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In-vitro Inhibitory effect of algae crude extracts against some gram-positive bacterial pathogens

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Abstract

Antibacterial activity of five crude algae extracts of *Codium tomentosum* (Chlorophyceae), *Laurencia papillosa* and *Hypnea musciformis* (Rhodophyceae), *Dictyota dischotoma* and *Padina pavonica* (Phaeophyceae) collected from the Syrian coast of the Mediterranean Sea, was investigated against six Gram-positive bacterial (*Staphyloccocus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis* and *Micrococcus luteus*) pathogens using aqueous, methanol, ethanol, chloroform, acetone, ethyl acetate and hexane solvents. Algal inhibitory activity has been screened by measuring zone of inhibition (ZI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Data showed that methanol *H. musciformis* exhibited the highest ZI of 19 mm against *E. faecalis*, followed by methanol *C. tomentosum* and *H. musciformis* extracts against *S. aureus* (17 mm). Otherwise, methanolic *C. tomentosum* was the most effective by exhibiting the lowest MIC value of 1.1 mg/mL against *S. aureus*, followed by methanolic *P. pavonica* (1.5 mg/mL) against the same pathogen. Moreover, the lowest MBC value was recorded to be 2.1 mg/mL with methanolic *C. tomentosum* and *P. pavonica* extracts against *S. aureus*. The current study proved that *C. tomentosum* and *P pavonica* could be serving in the future as a cheap and potent antibacterial agent.

Keywords: Algae, Inhibitory activity, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC)

Introduction

Many reports stated the algae importance and their multiuser for many purposes. Macroalgae are one of the potential and useful sources with low cost through various manners such as: Marine culture, food,feed, fuel, medicine, industry and heavy metal removing [1-4].

In the recent years, great efforts deal with utility of plants and algae as a potent and cheap source for human pathogens treatment. Algae among them, have been successfully used in pharmacology researches due to their abundance worldwide, richness in bioactive compounds and their availability with low cost. It has been demonstrated for long time that macroalgae displayed wide board range in human antibacterial treatment. Their biological activity could be related to their content of different bioactive constituents (phenols, carotenoids, saponins, tannins and flavonoids compounds) that act as secondary metabolites [5-6].

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Due to their importance in pharmacology studies, many reports worldwide demonstrated their potent as antimicrobial agent e.g. in India [7-10]; Turkey [11] Libya [2, 12]; Morocco [3, 13]; Egypt [14]; Palestine [15] and Andaman Islands [16] and more recently in Syria [17-18].

However, information available about algal inhibitory activity in Syria has not yet been examined in detail so far. Therefore, the current investigation was conducted to screen algae for their antibacterial effect against some selected Gram-positive bacteria using water and six examined solvents. Thereby, the present study will be allow somewhat to determine the most active algae and solvents. So, the most active extract will be handled with performance in future study.

Material and Methods

Algal SamplesCollection's

Sampling of *C. tomentosum*, *L. papillosa*, *H. musciformis*, *D. dischotoma* and *P. pavonica* algae species was carried out from the Syrian coast of the Mediterranean Sea at 4 km North Lattakia – Syria (Table 1). Algae identification has been done by

taxonomical study in the Division of Plant Biotechnology at the AECS in Damascus-Syria. Sampling was carried out manually using disposable gloves and algae were washed with seawater followed by two successive washing with ddH_2O. Then, they were placed over Whatman filter papers for facilitating their drying. Algal samples were shade dried for two weeks, and milled using special electric mill and then stored separately in polyethylene bags until used.

Preparation of Algal Extracts

Algal extracts preparation has been done by using aqueous, methanol, ethanol, chloroform, acetone, ethyl acetate and hexane as solvents. One g of shade-dried milled algae material was extracted in 100 mL solvent, until complete solubility. Then, filtration of extracts has been done by Whatman filter papers. Then, extracts kept under laboratory temperature for 2 h allowing evaporation of solvents. Final extracts were kept in tightly fitting stopper bottles and stored in 4°C. The final extract concentrations were considered as 10 mg/mL.

Phytochemical Assay

Bioactive compounds (Tannins, Flavonoids, Saponins, Alkaloids, Steroids,Carbohydrates, Terpenoids, Phenols and Proteins) were determined as described by many researches [19-21].

Pathogens and Growth Conditions

Six pure clinical pathogens of Gram-positive (*S. aureus, E. faecalis, L. monocytogenes, B. cereus, B. subtilis* and *M. luteus*) were obtained from the Microbiology and Immunology division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus - Syria. Pathogens were cultured in trypticase soy broth (TSB, Difco, BD, Spars, MD) at 37°C for 24 h. Samples were then centrifuged (1000 xg/15 min/4°C), and resuspended in sterile phosphate-buffered saline (PBS). Prior to antibacterial sensitivity test, a bacterial suspension was obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). Bacterial cultures standardize to approximately 10^6 CFU/mL[22]. The exact counts were assessed retrospectively by viable counts on trypticase soy agar plates (TSA, Difco, BD, Spars, MD) at 37°C for 18 h.

