibited important biomedical features described in Table 8.

Figure 3
Chromatogram of SD-6 ED ethyl acetate extract sample

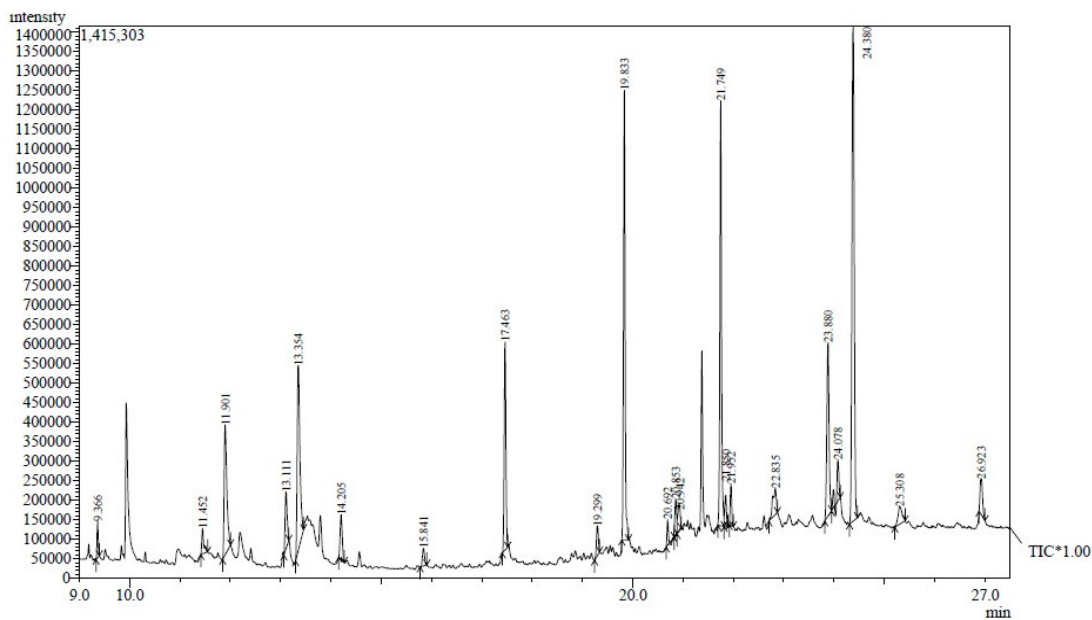


Table 7
GC-MS analysis of bioactive compound of Strain S6-EP

S.No	Peak Value	Compound name	Molecular formula	Molecular weight
1	9.366	Phenol, 2,4-Bis(1,1-Dimethylet	$C_{33} H_{56}N_{40}S_2$	588.95
2	11.452	n-Pentadecanol	$C_{15}H_{32}O$	228.41398
3	11.901	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexah	$C_8H_{16} O_2$	144.211395
4	14.205	Nonadecanol-1	$C_{19}H_{38}$	266.5050
5	15.841	1-Octadecanol	$CH_3(CH_2)_{17} OH$	270.49
6	17.463	1-Heptacosanol	$C_{27}H_{56}O$	396.73
7	19.299	3-Benzoyl-2-Tert-Butyl-4-Isop	C_6H_6	189.05
8	19.833	Nonadecyl trifluoroacetate	CF_3COOA	220.88
9	20.692	Hexadecanoic acid, 2,3-bis[(trimethylsil	$C_{25} H_{54} O_4S_{12}$	474.8649
10	20.853	Pregnane, Silane Deriv	SIH_4	534.09
11	20.942	Docosyl trifluoroacetate	$C_2HF_3O_2$	114.02
12	21.850	9-Tricosene, (Z)-	$C_{23} H_{46}$	322.61
13	21.952	5-Fluoro-2-trifluoromethylbenzoic acid,	$C_8 H_4 F_4 O_2$	208.11
14	22.835	Nonadecyl pentafluoropropionate	$C_{13}H_{22}O$	194.076
15	23.880	Heptacosyl heptafluorobutyrate	$C_{11} H_{18}N_2O_2$	210.2728
16	24.078	Decanedioic acid, bis(2-ethylhexyl) este	$C_{12}H_{11}N$	169.23
17	24.380	2,6,10,14,18,22-Tetracosahexaene, 2,6,	$C_{20}H_{40}O$	206.43
18	25.308	Triacontyl pentafluoropropionate	$C_{15}H_{24}$	204,657

