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Phytochemical profiling and bactericidal activity of marine sea weed *Chymodacea serrulata* against ocular pathogens

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Abstract
Assessment of marine life forms for their bioactive potential, being a significant part of marine biological system, has gotten the cadence in modern years with making recognition of their significance in existence of human. In the current investigation, antimicrobial efficacy of the sea grass *Chymodacea serrulata* was tested against isolated ocular pathogenic bacteria species such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Escherichia coli*, using polar solvents (water and Ethanol) and non-polar solvents (hexane and chloroform) were examined specifically. Maximum activity was noted in chloroform and Ethanol extracts. On account of phytochemical investigation, the polar and non-polar solvent extracts showed positive action with phytoconstituents like steroids, Phenols, Glycosides, terpenoids, alkaloids, saponins, tannins and flavonoids excluding sugars and quinines. However, additional investigations are in progress to disengage dynamic mixtures in controlling the growth of ocular pathogenic bacterial strains.

Keywords: *Chymodacea serrulata, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli*

Introduction
In everyday existence of human, the eye is perhaps the important sense organ. The care given to eye prosperity and tidiness is major. Due to dust, temperature fluctuations, microbial colonization and various other factors the incidence of ocular diseases may occur which will incite loss of sight (Mohammed *et al*, 2020) [16]. Other factors that influence the occurrence of ocular infections in the population are: contact lenses, trauma, surgery, previous eye infections, nasolacrimal duct obstructions and become older. Although fungi, viruses and parasites involved in the development of this disease, bacteria are the main causative agent of ocular problems, this is because the bacterial pathogens inhabit the ocular surfaces. Globally, 32-74% of ocular infections due to these microbial agents (Petrillo *et al*, 2020) [17]. In children *Haemophilus influenza* and *Sreptococcus pneumonia* and in adults *Staphylococcus aureus* are predominant pathogens. Now multi drug resistant bacterial species like Methicillin resistant *Staphylococcus aureus* (MRSA) are emerging as most important agents for causing this disease. However, Gram-positive bacterial species are responsible for 60-80% of acute infections (Aklilu *et al*, 2018) [1].
In spite of the defensive guard, the resident bacteria of the conjuctival sac or the surrounding bacteria can develop infection and should be treated with appropriate antibiotics. Notwithstanding, substantiation is gathering that recommends the development of resistant bacteria in the eye inferable from earlier antibiotic treatment of the eye. This emergence of antimicrobial resistance towards the ocular pathogens leads to failure of treatment. In our nation, the capricious utilization of antimicrobials without the specialists' medication may contribute the higher incidence of drug resistance (Sharma, 2011) [23]. Due to this emergence of antibiotic resistance among the ocular pathogens many traditional herbal medicines were prepared using various medicinal plants which cures ophthalmic problems (Sangeetha and Asokan, 2016) [21].
Seagrass are submerged blossoming plants found in shallow marine waters, for instance, Bays, estuaries, lagoons and along the territory rack moreover, accept a critical part in staying aware of the biodiversity and overall prosperity of coastal frameworks (Geevarghese *et al*, 2018) [8]. *Chymodocea* is a genus of the family Chymodoceaceae described as a genus in 1805 and it includes the 4 species of seagrass including *Chymodocea serrulata* distributed in warm marine ecosystem (Koenig, 1805) [15]. *Chymodocea* sp. is utilized as a sedative for children, as alleviating help during pregnancy and against cough and malaria. Generally seagrasses are rich source of discretionary metabolites which is acknowledged to be a shield segment to these plants. However, only very few studies are available regarding the antimicrobial activity of crude solvent extract of these plants (Kannan *et al*, 2010). So, the current study was aimed to investigate the presence of phytochemical constituents and antimicrobial activity of polar and non-polar solvent extracts of *C. serrulata* sea grass against the isolated ocular pathogens.

Materials and Methods
Sample collection- Eye swabs collection and isolation of ocular pathogens
A topical anesthetic was added over the eye and waited until the development of anesthetic effect (within 120-180 seconds). A sterile cotton swab, moistened with sterile saline was rotated along the inferior conjunctiva while the lower lid was retracted. Thus obtained swabs were inoculated into the sterile transport media and safely brought to the clinical laboratory in order to isolate the ocular pathogens. Each and every sample was inoculated into the culture media and incubated at 37°C for 3-5 days. After the appearance of pathogenic colonies on culture media, the pathogens were identified using the standard identification protocols (Holt *et al*, 1994) [11].

Sea grass collection

The fresh seag rass sample was collected from the intertidal zone of the Manora village, Thanjavur District, Pattukottai Taluk, Tamilnadu (Lat. 10. 2657°N; long. 079. 3042°E) during summer season. The seagrass thus collected were identified and authentified by Dr. Soosairaj, Associate Professor, Department of Botany, St. Joseph′s College, Trichy, Tamil Nadu, India. The extraneous matters were removed from the plant by proper washing with sea water and then with sterile distilled water. After proper washing the plants were shade dried for two weeks. The dried plant material were powdered and sieved through 0. 8nm^2 sieve plate and stored at air tight sterile containers.