Antimicrobial Activity Assay

The disc-diffusion assay

To examine the antibacterial activity, the disc-diffusion method was carried out as previously reported [23]. Ciprofloxacin (10 mg/mL) (Bayer, Istambul, Turkey) antibiotic was used as standard for antibacterial activity. Experiment design including bacterial culture, positive and negative control and ZI determination has been performed as reported by Saleh and Al-Mariri [17].

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) measurement

Microdilution broth susceptibility assay was investigated as reported by Ríos-Dueñas et al. [24]. Serial dilutions of extract (50 mg/mL) or of antibiotic (128 mg/mL) repeated three time in LB broth medium in 96-well microliter plates, by using a range of concentrations (0.625, 1.25, 2.5, 5, 10 and 20) for the 6 examined solvents of the examined algae. Experiment design including bacterial culture, positive and negative control, MIC and MBC determination has been performed as reported by Saleh and Al-Mariri [17].

Statistical Analysis

Statistical analyses were performed by using Statview 4.5 statistical package [25] at the 5%significance level (P = 0.05). Analysis of variance (ANOVA) to determine differences means between various examined solvents via selected pathogens for each algal species has been carried out. Significant differences between means were examined by Fisher's least significant difference (PLSD) test. Experiment has been repeated three times and data areexpressed as mean of three replicates.

Results and Discussion

Algal chemical qualitative analysis for the five examined algae species showed that, the chemical bioactive components were differed according to the examined algae species and tested solvents (Table 2). In this respect, *e.g.* flavonoids, alkaloids, carbohydrates, tannins and saponins were presented in methalonic *C. tomentosum* extract. As described in Table 2, flavonoids were presented in all methanolic algae extracts. While, proteins were absent in all algae extracts regardless of tested solvents. Whereas, phenols were presented with all tested solvents for both the *D. dischotoma* and *P. pavonica* extracts regardless tested solvents (Table 2).

Algal qualitative phytochemical test revealed that alkaloids were presented only with methalonic *C. tomentosum* extract and absent with all other tested solvents in the case of the mentioned algae. Whereas, these bioactive components were absent with all tested solvents for *D. dischotoma*. Alghazeer et al. [12] reported that the alkaloid contents (%) varied according to the algal species examined. In this regards, alkaloid contents (%) followed the following

order: *Dictyopteris membranacea* (Phaeophyta) > *C. tomentosum* (Chlorophyta) > *Gelidium latifolium* (Rhodophyta).Recently, bioactive componds of the *H. musciformis* red algae and thier antioxidant activity using FT-IR and GC-MS techniques have been investigated [4]. The previous study revealed that algal methanolic extract exhibited the strongest phenolic content followed by butanol, chloroform and ethyl acetate. More recently, Saleh and Al-Mariri [17] reported phytochemical compounds of three algae species [*Ulva lactuca* (green), *Dilophus spiralis* (brown) and *Jania rubens* (red)] using similar solvents tested in the current study. Data presented herein, were in accordance of the previous study, who reported phenols presence with proteins absence for algal extracts regardless tested solvents.

Algal crude extracts effect on an inhibition zone (ZI) has been investigated (Table 3). As shown in Table 3, methanolic *C. tomentosum* extract revealed the highest ZI of 17 mm recorded against *S. aureus*, followed by *B. subtilis* (16 mm). Whereas, little activity has been recorded with hexane extract against *M. luteus.* For *L. papillosa*, the highestZI value was recorded to be 12 mm with methanol against *S. aureus, B. cereus* and *M. luteus*. Whereas, little activity has been recorded with hexane against*E. faecalis, B. cereus, B. subtilis* and *M. luteus* and with ethyl acetate against *S. aureus, L. monocytogeneses* and *B. cereus* pathogens.

In the case of *H. musciformis*, ZI value varied between 9 mm with hexane against *M. luteus* and 19 mm with methanol against *E. faecalis.* As for *D. dischotoma*, the highest activity was recorded to be 11 mm with methanol against *L. monocytogeneses* and *B. cereus* pthogens. Whereas, little activity has been recorded with chloroform and acetone against *B. subtilis* and *M. luteus* and with ethyl acetate against *E. faecalis, L. monocytogeneses, B. cereus, B. subtilis* and also with hexane against *L. monocytogeneses* and *B. cereus* pthogens (Table 3). Whereas, for *P. pavonica*, it was varied between 6 mm for both ethyl acetate against *B. cereus* and chloroform against *B. subtilis;* and 16 mm for methanol against *L. monocytogeneses.*

