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COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF SEAWEED *(Sargassum muticm)* AND FRESHWATER WEED (*Spirodela polyrrhiza)*

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ABSTRACT

Development of new drugs is needed to resist the situation of diseases caused by drug resistant bacteria for public health safety. Natural resource is a big source to find candidates having antibacterial activity and aquatic weed is such a natural resource possessing such activity. The current study was aimed to determine the effectiveness of sea weed (*Sargassum muticum*) and fresh water weed/duckweed (*Spirodela polyrrhiza)* against six bacterial isolates *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas luteola* and *Bacillus subtilis.* The potency of methanol and ethanol extracts of these weeds was compared to determine the best candidate of weeds in inhibiting bacteria. Both agar well diffusion method and micro dilution was done to observe the antibacterial activity. Ethanol extract of *Sargassum muticum* worked best against *Pseudomans aeruginosa* (30mm zone of inhibition) and no activity against *Bacillus subtilis*. Methanol extract of the same *Sargassum muticum* showed less activity compared to ethanol extract except for *Bacillus subtilis* where it showed 21mm zone of inhibition. Ethanol and methanol extracts of *Spirodela polyrrhiza* showed less antibacterial activity against the bacteria compared to *Sargassum muticum.* They showed no antibacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus*. On average, the extracts impart a significant antibacterial activity against these six bacteria which are resistant to several antibiotics. Even one of them (*Escherichia coli*) is resistant to 4th generation cephalosporin but still fairly susceptible for extracts. The antibacterial properties of these marine and freshwater weeds can be subjected to develop new therapeutics to inhibit the resistant bacteria.

Keywords: Seaweed; freshwater weed; resistance; antibacterial activity

INTRODUCTION

Scientific community is searching for alternative components which can fight against antibiotic resistant infectious microorganisms **(Das etal., 2012; Jeyasree et al., 2012).** Their interest is now rising towards the medicinal plants and herbs again to seek for bioactive components for treatment of infections. Plant originated medicines are safer for patients, better bioavailability,no significant side effects with minimal toxicity **(Pradhan et al., 2009; Singh et al., 2010; Ekpo et al., 2011; Thanigaivel et al., 2015;).** Water originated plants contain antimicrobial and phytochemical components like phenols, phycobiline, phenolic compounds, phlorotannins, acrylic acid, halo-genated ketones and alkanes, fatty acids,steroids, flavonoids, terpenoids, cyclic polysulphides, polysaccharide etc. **(Abd El-Baky et al., 2008; Khan et al., 2014; Kavita et al., 2014; Hossain et al., 2018).** Freshwater plants often are used for treating skin diseases and inflammation with potent antioxidant activity. Marine weeds (seaweeds) often impart antibacterial, antiviral, antitumor and antioxidant properties according to different studies **(Abulude et al., 2007; Patra et al; 2008; De Fallcio et al., 2010; Kim et al., 2011; Devi et al., 2011).**They also contain vitamins like A, B1, B12, C, D, E, panthothanic acid, riboflavin, folic acid niacin, including minerals like P, Ca, K, Na **(Prakash et al., 2018).** In the current study, both seaweed (*Sargassum muticum*)and fresh water weed (*Spirodela polyrrhiza*) was subjected to the determination of antibacterial efficacy against several pathogenic bacteria and then their result was compared to understand which weed possesses the better antibacterial activity. *Sargassum muticum*, a brown algae contains higher phlorotannin contents among the marine phenolic compounds which have been found during a study **(Kostić et al., 2012).** Fresh water weed, *Spirodela polyrrhiza* as already been known in the scientific community for its bioremediating ability by removing heavy metal, arsenic as well excess nutrients **(Rahman et al., 2007; Devaleena et al., 2011; Loveson et al., 2013).** So this study was aimed to find out antibacterial activity of both *Sargassum muticum* and *Spirodela polyrrhiza* from marine and freshwater origin and compare their results accordingly Similar studies with *Spirodela polyrrhiza* had also been conducted in India and China **(Das et al., 2010; Qiao et al., 2011; Daboor et al., 2012).**

MATERIAL AND METHODS

Collection of samples

Brown algae (*Sargassum muticum*) was selected to study in the current experiment and was collected from the Saint Martin Island of Bangladesh in South Asia. And *Spirodela polyrrhiza* was selected as a candidate from the freshwater weed which is commonly known as duckweed. Both of these samples were collected in the month of January, 2019 in sterile bags along with some water to make them alive and fresh and taken back to the laboratory as soon as possible. The name of these water weeds were confirmed after close observation of their physical features.