Crude Extract Preparation

The extraction of sieved powder was done by using both polar (water and Ethanol) and non-polar solvents (chloroform and Hexane) as previously described by Handa *et al* (2008) [9]. 500 grams of dried seagrass powder was mixed with 2L (1:4w/v) of the solvents such as chloroform, hexane, water and ethyl acetate and kept it for 10 days in a shaker. The obtained extract was filtered through the Whatmann No. 1 filter paper and concentrated the filtrate using rotary evaporator. In case of aqueous extract, it was concentrated using the lyophilizer. Thus obtained concentrated crude extracts were stored in refrigerator and used for further *in vitro* studies.

Qualitative phytochemical analysis of sea grass extracts

The crude extract of the seag rass was subjected to phytochemical screening in order to detect the presence of phenols, steroids, terpenoids, flavonoids, alkaloids, glycosides, saponins and tannins by using the standard protocol previously stated by Kokate (2006).

Antibacterial Assay

Antibacterial activities of concentrated extract (chloroform, hexane, aqueous and Ethanol) of seagrass were tested against the selected ocular pathogens such as *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia* and *Escherichia coli*. Antibacterial activity determination was done by using Bauer's protocol (Bauer *et al*, 1966) [2]. Based on this method, the sterile filter paper disc containing 6mm diameter were loaded with 100µg, 200µg, 300µg and 400µg of crude extracts of the seagrass and allowed to air dry. The discs loaded with 5% DMSO (Dimethyl sulphoxide) were used as a negative control and the gentamycin antibiotic disc acts as a positive control (Standard). These discs were placed on the Mueller-Hinton agar inoculated with appropriate tested ocular pathogens and incubated at 37°C for 24 h. Zone of inhibition was noted in millimeters and the values of mean were reported and it was compared with the standard.

Minimum inhibitory concentration (MIC) determination

Broth micro-dilution method (NCCLS, 2003; Sangeetha and Asokan, 2016) [21] was used to determine the MIC values. Serial double dilutions are prepared with a solution of maximum active seagrass extracts: DMSO (95:5) in a 96 well micro-titer plate over the range of 7-3, 125µg/mL. The overnight broth culture of each tested strains were prepared and the final concentration of microbial inoculums in each well was adjusted to 2×10^5cfu/ml, from this 5µL of culture was added into the each well of micro-titer plate and the plates were incubated at 37°C for one day (24 hours). The lowest concentration of the sea grass extract at which the microbes does not show any visible growth and the absorbance of each well determined using an automatic ELISA reader at 630nm was considered as MIC value of the appropriate extracts of the sea grass. Replicates were maintained in all the tests. Gentamycin can acts as a positive control. The wells showing no visible growth was detected and 10µl of each well was transferred to Mueller-Hinton Agar plates for checking the appearance of bacterial growth. Values are expressed as mean, and standard error was calculated.

Results

Identification of pathogenic bacteria

The isolated ocular pathogens were identified based on the staining, motility test and biochemical analysis such as indole, Methyl-red test, Voges-Proskauer, Citrate utilization test, oxidase, catalase, carbohydrate fermentation ability. Based on the above identification procedures the isolated pathogens were identified as following bacterial species: *Bacillus subtilis, Corynebacterium xerosis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus epidermidis* were depicted in table 1. The morphology of the isolated strains by Gram-staining was noted (Plate 1). Among the identified strains only four were selected for further studies because of their predominant role for causing conjunctivitis.

Antibacterial activity of non-polar extracts of *Chymodocea serrulata* against ocular pathogens

Chymodocea serrulata extraction was done by using non-polar solvents such as chloroform and hexane according to the previous protocol given by Handa *et al* (2008) [9]. The concentrated extract was used for antimicrobial activity with various concentrations against *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,* and *Escherichia coli*. Gentamycin antibiotic used as positive control and the negative control was the DMSO. The antibiotic gentamycin inhibits all the pathogens and the negative control DMSO does not show any inhibition.

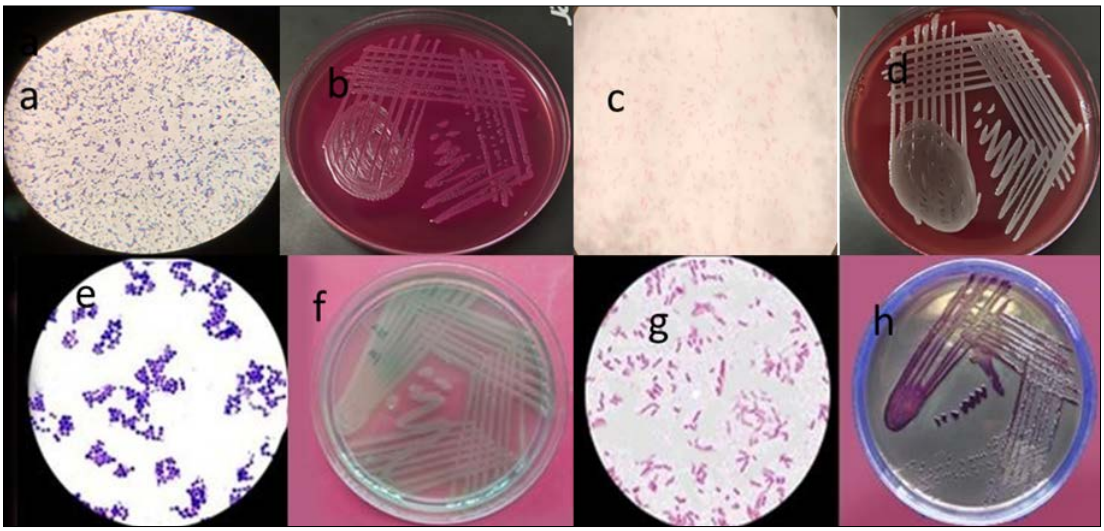
Table 1: Biochemical identification of ocular pathoges