Allover, algal aqueous extracts have no activity against all tested bacterial isolates regardless tested algae species. Variance analysis revealed that the effect of solvents, pathogens, and solvents x pathogens on ZI values, was significantly ($p < 0.001$) different for all tested algae extracts (Table 3). Previously, Ertürk and Taş [11] reported antibacterial activity of ethanolic extracts from 3 algae Chlorophyceae, 2 Phaeophyceae and 2 Rhodophyceae species collected from Vona coast's in Turkey against 6 bacterial pathogens. The previous study reaveled that ZI for *B. cereus* was found to be 9, 8, 10, 9, 10, 8 and 8 mm with *C. glomerata, E. linza, U. rigida, C. barbata, P. pavonica, C. officinalis* and *C. ciliatum,* respectively. Whereas, Hongayo et al. [5] investigated ethanolic *P. australis* Hauck extract effect against 4 bacterial pathogens. The previous study stated that their antibacterial inhibitory activity could be related to the occurrence of phenol and carotenoids compounds.

In the current study, the highest ZI was observed with methanol *H. musciformis* against *E. faecalis*. Anyway, the ZI could be classified according to the following order: *H. musciformis* (19 mm) >*C. tomentosum* (17 mm) >*P. pavonica* (16 mm) >*L. papillosa* (12 mm) >*D. dischotoma* (11 mm). Otherwise, hexane among the examined solvents showed the lowest ZI for all algae extracts against all tested pathogens.

All over, methanol followed by ethanol was the most active solvent for all examined algae against tested pathogens. In the case of *C. tomentosum* and *D. dischotoma*; it worth noting that *M. luteus* could be considered as the most tolerant pathogen by exhibiting the lowest ZI value with all tested solvents. Manilal et al. [7] investigated methanolic *L. brandenii* extract collected from the southwest coast of India (Indian Ocean) against 9 bacterial pathogens. The previous study showed that the highest ZI was found to be 213 mm^2 against *B. subtilis;* whereas, the lowest one was observed against *Salmonella typhi* (87 mm^2).

In our case study, ZI value were recorded to be 16 mm and 17 mm for methanolic*C. tomentosum* against *B. subtilis* and *S. aureus*, respectively. Similar findings were reported by Alghazeer et al. [12]. The previous study investigated alkaloids of 6 selected algae (2 Chlorophyta, 3 Phaeophyta and 1 Rhodophyta) collected from the western coast of Libya, against 4 Gram-positive bacterial pathogena. The previous study revealed that ZI was found to be 13, 20, 16 and 29 mm against *Bacillus subtilis*, *Bacillus* spp., *S. aureus* and *S. epidermidis*, respectively with *C. tomentosum*.

Our data showed that *C. tomentosum* extracts displayed the highest ZI value against *S. aureus* with all tested solvents. Other study, however, mentioned an inverse finding [16]. The previous study investigated *C. tomentosum* ethanolic, chloroform and diethyl ether extracts against three Gram-positive bacteria. The previous study mentioned that the highest ZI was recorded with chloroform against

Streptococcus sp. Whereas, *S. aureus* showed the lowest ZI with all tested solvents. Recently, Kausalya and Rao [10] investigated the antibacterial effect of *Sargassum tenerrimum* against 6 Gram-positive bacteria.The previous study showed that the highest ZI of 15 mm was recorded with ethanolic extract against *S.aureus.* Recently, Karthick et al. [8] studied the antibacterial effect of methanolic extracts from 5 algae (2 green 2 red and 1 brown species collected from South Andaman, India) against 5 bacterial pathogens. The previous study showed that *Dictyosphaeria cavernosa* of the 5 algae species showed the highest ZI of 18 mm against *S. aureus.*

Whereas, Hamzaet al. [14] studied the antibacterial activity of 2 green(*C. tomentosum* and *U. lactuca*) and 1 red (*H. musciformis*) algae species (collected form the Suez Canal, Egypt) using methanol /methylene chloride agent against 5 bacterial pathogens. The previous study showed that *C. tomentosum* had no activity against examined bacteria except against *S. typhimurium* and *S. boydii*. Whereas, *H. musciformis* and *U. lactuca* extracts showed inhibitory effect against the 5 tested bacteria. In this regards, the highest activity was recorded with *H. musciformis*, and *U. lactuca* against *K. pneumoniae*. Moreover, Kausalya and Rao [9] reported the inhibitory effect of *G. pusillum* and *Centroceros clavatum* algae collected from Visakhapatnam coast, India; against 6 Gram- positive bacteria (*B. subtilis, M. luteus, S. aureus, S. mutans, Streptococcus anginosus* and *Lactobacillus acidophilus*) using chloroform, ethanol, methanol and water solvents. They reported that the ethanolic *G. pusillum* extract exhibited the highest ZI of 19, 18, 17, 16, 16 and 14 mm against *S. aureus, L. acidophilus, M. luteus, S. anginosus, B. subitilis* and *S. mutans*, respectively withconcentration of 500 mg/mL.