Sample processing

After taking to the laboratory, the samples were washed thoroughly first with tap water and then with distilled water several times to wash out the salt, mud, dirt or any other impurities. After that the samples were shed dried for three to four days to make it all dry followed by blending to get fine powder of these samples. These dried powder samples were then stored in airtight jars until further processing for the assessment of antibacterial activity.

Preparation of extracts

20g of each seaweed and duckweed powdered samples were taken and mixed with 80ml of 95% ethanol and methanol separately in sterilized glass bottles and incubated at 37^{o}C in shaking condition for 48 hours. After 48 hours shaking, the ethanol and methanol extracts of both samples were filtered through sterilized cheesecloth and then through Whatman filter paper respectively. Extracts were then kept in evaporator for evaporation of the alcohol and the concentrates were then collected as stock solution and kept at 4^{o}C until use.

Test organisms

Six different bacterial isolates were collected from different sources to analyze the antibacterial activity of the seaweed and freshwater weed. The bacteria isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, Klebsiella pheumoniae (collected from clinical laboratory), *Staphylococcus aureus* (collected from the collection of clinical freeze dried laboratory isolates from Department of Microbiology, Stamford University Bangladesh), *Bacillus subtilis* (collected from environmental soil sample). All the microorganisms were biochemically identified by standard biochemical tests.

Antibiotic susceptibility of the tested organisms

Susceptibility of the bacterial isolates to the antibiotics was determined by agar disc-diffusion method called the Kirby Bauer method. Antibiotics used in this study included 25 antibiotics like Amikacin 30 µg.disk^{-1}, Cefepime 30 µg.disk^{-1},

Gentamycin 10 µg.disk^{-1}, Colistin 10 µg.disk^{-1}, Nitrofurantoin 100 µg.disk^{-1}, Cephradine 30 µg.disk^{-1}, Ceftriaxone 30 µg.disk^{-1}, Rifampin 5 µg.disk^{-1}, Novobiocin 30 µg.disk^{-1}, Nalidixic Acid 30 µg.disk^{-1}, Amoxicillin 30 µg.disk^{-1}, Ampicillin 10 µg.disk^{-1}, Cefepime 30 µg.disk^{-1}, Cefoperazone, Tigecycline, Piperacillin/Tazobactam 100/10 µg.disk^{-1}, Meropenem 10 µg.disk^{-1}, Imipenem 10 µg.disk^{-1}, Ciprofloxacin 5 µg.disk^{-1}, Trimethoprim/Sulfamethoxazole, Entrapenem 10 µg.disk^{-1}, Cefpodoxime 30 µg.disk^{-1}, Neomycin 30 µg.disk^{-1}, Erythromycin 15 µg.disk^{-1}, Tetracycline 30 µg.disk^{-1}. A suspension of *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, Klebsiella pheumoniae, *Staphylococcus aureus*, *Bacillus subtilis* were prepared after standardizing with 0.5 McFarland solution for the study. Lawn of the bacterial suspension was prepared using sterile cotton swab evenly over the entire surface of Mueller-Hinton agar plates separately for each bacteria. Using sterile forceps antibiotic discs were placed aseptically over the surface of the inoculated plates and incubated at 37ºC for 8 hours. After incubation the plates were examined for the presence of the zones of inhibition and measured in mm.

Antibacterial activity of the extracts

Bacterial suspensions were prepared by inoculating the isolates into normal saline and incubated at 37ºC. The cultures were ready when they matched with the McFarland turbidity standard (10^8 CFU/ml) (Jorgensen et al.,1999). Bacterial lawn was prepared on the Muller Hinton agar media using sterile cotton swab separately for each kind of bacteria. Ethanol and methanol extracts of *Sargassum muticum* and Spirodelapolyrrhiza placed over the media. 10 µl, 30 µl extracts (impregnated in sterile discs), 50 µl and 100 µl extracts (in well on the media) were used for antibacterial study. Plates were then kept in refrigerator for better absorption for 20 to 30 minutes in upright position and then incubated at 37ºC for 24 hours. After incubation plates were observed for the presence of zone of inhibition and measured in mm.