S.no	G	M	SP	I	MR	VP	CI	TSI	OX	CAT	Co	Sugar	SH	Selective media	Name of the Organism
1	GPR	-	SP	-	-	P	P	-	N		-	-	P	-	*Bacillus subtilis*
2	GPR	-	NSP	-	-	-	-	-	-	P	-	-	N	-	*Corynebacterium xerosis*
3	GPC	-	NSP	N	N	P	N	A/A	N	N	-	LF	-	-	*Enterococcus faecalis*
4	GNR	M	-	P	P	N	N	-	-	-	-	LF	-	EMB-Metallic sheen	*Escherichia coli*
5	GNR	-	-	N	N	P	P	A/AG+	-	-	-	LF	-	MC- Pink Mucous colony	*Klebsiella pneumoniae*
6	GNR	M	NSP	N	N	N	P	-	P	P	N	MAN-F	-	Cetrimide agar–Blue green	*Pseudomonas aeruginosa*
7	GPCRCL	-	-	-	-	-	-	-	N	P	P	MAN-F	-	MSA-YELLOW	*Staphylococcus aureus*
8	GPCRCL	-	-	-	-	-	-	-	N	P	N	-	-	MSAP–Y	*Staphylococcus epidermidis*
9	GPCRCL	-	-	-	-	-	-	-	N	P	N	-	-	MSA-NG	*Staphylococcus saprophyticus*

Table 2

G-Gram staining	M–Motile SP–Sporulated	NSP–Non Sporulated
I–Indole	MR–Methyl Red	VP–Voges Proskauer
CI–Citrate	TSI–Triple Sugar Iron	Ox–Oxidase
Cat–Catalase	Co–Coagulase	Sug-Sugar Fermentation
SH-Starch Hydrolysis	MAN-Mannitol	F–Fermentation
NLF–Non Lactose Fermenter	LF-Lactose Fermenter	NG–No Growth
GLU–Glucose	N–Negative	P–Positive
Ak –Alkaline	A–Acid	G–Gas
P-Y–Pink yellow	Heamolysis-Haemolysis on BloodAgar	MC–MacConkey Agar
MSA–Mannitol Salt Agar	EMB-Eosin methylene blue agar	

GPC RCL- Gram positive cocci in regular cluster; GPCPS- Gram positive cocci in pairs; GPCCH- Gram positive cocci in chain; GNR-Gram negative rods; GPR- Gram positive rods; GPRCH- Gram positive rod in chain



a- Gram positive cocci (*S. aureus*) b- macroscopic image (*S. aureus)*; c- Gram negative Rods (*K. pneumoniae*) d- macroscopic image (*K. pneumoniae);* e- Gram positive cocci (*E. faecalis)* f- macroscopic image (*E. faecalis*); g- Gram negative rods (*E. coli)* h- macroscopic image (*E. coli*)

Plate 1: Microscopic and macroscopic observation of ocular pathogens

The extract obtained using chloroform showed maximum inhibition on Staphylococcus aureus at following concentrations 400µg (13mm), 300 µg (11mm), 200 µg (9mm) and 100 µg (8mm) subsequently E. coli 11mm, 10mm, 9mm and 8mm at 400 µg, 300 µg, 200 µg and 100 µg respectively. There was no inhibitory zones were recorded in remaining two pathogens such as Klebsiella pneumoniae and Enterococcus faecalis. In case of hexane extracts there were no inhibition on all the tested pathogens (Table 2 and Plate 2 &3).