The current study assumed that methanol followed by ethanol was the most active extract against all tested pathogens. Indeed, *S. aureus* was the most sensitive isolate with all examined algae and solvent extracts. Similar findings were reported previously by Oumaskour et al. [13] with marine red algae. The previous study showed that methanolic and methanol-Dichloromethan (50:50) were the most potent with ZI > 10 mm. Similarly, Srikong et al. [26] reported that *S. aureus* was the most sensitive pathogen among all tested pathogens with dichloromethane *G. fisheri* extract. Indeed, other study [27] reported that methalonic *L. papilosa* extract among 11 algae species had the highest antibacterial activity with ZI of 14.33 and 13.33 mm against *S. aureus* and *B. subtilis* bacteria, respectively. Recently, Srikong et al. [26] investigated methanol, ethanol, dichloromethane and hexane antibacterial activity of *U. intestinalis* (green) and *G. fisheri* (red) algae species, against 5 positive-Gram bacteria. They reported that the hexane *U. intestinalis* extract exhibited the highest ZI (16.45 mm) against *S. aureus* NPRC 001R (MRSA 001R).

More recently, Saleh and Al-Mariri [17] reported antibacterial activity of three algae species [*U. lactuca* (green), *D. spiralis* (brown) and *J. rubens* (Red)] using similar solvents tested in the current study, against 2 Gram-positive bacterial (*S. pyogenes* and *M. luteus*) pathogens. They reported that the ZI ranged between 6-17 mm. In the regards, the highest ZI value was recorded to be 17 mm with methanolic *D. spiralis*, followed by methanolic *J. rubens* (15 mm) and methanolic *U. Lactuca* (10 mm) against *M. luteus* pathogen.

Algal antibacterial activity has been also evaluated by MIC (Table 4) and MBC (Table 5) values estimation. In this regards, the lowest MIC value was found with methanolic *C. tomentosum* against *S. aureus* (1.1 mg/mL) followed by methanolic *P. pavonica* extract against the same pathogen (1.5 mg/mL) and methanolic *C. tomentosum* against *E. faecalis* (1.5 mg/mL). Statistical analysis revealed that the solvents effect and isolates on MIC values, was significantly ($p < 0.001$) different for all tested algae extracts (Table 4).

As for MBC values (Table 5), the lowest MBC value was recorded to be 2.1 mg/mLwith methanolic *C. tomentosum* and *P. pavonica* extracts against *S. aureus*. Indeed, chloroform, acetone, ethyl acetate and hexane *D. dischotoma* extracts were inactive against all tested pathogens. From Table 5, the effect of solvents on the mentioned parameter, was significantly ($p < 0.001$) different for all tested algae extracts.

Ertürk and Taş [11] reported that MIC value ranged between >1.25 and >10 mg/mL Otherwise, *B. cereus* was the most resistant bacteria by exhibiting the highest MIC value (>10 mg/mL). Whereas, Dulger and Dulger [28] investigated aqueous and ethanolic *P. pavonica* and *Cystoseira compressa* extracts against Methicillin-Resistant *S. aureus*. The previous study showed that ethanolic *C. compressa* extracts were the most potent with MIC of 3.2-6.3 mg/mL and MBC of 6.3-25 mg/mL. While, aqueous extracts have an inhibitory activity with MIC of 6.3-12.5 mg/mL and 12.5-25 mg/mL; whereas, MBC value was recorded to be 12.5-25 mg/mL and 25-50 mg/mL for *C. compressa* and *P. pavonica*, respectively. Moreover,

Kavita et al. [27] reported that the methalonic *L. papilosa* extract was the most potent among 11 algae species with MIC50 of 0.00053 and 0.00106 mg/mL against *S. aureus* and *B. subtilis* bacteria, respectively. Recently, Selim et al. [29] investigated inhibitory effect of *H. esperi* (red) and *Caulerpa prolifera* (green) algal species against *B. subtilis* and *S. aureus* pathogens. The previous study showed that algal *H. esperi* and *C. prolifera* extracts C, exhibited an antibacterial activity with MIC/MBC value of 0.3/0.4 and 0.5/0.5 mg/mL, respectively against *B. subtilis*. Whereas, it was recorded to be 0.5/0.7 and 0.6/0.6 mg/mL with H. esperi and C. prolifera extracts, respectively against *S. aureus*. Whereas, Srikong et al. [26] studied methanol, ethanol, dichloromethane and hexane antibacterial activity of *U. intestinalis* (green) and *G. fisheri* (red) algae species, against 5 positive-Gram bacteria. They reported that the lowest MIC/MBC value of 0.256/0.001024 mg/mL was recorded with dichloromethane *G. fisheri* extract against *S. aureus* ATCC 29213 and hexane against *B. cereus* TISTR 687.More recently, Saleh and Al-Mariri [17] reported antibacterial activity of three algae species [*U. lactuca* (green), *D. spiralis* (brown) and *J. rubens* (Red)] using similar solvents tested in the current study, against 2 Gram-positive bacterial (*S. pyogenes* and *M. luteus*) pathogens. The previous study suggested that *M. luteus* as the most sensitive pathogen by exhibiting the lowest MIC/MBC value of 26.7/53.3 µg/mL with chloroform *D. spiralis* extract.