Table 8
Application of the Chromatogram compounds

S.No	Compound Name	Applications
1	Phenol, 2,4-Bis(1,1-Dimethylet	Antimicrobial activity
2	n-Pentadecanol	Industrial Chemicals: Alcohols
3	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexah	Chemical Research
4	Nonadecanol-1	Anti-inflammatory activity
5	1-Octadecanol	Surfactant for the Functionalization of Carbon Nanotubes.
6	1-Heptacosanol	Antimicrobial and antioxidant
7	3-Benzoyl-2-Tert-Butyl-4-Isop	Anti-tuberculosis , insecticidal
8	Nonadecyl trifluoroacetate	Antioxidant and anti-inflammatory
9	Hexadecanoic acid, 2,3-bis[(trimethylsil	Anti-inflammatory, antiallergic, anticancer,
10	Pregnane, Silane Deriv	Nematicidal , anticancer
11	Docosyl trifluoroacetate	Anti-inflammatory, anti-atherosclerotic
12	9-Tricosene, (Z)-	Immune Co-stimulatory enhancer
13	5-Fluoro-2-trifluoromethylbenzoic acid,	Antioxidant
14	Nonadecyl pentafluoropropionate	Surfactant to the functionalization of carbon nanotubes.
15	Heptacosyl heptafluorobutyrate	Anti inflammatory
16	Decanedioic acid, bis(2-ethylhexyl) este	Anti microbial activity
17	2,6,10,14,18,22-Tetracosahexaene, 2,6,	Anti-oxidant, antibacterial activity
18	Triacontyl pentafluoropropionate	Anti tumor activity

DISCUSSION

UTI are one of the dangerous issues today. The untreated lower urinary tract infections have the danger of developing into an upper urinary tract infection. The complications increases further if upper tract infections cannot be managed, leading to sepsis or systemic disease which may become life threatening. In this study the organisms isolated from UTI patients included *E. coli*, *Klebsiella*, *Enterococci* and *Pseudomonas* which were also the most commonly isolated pathogens in similar studies by others. CAUTI pathogens in this study showed Multiple antibiotic resistance (MAR) index of ≥ 0.5. Usually the antibiotic resistant nature and biofilm forming ability of CAUTI pathogens pose problems during treatment. All isolated pathogens in this study showed varying degrees of resistance to antibiotics. CLSI guidelines recommend that antibiotics loosing sensitivity to > 10% of local UTI causative agents should not be considered for empirical therapy. From this point of view most of the tested antibiotics in this study could be ruled out. None of the antibiotics tested showed 100% sensitivity. Most tested antibiotics showed a multiple antibiotic resistance value near to 1 that indicates that the antibiotic is inefficient. Compared to other antibiotics Fosfomycin was found much inhibitory to 0.9% of the tested pathogens in this study. The inhibitory mechanisms of antibiotics vary *viz.,* inhibiting the cell wall synthesis, hindering transcription or translation, and nucleic acid binders etc. But pathogens have developed counter mechanisms to overcome antibiotic effects by way of mutations or gene transfers. Hence antibiotics with altered antibacterial mechanisms are the need of the hour. The highly potential antibiotics in reserve for UTI are very few and costly too. Many of them are not free from side effects and do not confirm to 100% safety index. Pharmaceutical industries are giving importance to the compounds derived from marine algae and associated organisms[20]. When compared to cumbersome contamination risk associated mariculturing, the sea