Determination of MIC (Minimum Inhibitory Concentration)

Minimum inhibitory test was done using 96 well plates. Nutrient broth was taken as base for dilution. At first 100 µl broth was added equally in wells from 1 to 11. From well number 2 to 11, extracts were added sequentially from 10 µl to 100 µl. Number 1 well was kept free of any extract solution to compare the growth of bacteria without any extracts as positive control. After that, 100 µl of bacterial suspension was added equally in all the wells from 1 to 11. Ethanol and methanol extracts were applied in two separate 96 well plates and for these two extracts five bacterial suspensions (*Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, Klebsiella pheumoniae, *Staphylococcus aureus*, *Bacillus subtilis*) were inoculated into the wells of separate rows. The plates were then covered and incubated at 37ºC for 24 hours. In each plate different concentrations (10 µl to 100 µl) of the extracts were used for all of the five bacteria. After incubation our aim was to determine the well where no visible growth of bacteria was found after comparing with the growth of bacteria in well 1.

Determination of MBC (Minimum Bactericidal Concentration)

For minimum bactericidal concentration, loop full sample from the wells starting from the MIC concentration to the last well (well no. 11) were taken and streaked over nutrient agar media. After 24 hours incubation at 37ºC, the presence of the growth of bacteria on the streaking line was observed. The concentration of extract where no growth bacteria was first appeared can be determined as the minimum bactericidal concentration.

RESULTS

Bacteria from different origin were subjected to know the effects of the extracts on both clinical pathogenic bacteria and environmental bacteria (Table 01).

Table 01 Biochemical identification of bacteria collected from different sources.

Test	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Pseudomonas luteola*	*Escherichia coli*
Gram negative bacteria				
APPA	-	-	-	-
H_2S	-	-	-	-
BGLU	(-)	+	-	-
ProA	+	+	+	-
SAC	-	+	-	-
ILATk	+	+	+	+
GlyA	+	+	-	-
O129R	+	+	+	ND
ADO	-	+	-	-
BNAG	-	-	-	-
dMAL	-	+	-	+
LIP	-	-	-	-
dTAG	-	-	-	-
AGLU	-	-	-	-
ODC	-	-	-	+
GGAA	+	-	-	ND
PyrA	+	+	-	-
AGLTp	-	(+)	-	-
dMAN	-	+	-	+
PLE	-	+	-	-
dTRE	-	+	-	+
SUCT	+	+	+	+
LDC	-	+	-	+
IMLTa	+	+	+	ND
IARL	-	-	-	-
dGLU	+	+	+	+
dMNE	+	+	+	+
TyrA	+	+	+	-

Test	*Bacillus subtilis*	*Staphylococcus aureus*
Gram positive bacteria		
APPA	-	-
H_2S	-	-
BGLU	-	-
ProA		+
SAC	+	+
ILATk	+	+
NAG	ND	+
O129R	ND	+
NOVO	ND	-
LAC	-	-
dMAL	ND	+
BGURr	ND	-
AGLU	-	-
dGAL	ND	+
dRIB	+	-
PyrA	-	+
dRAF	-	-
dMAN	-	+
dXYL	-	ND
dTRE	+	+
dMNE	-	+
TyrA	ND	-
URE	+	-
AGAL	-	-
BGAL	+	-
dSOR	-	-
PHOS	+	+
BGUR	+	-

CIT	+	+	+	-
NAGA	-	+	-	-
IHISa	-	+	+	-
ELLM	-	-	-	ND
dCEL	-	+	-	-
GGT	+	+	+	-
BXYL	-	+	-	-
URE	-	+	-	-
MNT	+	+	+	-
AGAL	-	+	-	+
CMT	+	-	+	+
ILATa	+	+	+	ND
BGAL	-	+	-	+
OFF	-	+	-	+
BAlap	+	ND	ND	-
dSOR	-		-	+
5KG	-	+	-	+
PHOS	-	-	-	-
BGUR	**(-)**	-	-	-