In summary of the current investigation, alga phytochemical assay revealed the presence of flavonoids in all methanolic algal extracts. Whereas, proteins were absent in all algal extracts regardless of tested solvents. While, phenols were presented with all tested solvents for both the *D. dischotoma* and *P. pavonica* extracts regardless tested solvents. This observation was in agreement of Saleh and Al-Mariri [17] who reported similar results in three algae species [*Ulva lactuca* (green), *Dilophus spiralis* (brown) and *Jania rubens* (red)] using similar solvents tested in the current study. Antibacterial activity test revealed that methanol *H. musciformis*displayed the highest ZI of 19 mm against *E. faecalis*, followed by methanol *C. tomentosum* and *H. musciformis* extracts against *S. aureus* (17 mm). Overall, methanolic *C. tomentosum* was the most effective by showing the lowest MIC/MBC value of 1.1/2.1 mg/mLfollowed by methanolic *P. pavonica* (1.5/2.1 mg/mL) against *S. aureus*.

Conclusion

In conclusion, algal antibacterial activity against six Gram-positive bacterial pathogens has been evaluated based on ZI, MIC and MBC values estimation. The current study could be suggest that *M. luteus* as the most resistant pathogen by exhibiting the lowest ZI and highest MIC and MBC values. All over, the current study could suggest that the inhibitory effect was in the following order: Chlorophyta > Phaeophyta > Rhodophyta. Due to the highest observed antibacterial activity of *C. tomentosum* and *P. pavonica* extracts; further and performance studies on isolation, characterization and function of bioactive components of these algae species are needed.

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anticoagulant activities from *Hypnea esperi* and *Caulerpa prolifera* marine algae. Pak J Pharm Sci, 28: 525-530.

Table 1: Description of original sites where algae species were collected

Algae phyla	Algae species	Latitude	Longitude
Chlorophyta	*C. tomentosum*	35°33′990″N	35°44′288″E
Rhodophyta	*H. musciformis*	35°33′786″N	35°43′992″E
	L. papillosa	35°33′786″N	35°43′992″E
Phaeophyta	*D. dischotoma*	35°33′917″N	35°44′179″E
	P. pavonica	34°37′734″N	38°29′766″E

Table 2: Algal phytochemical analysis using different examined solvents

Chemical components	Aqueous	Methanol	Ethanol	Chloroform	Acetone	Ethyl acetate	Hexane
C. tomentosum							
Alkaloids	-	+	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+
Saponins	+	+	+	+	-	+	+
Terpenoids	-	+	+	+	-	+	+
Tannins	+	+	+	-	-	-	+
Steroids	-	-	+	-	+	+	-
Carohydrates	-	+	-	+	-	-	-
Proteins	-	-	-	-	-	-	-
Phenols	+	-	+	+	+	+	+
L. papillosa							
Alkaloids	+	+	+	-	+	+	+
Flavonoids	+	+	+	+/-	-	-	+
Saponins	+	++	+	-	+	+	+
Terpenoids	-	-	-	-	-	-	-
Tannins	+	+	++	+/-	+	+	-
Steroids	+	+	+	+	-	-	+
Carohydrates	+	+	+	+	-	+	+
Proteins	-	-	-	-	-	-	-
Phenols	+	+	+	+	-	+	+
H. musciformis							
Alkaloids	+	+	+	-/+	+	+	+
Flavonoids	+	+	+	+	-	-	-
Saponins	+	+	+	-	+	+	+
Terpenoids	-	-	-	-	-	-	-
Tannins	+	++	+	+	-/+	+	+
Steroids	+	++	++	+	-	+	+
Carohydrates	+	+	+	+	-	-	+
Proteins	-	-	-	-	-	-	-
Phenols	+	+	+	+	-	+	+
D. dischotoma							
Alkaloids	-	-	-	-	-	-	-
Flavonoids	-	+	+	+	-	+	-

Saponins	-	-	-	++	-	++	+
Terpenoids	-	+	+	+	-	+	+
Tannins	++	+	+	-	+	-	-
Steroids	-	-	-	++	+	+	-
Carohydrates	-	+	+	+	-	-	-
Proteins	-	-	-	-	-	-	-
Phenols	+	++	++	+	+	+	++
P. pavonica							
Alkaloids	-	+	+	-	-	-	-
Flavonoids	-	+	+	-	-	-	-
Saponins	-	-	-	++	-	+++	-
Terpenoids	-	-	-	-	-	+	+
Tannins	+	+	++	+	+	+	+
Steroids	-	-	-	-	+	-	-
Carohydrates	-	+	+	-	-	+	-
Proteins	-	-	-	-	-	-	-
Phenols	+	++	++	+	+	+	+

Legend: (-) Absent; (+) Present; (++) Higher presence.