Table 3: Antibacterial activity of non-polar extracts of *Chymodocea serrulata* against ocular pathogens

Zone of inhibition (mm in diameter)											
S. No	**Bacterial pathogens**	**Chloroform(µg)**					**Hexane(µg)**				
		Gentamycin	**100**	**200**	**300**	**400**	**Gentamycin**	**100**	**200**	**300**	**400**
1	*Klebsiella pneumoniae*	13.0±0.0	-	-	-	-	15.0±0.0	-	-	-	-
2	*Staphylococcus aureus*	18.0±0.0	8.0±0.0	9.0±0.0	11.0±0.0	13.0±0.0	20.0±0.0	-	-	-	-
3	*Enterococcus faecalis*	15.0±0.0	-	-	-	-	16.0±0.0	-	-	-	-
4	*Escherichia coli*	12.0±0.0	8.0±0.0	9.0±0.0	10.0±0.0	11.0±0.0	14.0±0.0	-	-	-	-

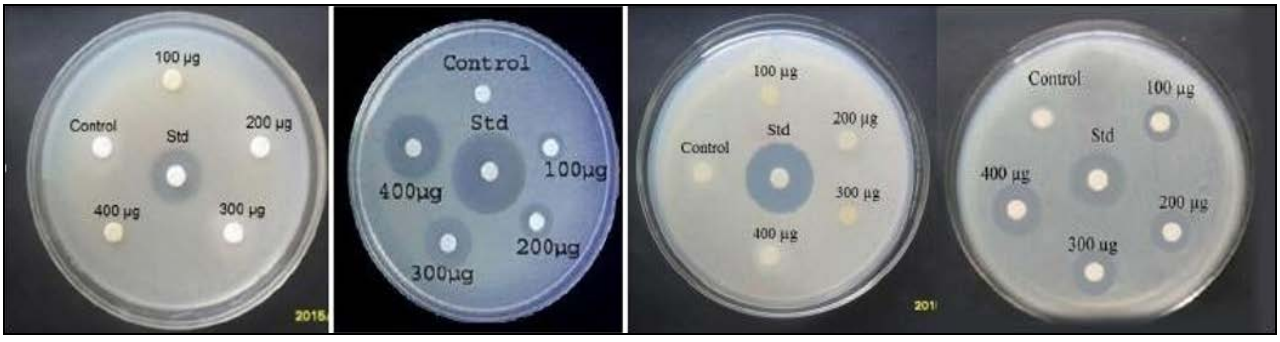


Plate 2: Antibacterial activity of chloroform extracts of *Chymodocea serrulata* against ocular pathogens

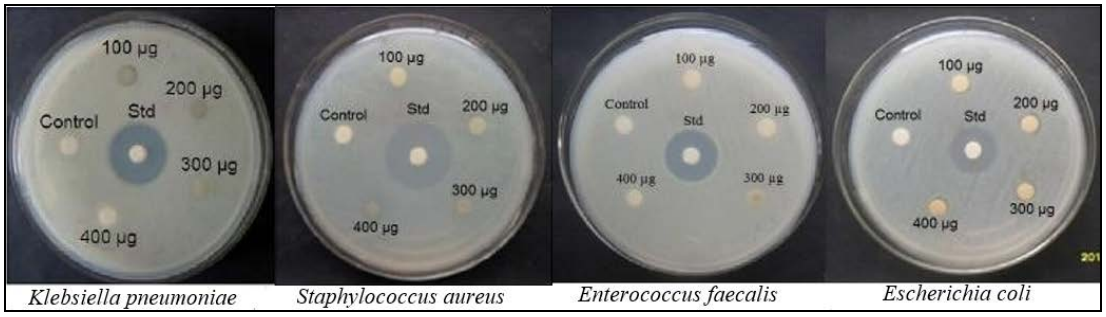


Plate 3: Antibacterial activity of hexane extracts of *Chymodocea serrulata* against ocular pathogens

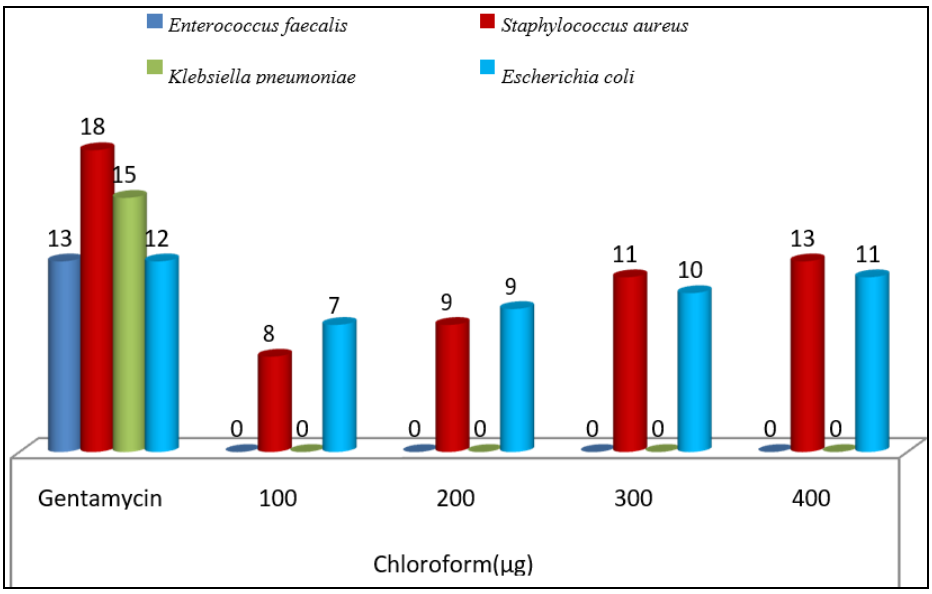


Fig 1: Antibacterial activity of chloroform extracts of *Chymodocea serrulata* against ocular pathogens