ADONITOL=ADO, L-Pyrrolydonyl-ARYLAMIDASE=PyrA, L-ARABITOL=IARL, D-CELLOBIOSE=dCEL, BETA-GALACTOSIDASE=BGAL, H_2S production=H_2S, BETA-N-ACETYL-GLUCOSAMINIDASE=BNAG, GlutamylArylamidasepNA=AGLTp, D-GLUCOSE=dGLU, GAMMA-Glutamyl-TRANSFERASE=GGT, FERMENTATION/GLUCOSE=OFF, BETA-GLUCOSIDASE=BGLU, D-MALTOSE=dMAL, D-MANNITOL=dMAN, D-MANNOSE_dMNE, BETA-XYLOSIDASE=BXYL, BETA-Alanine arylamidasepNA=BALap, L-Proline ARYLAMIDASE=ProA, LIPASE=LIP, PALATINOSE=PLE, Tyrosine ARYLAMIDASE=TyrA, UREASE=URE, D-SORBITOL=dSOR, D-TAGATOSE=dTAG, D-TREHALOSE=dTRE, CITRATE(SODIUM)=CIT, MALONATE=MNT, 5-KETO-D-GLUCONATE=5KG, L-LACTATE alkanization=ILATk, ALPHA-GLUCOSIDASE=AGLU, SUCCINATE alkanization=SUCT, Beta-N-ACETYL-GALACTOSEAMINIDASE=NAGA, ALPHA-GALACTOSIDASE=AGAL, PHOSPHATASE=PHOS, Glycine ARYLAMIDASE=GlyA, ORNITHINE DECARBOXYLASE=ODC, LYSINE DECARBOXYLASE=LDC, L-HISTIDINE assimilation=lHISa, COUMARATE=CMT, BETA-GLUCORONIDASE=BGUR, O/129 RESISTANCE (comp. vibrio.)=O129R, Glu-Gly-Arg-ARYLAMIDASE=GGAA, L-MALATE assimilation=lMLTa, L-LACTATE assimilation=lLATa, D_XYLOSE=dXYL, BETA GLUCORONIDASE=BGURr, D-GALACTOSE=dGAL, LACTOSE=LAC, N-ACETYL-D-GLUCOSAMINE=NAG, NOVOBIOCIN RESISTANCE=NOVO, D-RAFFINOSE=dRAF, D-TREHALOSE=dTRE, D-RIBOSE=dRIB.
ND= Not done

To know the antibiotic susceptibility towards the commonly prescribed antibiotics, Kirby-Bauer antibiotic susceptibility test was performed. 25 antibiotics from different groups were selected for antibiotic susceptibility test of the six selected bacterial isolates. For each bacterium separate antibiotics were used upon the availability of antibiotics. Amikacin, Cefoperazone/Sulbactam, Imipenem, Piperacillin/Tazobactam, Meropenem antibiotics were tested for four isolates among six and showed to be effective against all of the four isolates. Gentamicin was effective for all of the isolates. Cefpodoxime, Neomycin, Tetracycline, Erythromycin were used only for environmental and laboratory isolates *Bacillus subtilis* and *Staphylococcus aureus* and found to be positive in producing clear zone of inhibition. *Bacillus subtilis* and *Staphylococcus aureus* were also susceptible for Cephradine, Rifampicin, Ampicillin. The pathogenic isolates *Pseudomonas aeruginosa* (Tigecycline), *Pseudomonas luteola* (Colistin), *Klebsiella pneumonia*(Ampicillin), *Escherichia coli* (Cefepime, Nalidixic acid, Ceftriaxone, Ciprofloxacine, Cefuroxime) showed resistance to various antibiotics. Among them only *Escherichia coli* showed resistance against five antibiotics which include 3rd and 4th generation cephalosporins (Table 02).

Table 02 Antibiotic susceptibility test of the bacterial isolates.