Table 3: Algal antibacterial activity using disc-diffusion method (zone of inhibition in mm).

Zone of inhibition (ZI) (mm)							
Microorganisms	**Methanol**	**Ethanol**	**Chloroform**	**Acetone**	**Ethyl acetate**	**Hexane**	**Control**
C. tomentosum							
S. aureus	17±0.45Aa	15±0.25Ba	13±0.19Da	15±0.35Ba	14±0.25Ca	12±0.34Ea	25±0.3
E. faecalis	15±0.4Ac	13±0.5Bc	12±0.3Cb	15±0.22Aa	13±0.09Bb	11±0.3Db	23±0.28
L. monocytogeneses	14±0.29Bd	14±0.33Bb	12±0.26Cb	15±0.35Aa	14±0.22Ba	12±0.15Ca	18±0.19
B. cereus	15±0.17Ac	13±0.26Cc	13±0.5Ca	15±0.28Aa	14±0.35Ba	11±0.27Db	20±0.12
B. subtilis	16±0.27Ab	14±0.35Bb	13±0.25Ca	14±0.4Bb	13±0.38Cc	12±0.26Da	20±0.17
M. luteus	11±0.17Ae	9±0.13Cd	10±0.08Bb	8±0.09Dc	7±0.11Ed	+	23±0.32
L. papillosa							
S. aureus	12±0.4Aa	10±0.27Bb	7±0.17Db	10±0.2Ba	+	8±0.29Ca	25±0.3
E. faecalis	11±0.3Ab	11±0.45Aa	7±0.42Cb	10±0.46Ba	-	+	23±0.28
L. monocytogeneses	10±0.33Bb	11±0.37Aa	7±0.45Cb	+	+	-	18±0.19
B. cereus	12±0.4Aa	10±0.39Bb	9±0.55Ca	8±0.41Db	+	+	20±0.12
B. subtilis	9±0.31Ad	11±0.53Ba	+	6±0.26Dc	7±0.19Ca	+	20±0.17
M. luteus	12±0.18Aa	10±0.36Bb	9±0.41Cb	8±0.26Db	-	+	23±0.32
H. musciformis							
S. aureus	17±0.35Ab	15±0.25Bb	13±0.24Da	11±0.3Ed	10±0.27Fc	14±0.35Ca	25±0.3
E. faecalis	19±0.45Aa	16±0.22Ba	12±0.19Db	13±0.22Cb	11±0.23Eb	10±0.15Fe	23±0.28

L. monocytogeneses	16±0.35Ac	16±0.27Aa	13±0.22Ca	14±0.37Ba	12±0.37Da	11±0.24Ed	18±0.19
B. cereus	17±0.2Ab	16±0.45Ba	12±0.29Db	13±0.27Cb	11±0.3Eb	12±0.29Dc	20±0.12
B. subtilis	16±0.37Ac	14±0.4Bc	13±0.25Ca	11±0.35Ed	12±0.27Da	13±0.2Cb	20±0.17
M. luteus	15±0.09Ad	13±0.24Bd	13±0.15Ba	12±0.13Cc	11±0.07Db	9±0.19Ef	23±0.32
D. dischotoma							
S. aureus	9±0.15Ac	7±0.19Cc	5±0.2Ec	8±0.25Ba	6±0.19Da	6±0.27Da	25±0.3
E. faecalis	10±0.35Ab	8±0.27Bb	6±0.17Cb	6±0.28Cb	+	6±0.15Ca	23±0.28
L. monocytogeneses	11±0.38Aa	10±0.29Ba	7±0.25Da	8±0.19Ca	+	+	18±0.19
B. cereus	11±0.25Aa	10±0.35Ba	7±0.2Ca	6±0.19Db	+	+	20±0.12
B. subtilis	9±0.22Ac	7±0.13Bc	+	+	+	6±0.19Ca	20±0.17
M. luteus	6±0.17Ad	6±0.14Ad	+	+	-	-	23±0.32
P. pavonica							
S. aureus	12±0.15Ac	13±0.14Ab	10±0.08Cc	10±0.17Cc	7±0.09Dd	11±0.12Ba	25±0.3
E. faecalis	15±0.28Ab	13±0.29Bb	11±0.11Db	12±0.35Ca	13±0.33Bb	9±0.17Eb	23±0.28
L. monocytogeneses	16±0.27Aa	14±0.11Ca	13±0.19Da	11±0.22Eb	15±0.27Ba	9±0.09Fb	18±0.19
B. cereus	11±0.19Ad	9±0.08Bd	11±0.07Ab	9±0.18Bd	6±0.06Ce	7±0.04Dc	20±0.12
B. subtilis	9±0.1Ae	7±0.09Ce	6±0.14Dd	8±0.13Be	-	-	20±0.17
M. luteus	11±0.13Bd	10±0.09Cc	13±0.2Aa	7±0.07Ef	9±0.22Dc	-	23±0.32