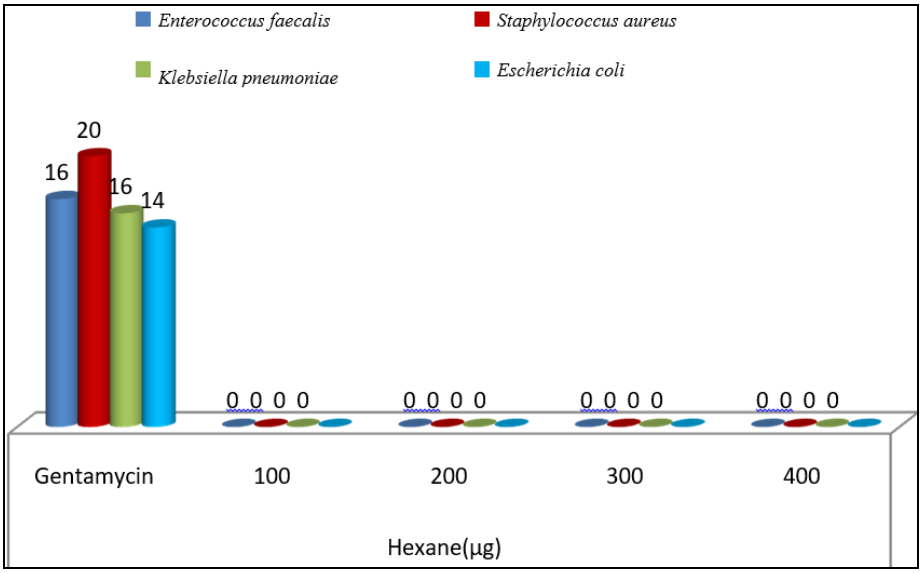


Fig 2: Antibacterial activity of hexane extracts of *Chymodocea serrulata* against ocular pathogens

Antibacterial activity of polar solvent extracts of *Chymodocea serrulata*

The polar solvents like water and ethanol extracts of *Chymodocea serrulata* were subjected to antimicrobial activity against the ocular pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Escherichia coli* with varying concentrations and the results were tabulated (Table 3). The aqueous extracts of *Chymodocea serrulata* showed no inhibition on the growth of *Klebsiellka pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* whereas in *E. coli* the 400µg concentration showed 6 mm zone and other concentrations were not inhibit the growth of *E. coli*. The ethanol extracts of *Chymodocea serrulata* showed maximum activity against *Staphylococcus aureus* like 12mm, 10 mm, 8 mm and 6mm inhibitory zones at 400 µg, 300 µg, 200 µg and 100 µg respectively. *E. coli* showed inhibitory zone at 400 µg (8mm) and 6 mm was recorded at other three concentrations

(300 µg, 200 µg and 100 µg). Whereas, the other two pathogens such as *Klebsiella pneumonia* and *Enterococcus faecalis* showed no response (Table 3; Plate4 and 5).

Table 4: Antibacterial activity of polar extracts of *Chymodocea serrulata* against ocular pathogens

Zone of inhibition (mm in diameter)											
S.no	Bacterial pathogens	Water(µg)					Ethanol(µg)				
		Gentamycin	100	200	300	400	Gentamycin	100	200	300	400
1	*Klebsiella pneumoniae*	13.0±0.0	-	-	-	-	18.0±0.0	-	-	-	-
2	*Staphylococcus aureus*	18.0±0.0	-	-	-	-	15.0±0.0	6.0±0.0	8.0±0.0	10.0±0.0	12.0±0.0
3	*Enterococcus faecalis*	14.0±0.0	-	-	-	-	15.0±0.0	-	-	-	-
4	*Escherichia coli*	15.0±0.0	-	-	-	6.0±0.0	14.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	8.0±0.0

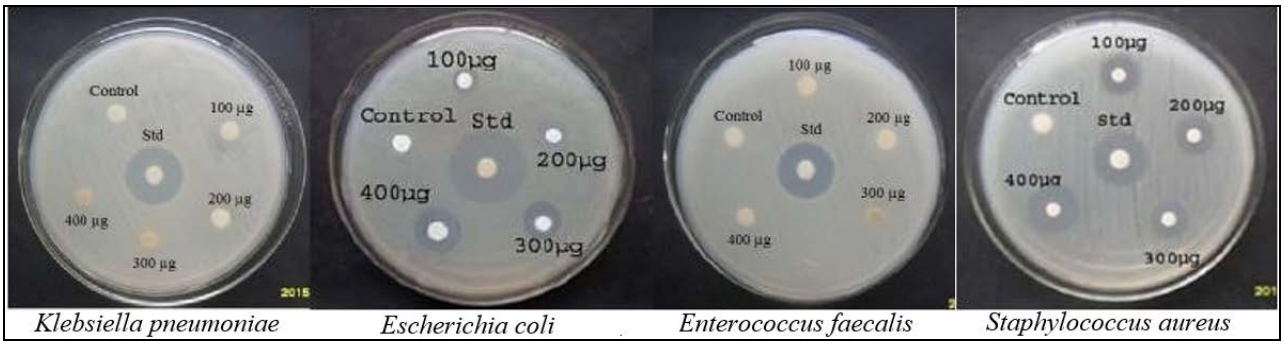


Plate 4: Antibacterial activity of Ethanol extracts of *Chymodocea serrulata* against ocular s pathogens