Antibiotics	**Group of antibiotic**	***Escherichia coli***	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Pseudomonas luteola***	***Klebsiella pneumoniae***	***Staphylococcus aureus***
Nitrofurantoin (100 µg)	Macrobid	S	-	-	-	S	-
Cefepime (30µg)	Cephalosporins (4th)	R	-	S	S	S	-
Gentamycin (10µg)	Aminoglycosides	S	S	S	S	S	S
Piperacillin/Tazobactam (100/10µg)	Piperacillin/βlactamase inhibitor	S	-	S	S	S	-
Cefuroxime (30µg)	Cephalosporins (2nd)	R	-	-	-	S	-
Cephradine (30µg)	Cephalosporins	-	S	-	-	-	S
Colistin (10µg)	Polymixins	S	-	S	R	S	-
Amoxicillin (30µg)	Aminobenzyl penicillin	S	-	-	-	S	-
Amikacin (30µg)	Aminoglycosides	S	-	S	S	S	S
Ampicillin (10µg)	Aminobenzyl penicillin	-	S	-	-	R	S
Meropenem (10µg)	Carbapenems	S	-	S	S	S	-
Ertapenem (10µg)	Carbapenems	S	-	-	-	S	-
Cefoperazone/Sulbactam (75/30µg)	βlactamase inhibitor	S	-	S	S	S	-
Trimethoprim/Sulfamethoxazole	Trimethoprim/Sulfonamide	S	-	-	S	S	-
Ciprofloxacin (5µg)	Quinolones (2nd)	R	-	S	S	S	-
Imipenem (10µg)	Carbapenems	S	-	S	S	S	-
Neomycin (30µg)	Amynoglycoside	-	S	-	-	-	S

Tetracycline (30µg)	Tetracyclines	-	S	-		-	S
Rifampicin (5µg)	Ansamycins	-	S	-	-	-	S
Ceftriaxone (30µg)	Cephalosporins (3rd& 4th)	R	-	-	S	S	-
Erythromycin (15µg)	Macrolides	-	S	-	-	-	S
Cefpodoxime (30µg)	Cephalosporins (3rd& 4th)	-	S	-	-	-	S
Tigecycline (15µg)	Glycylcyclines	S	-	R	S	S	-
Nalidixic Acid (30µg)	Fluoroquinolones (1st)	R	-	-	-	S	-
Novobiocin (30µg)	Aminocoumarin	-	S	-	-	-	-

Antibacterial activity of seaweed (*Sargassum muticum*) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, Klebsiella pheumoniae, *Staphylococcus aureus*, *Bacillus subtilis* were determined. Four different concentrations were used to find visible clear zone. 10µl, 20 µl suspension was absorbed into sterile filter paper and soaked on media. 50 µl, 100 µl suspension was added inside the well made on the media. For *Sargassum muticum*ethanol extracts showed better effectiveness than methanol extraction (Figure 01). For ethanol extraction, 10µl extract showed no activity and 20 µl extract showed a little activity against *Pseudomonas luteola*but the activity increased as the concentration rose to 50 µl, 100 µl. *Klebsiella pneumonia* was showed very little zone of inhibition with 100 µl extract whereas *Pseudomonas aeruginosa*and Staphylococcus aureu shoed moderate activity at 100 µl extract. On the other hand, for methanol extraction, *Pseudomonas luteola* showed no inhibition at all and slightly inhibition occurred for *Escherichia coli* and *Staphylococcus aureus*. *Pseudomonas aeruginosa* and *Bacillus subtilis* showed moderate inhibition and *Klebsiella pneumonia* showed the best results for highest inhibition among all the bacteria against methanol extraction (Table 03).

Table 03 Antibacterial activity of *Sargassum muticum* against selected bacterial isolates.

Bacterial isolates	**Ethanol extract**				**Methanol extract**			
	10 µl	**20 µl**	**50 µl**	**100 µl**	**10 µl**	**20 µl**	**50 µl**	**100 µl**
Pseudomonas luteola	-	+	++	+++	-	-	-	-
Pseudomonas aeruginosa	-	-	-	++	-	-	-	++
Escherichia coli	-	-	-	-	-	-	-	+
Staphylococcus aureus	-	-	+	++	-	-	-	+
*Klebsiella pneumonia*e	-	-	-	+	-	-	-	+++
Bacillus subtilis	-	-	-	-	-	-	-	++

Antibacterial activity of freshwater weed (*Spirodela polyrrhiza*) against thee similar bacterial isolates were determined. For *Spirodela polyrrhiza* ethanol and methanol extracts showed similar effectiveness like *Sargassum muticum*. *Pseudomonas luteola* showed no zone of inhibition against any extracts. Both extracts had only slight and moderate antibacterial activity against *Bacillus subtilis* and *Escherichia coli* respectively with 100 µl concentration. Ethanol and methanol extract showed moderate and low antibacterial activity against *Pseudomonas aeruginosa* at 100µl concentration (Table 04).