Legend:Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at P = 0.05 probability by Fisher's PLSD test. LSD0.05 solvent and isolate 0.664 for *C. tomentosum*, *H. musciformis* and *D. dichotoma*; whereas, it was 0.636 for both *L. papillosa* and *P. pavonica*.
(-): Nil activity; (+): Trace activity (little)(< 6mm)

Table 4: Algal minimum inhibition concentration (MIC) values against tested pathogens

Minimum inhibitory concentration (MIC) (mg/mL)							
Microorganisms	**Methanol**	**Ethanol**	**Chloroform**	**Acetone**	**Ethyl acetate**	**Hexane**	**Control**
C. tomentosum							
S. aureus	1.1Bb	2.1Bc	2.1Bc	1.7Bc	2.1Bb	8.3Ab	0.5±0.1
E. faecalis	1.5Cb	2.1Cc	3.3Bb	2.9Ba	1.7Cc	8.3Ab	3±1
L. monocytogeneses	2.5Cb	3.3Bb	3.3Bb	3.3Ba	2.1Cb	10.0Aa	2±1
B. cereus	3.3Ba	3.3Bb	4.2Ba	3.3Ba	2.1Cb	10.0Aa	0.19±0.01
B. subtilis	3.3Ba	3.3Bb	4.2Ba	4.2Ba	2.9Cb	10.0Aa	0.15±0.01
M. luteus	4.2Ca	6.7Ba	5.8Ba	4.2Ca	5.8Ba	>10.0Aa	4±1
L. papillosa							
S. aureus	8.3Cb	10.0Cb	16.7Bb	16.7Bb	20.0Aa	20.0Aa	0.5±0.1

E. faecalis	8.3Db	10.0Db	13.3Cc	13.3Cc	16.7Bb	20.0Aa	3±1
L. monocytogeneses	10.0Db	11.7Cb	13.3Cc	16.7Bb	20.0Aa	20.0Aa	2±1
B. cereus	8.3Db	8.3Dc	13.3Cc	16.7Bb	20.0Aa	20.0Aa	0.19±0.01
B. subtilis	10.0Db	11.7Cb	13.3Cc	16.7Bb	20.0Aa	20.0Aa	0.15±0.01
M. luteus	16.7Ba	20.0Aa	20.0Aa	20.0Aa	>20.0Aa	>20.0Aa	4±1
H. musciformis							
S. aureus	5.8Bb	5.8Bc	10.8Ab	11.7Ab	10.8Ac	13.3Ab	0.5±0.1
E. faecalis	3.3Cc	5.4Bc	9.2Bb	13.3Ab	13.3Ab	16.7Ab	3±1
L. monocytogeneses	5.8Bb	8.3Bb	13.3Ab	16.7Ab	13.3Ab	16.7Ab	2±1
B. cereus	9.2Bb	10.0Bb	11.7Ab	13.3Ab	16.7Ab	13.3Ab	0.19±0.01
B. subtilis	8.3Bb	11.7Ab	13.3Ab	16.7Ab	16.7Ab	16.7Ab	0.15±0.01
M. luteus	16.7Ea	20.0Da	23.3Da	33.3Ca	40.0Ba	66.7Aa	4±1
D. dischotoma							
S. aureus	23.3Bb	30.0Ab	ND	ND	ND	ND	0.5±0.1
E. faecalis	16.7Ac	20.0Ac	ND	ND	ND	ND	3±1
L. monocytogeneses	16.6Bc	23.3Ac	ND	ND	ND	ND	2±1
B. cereus	13.3Bc	30.0Ab	ND	ND	ND	ND	0.19±0.01
B. subtilis	16.7Ac	13.3Ad	ND	ND	ND	ND	0.15±0.01
M. luteus	40.0Aa	40.0Aa	ND	ND	ND	ND	4±1
P. pavonica							
S. aureus	1.5Cc	1.7Cd	2.1Ce	4.2Bd	3.3Bb	8.3Ab	0.5±0.1
E. faecalis	2.9Cb	3.3Cc	4.2Bd	5.0Bc	2.1Dc	6.7Ac	3±1
L. monocytogeneses	3.3Db	6.7Cb	5.8Cc	8.3Bb	4.2Db	10.0Aa	2±1
B. cereus	8.3Bb	7.5Bb	7.5Bb	8.3Bb	10.0Aa	10.0Aa	0.19±0.01
B. subtilis	10.0Aa	10.0Aa	10.0Aa	>10.0Aa	10.0Aa	>10.0Aa	0.15±0.01
M. luteus	10.0Aa	10.0Aa	10.0Aa	10.0Aa	10.0Aa	10.0Aa	4±1

Legend:ND: Not determined. Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at $P = 0.05$ probability by Fisher's PLSD test. $LSD_{0.05}$ solvent and isolate 1.084, 2.597, 5.073, 5.790 and 1.107 for *C. tomentosum, L. papillosa, H. musciformis, D. dischotoma* and *P. pavonica,* respectively.