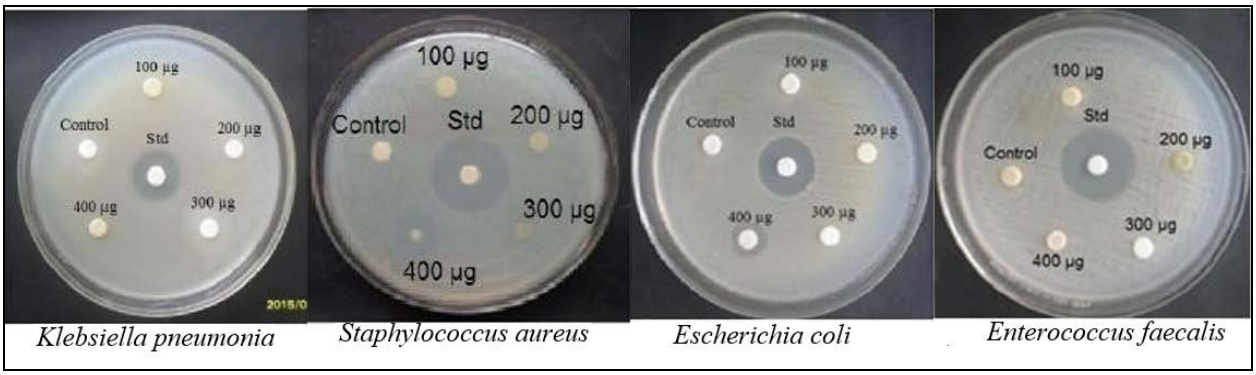


Plate 5: Antibacterial activity of water extracts of *Chymodocea serrulata* against ocular s pathogens

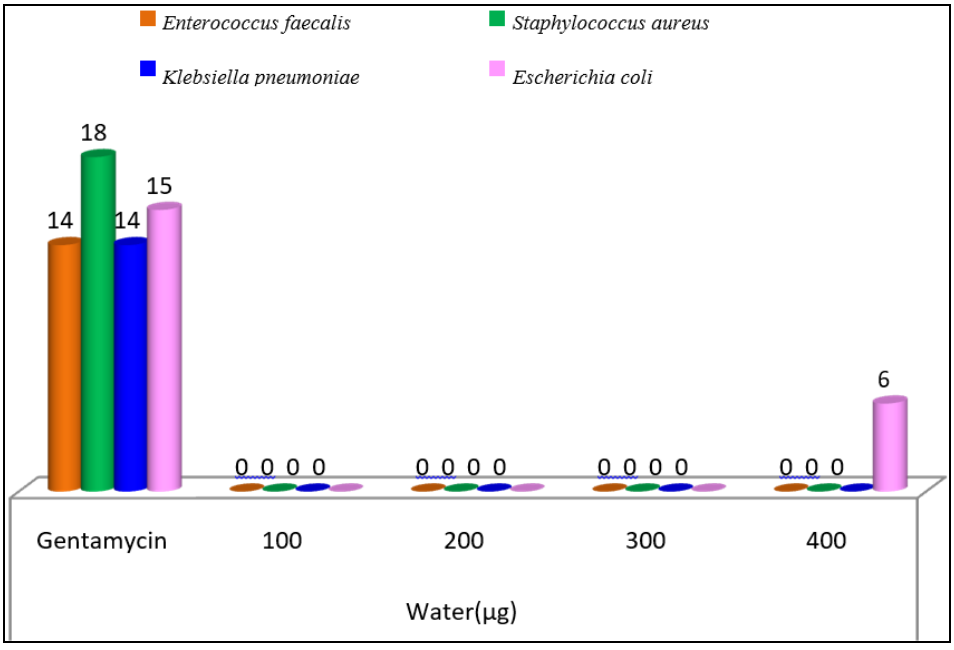


Fig 3: Antibacterial activity of water extracts of *Chymodocea serrulata* against ocular pathogens