Table 04 Antibacterial activity of *Spirodela polyrrhiza* against selected bacterial isolates by agar well diffusion.

Bacterial isolates	**Ethanol extract**				**Methanol extract**			
	10 µl	**20 µl**	**50 µl**	**100 µl**	**10 µl**	**20 µl**	**50 µl**	**100 µl**
Pseudomonas luteola	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	++	-	-	-	+
Escherichia coli	-	-	-	++	-	-	-	++
Staphylococcus aureus	-	-	-	-	-	-	-	-
Klebsiella pneumonia	-	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	+	-	-	-	+

After identifying the antibacterial activity of *Sargassum muticum*and *Spirodela polyrrhiza* against some pathogenic and environmental bacteria, the study aimed to determine the MIC and MBC of both ethanol and ethanol extracts of the aquatic weeds from both marine and freshwater origin. Apparently it can be seen from the table that *Sargassum muticum* has the ability to lower the growth of bacteria to stop visible growth both with ethanol and methanol extraction. *Pseudomonas luteola* (ethanol- 30 µl, methanol- 80 µl) showed the MIC in lowest concentration for ethanol extracts. Other isolates showed MIC at higher concentrations. MBC was found with both extracts only for *Escherichia coli* (methanol- 100 µl) and *Pseudomonas aeruginosa* (ethanol-90 µl, methanol-100µl). *Staphylococcus aureus* was unable to be killed with any of these extracts. *Bacillus subtilis* and *Klebsiella pneumonia* were killed with methnol extract at 70 µl and 100 µl respectively. In case of *Spirodela polyrrhiza*, *Staphylococcus aureus* showed no MIC and MBC within the range of concentrations of the extracts used in the study (10 µl- 100 µl). Ethanol and methanol extracts showed MIC and MBC for *Pseudomonas aeruginosa*, *Pseudomonas luteola* and *Escherichia coli*only. They were observed only to inhibit (90 µl) the growth of *Klebsiella pneumonia* and *Bacillus subtilis* (Table 05).

Table 05 MIC and MBC of *Sargassum muticum* and *Spirodela polyrrhiza* against different bacteria.

Bacteria	Solvents	*Sargassum muticum* extracts		Spirodelapolyrrhiza Extracts	
		MIC	MBC	MIC	MBC
Pseudomonas luteola	Ethanol	30 µl	100 µl	70 µl	90 µl
	Methanol	80 µl	-	90 µl	100 µl
Pseudomonas aeruginosa	Ethanol	70 µl	90 µl	60 µl	80 µl
	Methanol	90 µl	100 µl	40 µl	60 µl
Escherichia coli	Ethanol	-	-	60 µl	90 µl
	Methanol	100 µl	-	50 µl	80 µl
Staphylococcus aureus	Ethanol	80 µl	-	-	-
	Methanol	90 µl	-	-	-
Klebsiella pneumonia	Ethanol	90 µl	-	-	-
	Methanol	70 µl	100 µl	90 µl	-
Bacillus subtilis	Ethanol	90 µl	-	-	-
	Methanol	60 µl	70 µl	90 µl	-

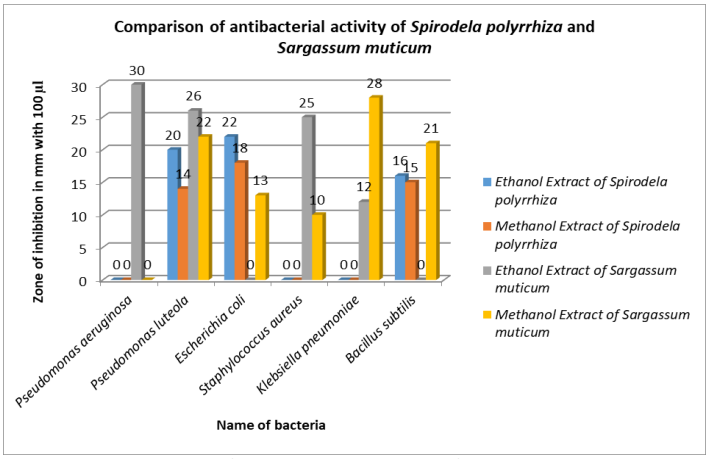

Figure 01 Comparison of antibacterial activity of *Spirodela polyrrhiza* and *Sargassum muticum*

DISCUSSION

As antibiotic drugs are getting to a situation where many pathogenic bacteria have become resistant, alternative resource is needed to combat such infectious pathogens with abundant, cost effective and consumer safe antibacterial products. With the same point of view, candidates of aquatic weeds (because of their high availability) from marine and fresh water region were chosen to investigate such properties.