Table 5: Algal minimum bactericidal concentration (MBC) values against tested pathogens

Minimum bactericidal concentration (MBC) (mg/mL)							
Microorganisms	**Methanol**	**Ethanol**	**Chloroform**	**Acetone**	**Ethyl acetate**	**Hexane**	**Control**
C. tomentosum							
S. aureus	2.1Cc	3.3Bc	4.2Bc	3.3Bc	4.2Bd	10.0Aa	1±0
E. faecalis	3.3Cb	3.3Cc	5.0Bc	4.2Bc	4.2Bd	10.0Aa	6±1
L. monocytogeneses	4.2Db	4.2Dc	6.7Cb	6.7Cb	8.3Bb	10.0Aa	4±1
B. cereus	4.2Db	5.0Db	6.7Cb	6.7Cb	8.3Bb	10.0Aa	0.4±0.1
B. subtilis	4.2Cb	5.0Cb	6.7Bb	6.7Bb	6.7Bc	10.0Aa	0.3±0.1

M. luteus	8.3Ba	10.0Aa	10.0Aa	10.0Aa	10.0Aa	>10.0Aa	8±0.1
L. papillosa							
S. aureus	13.3Bb	13.3Bc	20.0Aa	20.0Aa	20.0Aa	>20.0Aa	1±0
E. faecalis	13.3Cb	13.3Cc	16.7Bb	16.7Bb	20.0Aa	>20.0Aa	6±1
L. monocytogeneses	13.3Cb	16.7Bb	20.0Aa	20.0Aa	20.0Aa	>20.0Aa	4±1
B. cereus	13.3Cb	13.3Cc	16.7Bb	20.0Aa	20.0Aa	>20.0Aa	0.4±0.1
B. subtilis	13.3Cb	16.7Bb	20.0Aa	20.0Aa	20.0Aa	>20.0Aa	0.3±0.1
M. luteus	20.0Aa	20.0Aa	>20.0Aa	20.0Aa	>20.0Aa	>20.0Aa	8±0.1
H. musciformis							
S. aureus	6.7Bc	8.3Bd	15.0Ab	16.7Ab	16.7Ab	16.7Ab	1±0
E. faecalis	6.7Dc	8.3Dd	13.3Cd	20.0Aa	16.7Bb	20.0Aa	6±1
L. monocytogeneses	8.3Cc	13.3Bc	20.0Aa	20.0Aa	20.0Aa	15.0Bb	4±1
B. cereus	11.7Cb	13.3Cc	16.7Bb	20.0Aa	20.0Aa	20.0Aa	0.4±0.1
B. subtilis	13.3Cb	16.7Bb	16.7Bb	20.0Aa	20.0Aa	10.0Dc	0.3±0.1
M. luteus	16.7Ba	20.0Aa	20.0Aa	20.0Aa	>20.0Aa	>20.0Aa	8±0.1
D. dischotoma							
S. aureus	33.3Bb	40.0Ac	ND	ND	ND	ND	1±0
E. faecalis	33.3Ab	30.0Ad	ND	ND	ND	ND	6±1
L. monocytogeneses	26.7Bc	33.3Ad	ND	ND	ND	ND	4±1
B. cereus	26.7Bc	53.3Ab	ND	ND	ND	ND	0.4±0.1
B. subtilis	33.3Ab	33.3Ad	ND	ND	ND	ND	0.3±0.1
M. luteus	66.7Ba	80.0Aa	ND	ND	ND	ND	8±0.1
P. pavonica							
S. aureus	2.1Dc	2.9Dc	4.2Cb	4.2Cb	6.7Bb	>10.0Aa	1±0
E. faecalis	4.2Db	5.8Cb	8.3Ba	8.3Ba	7.5Bb	>10.0Aa	6±1
L. monocytogeneses	4.2Db	5.8Cb	8.3Ba	8.3Ba	7.5Bb	>10.0Aa	4±1
B. cereus	10.0Aa	10.0Aa	10.0Aa	10.0Aa	10.0Aa	>10.0Aa	0.4±0.1
B. subtilis	10.0Aa	10.0Aa	10.0Aa	>10.0Aa	10.0Aa	>10.0Aa	0.3±0.1
M. luteus	>10.0Aa	>10.0Aa	>10.0Aa	>10.0Aa	>10.0Aa	>10.0Aa	8±0.1

Legend:ND: Not determined. Figures sharing same lowercase letter (column) and capital letter (row) are not significantly different at $P = 0.05$ probability by Fisher's PLSD test. $LSD_{0.05}$ solvent and isolate 1.155, 2.305, 2.805 and 5.311 for *C. tomentosum, L. papillosa, H. musciformis* and *D. dischotoma*, respectively. Whereas, $LSD_{0.05}$ solvent 1.011 and isolate 1.669 for *P. pavonica.*

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