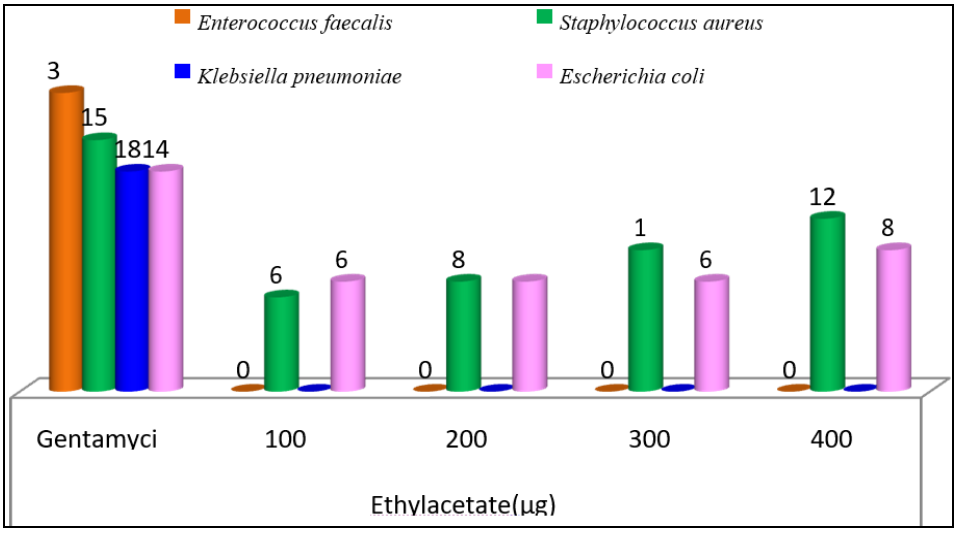


Fig 4: Antibacterial activity of Ethanol extracts of *Chymodocea serrulata* against ocular pathogens

Minimum inhibitory concentration studies of *C. serrulata* extracts against ocular pathogens

Minimum inhibitory concentrations of chloroform and ethanol of the sea grass *Chymodacea serrulata* which showed maximum activity against *S. aureus* and *E. coli* and other two solvent extracts showed no inhibition. And also all solvent extracts does not showed any inhibition on *Enterococcus faecalis* and *Klebsiella pneumonia* are depicted in table 4. The minimum inhibitory concentration such as, 850 µg/ml of chloroform extracts of *C. serrulata* on *S. aureus* was noted and on *E. coli* 90 µg/ml; whereas, the ethanol extracts of *C. serrulata*, the MIC value such as 875 µg/ml was recorded on *S. aureus* and on *E. coli* 75 µg/ml serves as MIC.

Table 5: Minimum Inhibitory Concentration studies of *Chymodocea serrulata* against ocular pathogens

S.No	Seagra ss	Solvents	Pathogens	MIC value (µg/ml)
1	*Chymodocea serrulata*	Chloroform	*Staphylococcus aureus*	850
2		Ethanol		875
3		Water		-
4		Hexane		-
5		Chloroform	*E.coli*	90
6		Ethanol		75
7		Water		-
8		Hexane		-
9		Chloroform	*Enterococcus faecalis*	-
10		Ethanol		-
11		Water		-
12		Hexane		-
13		Chloroform	*Klebsiella pnemoniae*	-
14		Ethanol		-
15		Water		-
16		Hexane		-

(-) no inhibition

Qualitative phytochemical analysis of seagrass using different solvents

Qualitative phytochemical analysis of seagrass (*Chymodocea serrulata*) was done by using different solvents like Hexane, chloroform, Ethanol and water. The hexane extracts of *Chymodocea serrulata* showed the presence of steroids and absence of flavonoids, saponins, terpenoids, tannins and phenols. The extract obtained using ethanol showed the presence of flavonoids, terpenoids, tannins and phenols whereas steroids, alkaloids, glycosides and saponins were absent. The ethanol extracts of *Chymodocea serrulata* showed the presence of glycosides, saponins and phenols but steroids, alkaloids, flavonoids, terpenoids and tannins were not noticed. And at the same time the aqueous extracts contains steroids, glycosides and saponins but alkaloids, flavonoids, terpenoids, tannins and phenols were absent (Table 5).

Table 6: Qualitative phytochemical analysis of seagrass using different solvents

Seagrasses	***Chymodocea serrulata***			
Compounds	H	C	E	AQ
Steroids	+	-	-	+
Alkaloids	-	+	-	-
Flavonoids	-	-	-	-
Glycosides	-	-	+	+
Saponins	-	-	+	+
Terpenoids	-	-	-	-
Tannins	-	-	-	-
Phenols	-	-	+	-

Discussion

However long haul research on bioactive compounds from marine species has given a general and better knowledge of marine natural products. Seagrasses are a rich basis of

structurally new and naturally dynamic metabolites which they produce with the purpose of maintaining their growth and development during the extreme environmental stresses existing under sea (Tangon *et al*, 2021) [24]. As there are number of evidence which exhibit the antimicrobial action of mangroves, sea weeds and other marine creatures and just restricted reports were accessible for the sea grasses in the global and surprisingly very little data accessible from our country. So this study was aimed to explore and analyze the capacity of seagrass *C. serrulata* extracts against ocular pathogens and to create novel mixtures which can be utilized for therapeutic applications.

In our study, *Escherichia coli* (11) and *Staphylococcus aureus* (9) were isolated from 84 patients. Similar results were obtained previously from 25 samples such as *Staphylococcus species (*6), *Staphylococcus aureus* and *Escherichia coli* (1) bacteria, (Sumathi and Preethi, 2016). Earlier reports identified the high frequency of *Staphylococcus epidermidis*, isolated from conjunctival secretion and it was confirmed by most authors (Elander *et al.*, 1991; Garg *et al.*, 1990; Jawetz *et al.*, 1995 and Miller, 1981) [4, 7, 13].