Firstly, we biochemically confirmed the collected bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas luteola*, Klebsiella pheumoniae, *Staphylococcus aureus*, *Bacillus subtilis*) and detected the antibiotic susceptibility of them toward different antibiotics. Here we have observed *Escherichia coli*was resistant to few 2nd, 3rd and even 4th generation cephalosporin drug with resistance to 1st generation Fluoroquinolones. Other bacterial isolates were somewhat sensitive towards the antibiotics we used for them with resistance towards a few antibiotics. From this part of study of understood the risk with infectious *Escherichia coli* which showed higher degree of resistance, and there is a need to discover newer agents to inhibit them. Environmental laboratory freeze dried isolates showed sensitivity toward the antibiotics we used for them. As they have not been encountered with antibiotics before, they have not started to get the resistance from other drug resistant isolates yet.

After studying the antibiotic resistance, we further attempted to determine the antibacterial activity of ethanol and methanol extracts of *Sargassum muticum* and *Spirodela polyrrhiza* towards those bacteria. *Pseudomonas aeruginosa*, Pseudomonas lutiola, *Staphylococcus aureus* showed satisfactory results with ethanol extracts of *Sargassum muticum* and *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* showed good results with methanol extracts of *Sargassum muticum*. As like *Escherichia coli* was most resistant to the advanced antibiotics, it showed little sensitivity to methanol extract of *Sargassum muticum*. Aqueous extract as well as raw extracts can be further studied to get the complete picture of its activity to *Escherichia coli* as it is of utmost importance to get an a alternative drug. Other isolates are satisfactorily sensitive to the extracts though they impart some resistance towards few antibiotics. So *Sargassum muticum* can also be an alternative drug of choice against *Pseudomonas aeruginosa*, Pseudomonas lutiola, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*.

Similarly, the antibacterial activity of both ethanol and methanol extracts of *Spirodela polyrrhiza* was quite satisfactory for *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*. It is great to know the extracts of the freshwater weed (duckweed) possess antibacterial activity to three of the common bacterial pathogens including one bacteria (*Escherichia coli*) with resistance to 4th generation cephalosporins. This finding definitely could be a great opportunity to the pharmaceutical industries to take initiatives for production of new drug of choice for resistant pathogenic bacteria (*Escherichia coli*) enabling to keep the public health safe by lessening mortality and morbidity rate by the infection of multi drug resistant *Escherichia coli*.

The MIC and MBC test for the isolates with ethanol and methanol extracts of *Sargassum muticum* and *Spirodela polyrrhiza* represents similar results like agar well diffusion test. During this part of study we determined the MIC and MBC to determine the dosage of the extracts to inihibit the visisble growth as well as to kill the bacteria. For ethanol and methanol extracts of those two aquatic weeds, higher concentrations were observed (80µl-100µl). *Bacillus subtilis* and *Pseudomonas aeruginosa* ware inhibited at 60µl and 70µl respectively, comparatively lower concentrations than others.

CONCLUSION

Modern age is facing problem to combat diseases using antibiotics as many pathogenic bacteria have become multidrug resistant to the most advanced antibiotics. As a consequence, the pathogens are able to cause life threatening conditions which were before very easy to treat with antibiotic use. So alternatives or new drugs are necessary to treat infected people with such resistant bacteria. In our current study we observed that *Sargassum muticum* and *Spirodela polyrrhiza* have the ability kill some of such bacteria which are pathogenic and also have some resistance to some antibiotics. The most significant result was found for *Escherichia coli* (having resistance to 4th generation antibiotics) towards *Spirodela polyrrhiza* extracts and little activity towards *Sargassum muticum*. This breakthrough information can be aimed to develop new drugs for treating 4th generation cephalosporin resistant bacteria.

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