weed associated heterotrophic marine bacteria with their simple and faster multiplication process, can be cultivated in large amounts inexpensively. Hence recently research is focused towards seaweed-associated bacteria instead of their hosts. In the effort of screening potential microbes from marine brown alga *Turbinaria conoides*, epiphytes were isolated more in numbers than endophytes. The possible reason could be the internal scavenging mechanism of the algal system that keeps endophyte numbers in check. Since the sea water is rich with microbial population, it is natural that the isolated epiphyte bacterial population outnumbered the endophytes[21]. The number of seaweed-associated bacteria exceeds those in the surrounding seawater by 100 – 10000 times. There is no single medium that supports the growth of all available bacteria and in this study only 36 bacteria were isolated on the whole. An epiphyte on brown alga *Turbinaria*, SP 6- EP, was screened to be a potential antagonizer of the human UTI pathogens in this study. The reason could be attributed to the fact that due to the prevailing competitive atmosphere, the seaweed associated epiphytic bacteria should produce antibiotic substances that prevent colonization of the algal surface by other bacterial competitors and pathogens[22]. In a similar attempt to screen natural bioactive compounds against UTI pathogens[23], had found crude clove extracts to be effective for pathogens like *E.coli*, Klebsiella and Pseudomonas, at a concentration of 5 mg/ ml where 50 µl of preparation containing 1 g in 10 ml was made. In the present study antibacterial effect of crude marine bacterial compounds are exhibited even at a lower concentration of 2 mg/ml against the pathogens like *E.coli*, Klebsiella and Pseudomonas. Marine bacterial compounds can be successful candidates in combating UTI pathogens provided they do not cause human cytotoxicity. From the MIC and MBC results obtained it was noted down that pronounced inhibition was conferred by SP 6- EP marine strain with MIC value of 250 µg to 500 µg /ml against the tested multidrug resistant UTI pathogens. Their inhibitory action against gram negative and positive bacteria is suggestive of best antibacterial potential of the bioactive principles of SP 6-EP *Turbinaria conoides* associated marine strain extract. When choosing Fosfomycin as an effective drug of this study, the MIC value of 250 to 500 µg/ml of tested marine bacterial extract is much higher than the Fosfomycin MIC value of ≤ 32mg/L based on EUCAST guidelines. MIC breakpoints are yet to be set by CLSI for Fosfomycin. However, the MIC value of the test extract can be reduced if the bioactive compound in extract is used in the purified form. If MIC value is lower, less amount of drug is required to inhibit/kill bacteria. Presence of pathogen inhibitory action in the S6-EP marine strain suggests that they may aid in providing a pharmacophore which could be used for the development of new drug with novel mechanisms of action. This strain was found to be a Gram negative pigment producer. Further identification of the organisms will be the future scope of this study. Majority of spectral compounds revealed in chromatogram show antimicrobial, antitumor and anticancer properties. In the present investigation GC-MS analysis of crude extract of active strain S6 strain demonstrated interesting 18 compounds. Majority of them shows antimicrobial, antiphrastic, antitumor and anticancer properties. Different phenols and fatty acids such as Phenol, 2, 4 – Bis (1, 1 - Dimethylet, Pyrrolo [1, 2 - A] Pyrazine - 1, 4 - Dione, 1 - Octadecanol, HexadecanoicAcid, 2, 3 - Bis [(Trimethylsil)] with antimicrobial activity and pharmaceutical importance were identified. Among the several compounds identified by gas chromatogram from marine brown algal associated bacterial strain, it is possible to carry out the screening of specific bioactive compounds with anti-pathogenic activity which will be taken up for further studies by the researchers.The GC-MS results indicate that such active marine microalgae can be used to get biocide for controlling infectious human pathogens. SD-6 EP strain could serve a source of potential biocide to be exploited further for safer application in therapeutics with reduced dosage related toxicity. But their efficacy has to be scientifically tested to the same degree as the drugs already in medical usage. A further study on these compounds can prove the relation between these metabolites and the antimicrobial properties of *Turbinaria conoides* associated SD-6 EP strain.

CONCLUSION

Due to the development of antibiotic resistance among the most common UTI pathogens there is an immediate demand for alternative treatments or natural remedies for Urinary Tract Infection. As resistance among uropathogens causing community acquired uncomplicated cystitis or pyelonephritis is increasing, algal associated bacterial extracts may become more useful, particularly if no other oral agents with in vitro activity are available. The identification of the strain and purification of the bioactive compound for structural elucidation will be the future scope of this research study.

ACKNOWLEDGEMENT

Authors express their thanks to S.K.S Hospital and Periyar University authorities for providing the facilities for this research work.

CONFLICT OF INTEREST

No conflict of interest.

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