Similarly in another report gram-negative organisms were isolated from normal conjunctiva such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacterium nitratum, Proteus morgani* and *Enterobacter cloacae* (Fahmy *et al*, 1975) [6]. The most common bacterial species, isolated from eyelids and conjunctival infections are *S. aureus* (23. 13%) followed by *S. pneumoniae* (21. 78%) and coagulase negative *Staphylococci* (18. 29%). Ramesh *et al* (2010) [19] reported that *S. aureus* was the most predominant bacteria isolated as ocular pathogens. But in our findings, *S. aureus* (7. 1%) was isolated in very little numbers. The present study also concurred with other studies, the potential bacterial isolates, from 89 conjunctival specimens of Taif University, Saudi Arabia, as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Klebsiella pneumoniae, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Micrococcus roseus, Enterobacter aerogenes* and *Pseudomonas aeruginosa* (Shahaby *et al.*, 2015) [22].

The present research revealed that chloroform and ethyl acetate of seagrass, such as *Cymodocea serrulata*, showed potential activity against most of the tested bacterial ocular pathogens and noticeable activity was found against *Staphylococcus aureus* and *Escherichia coli*. These findings were similar to the report of Rengasamy *et al* (2010), the hexane extract of *C. serrulata* does not show any inhibitory activity against the isolated ocular pathogens. The present results, in agreement with Ravikumar *et al.*, (2011) [20], found antibacterial activity of acetone extracts of *Cymodocea serrulata* root, The 6th acetone fraction from *Cymodocea serrulata* root extract to be maximum, (12mm, 12 mmand12 mm) against three, *Aeromonas hydrophila, Bacillus subtilis* and *Serratia species*, of the five fish pathogens tested and least amount of activity was observed against *Vibrio parahaemolyticus, Vibrio harveyii* (7mm, 8mm) and furthermore the hexane extract of *C. serrulata* root exhibited utmost antibacterial (10mm), against *Vibrio parahaemolyticus* and least activity of 8 mm, 7 mm were observed against *Bacillus subtilis* and *Serratia species*, correspondingly. Balakrishnan *et al.* (2013) reported that, the highest antibacterial activity was observed in methanol extract of *Cymodocea serrulata* against the UTI pathogens. In contrast, our present research confirms that the chloroform extract of *Cymodocea serrulata* showed activity against human eye pathogens. As well, the antibacterial and antifouling capacity of acetone, dichloromethane and methanol extracts of *C. serrulata* and other marine foliage such as *S. isoetifolium* were also reported (Iyapparaj *et al*, 2014) [12].

In the present research, ethanol and chloroform extract of the seagrass *C. serrulata* and their minimum inhibitory concentration against ocular pathogens were studied. Moderate level of minimum inhibitory activity was recorded by chloroform and ethanol extract of *Cymodocea serrulata* (850µg/ml and 875µg/ml) against *Staphylococcus aureus* and (75µg/ml and 90µg/ml) against *Escherichia coli*. But the organisms *Klebsiella pneumoniae* and *Enterococcus faecalis* were susceptible to the chloroform and ethanol extract of *Cymodocea serrulata*, and the above results were compared with positive control, gentamycin, in 25µg/ml. In earlier report, Ragupathi *et al*. (2013) [18] determined the minimum inhibitory concentrations of seagrasses, by serial broth dilution method. *Cymodocea serrulata* showed the maximum inhibitory effect, against *Salmonella dysenteriae* and *S. paratyphi*, with the MIC value of 130µg/ml.

In this study, phytochemical analysis of hexane, chloroform, ethanol and aqueous extracts of the sea grass, revealed the presence of phenols, steroids, terpenoids, flavonoids, alkaloids, glycosides, saponins, and tannins. Whereas, sugars and quinine were absent. Earlier report like Ergene *et al*. (2006) [5] studied the phyto constituents' analysis of *Cymodocea rotundata* and reported the presence of reducing sugar, acidic compounds, proteins, tannins, resins, terpenoids, alkaloids, saponins and glycosides. Similar to our results, the presence of phytochemical compounds such as alkaloid, terpenoid, polyphenol and flavonoids in the ethyl acetate extract of fresh sea grass (Hardoko *et al*, 2016) [10]. Another study reported the presence of flavonoids, phenol, steroids and glycosides in the *C. serrulata* extracts (Bharathi *et al*, 2019) [3].

Conclusion

The current research brings out satisfactory information on the antibacterial capability of seagrass such as *C. serrulata* extracts for the amalgamation of novel antimicrobial agents. Further exploration contemplates are being done on different types of seagrasses of various environments to give total information of the antimicrobial capability of these plants. It is furthermore essential for study the standard compound present in the seagrasses which is liable for antimicrobial turn of events; it might be refined by accomplished by utilizing progressed separation procedures.

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