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Research Article

Antifungal, antibacterial and insecticidal potential of *Chara schweinitzii* (A. Braun) Kützing in Charsadda, Pakistan

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Abstract
Various organic solvents were experimented for the screening out of the natural activities of *Chara schweinitzii* and crude ethanolic extracts. *In vitro* potential like antifungal activities, antibacterial activities, insecticidal activities and some extracts (n-hexane, chloroform, ethyl acetate, methanol and ethanol) were tested into the experiment. The results of our study showed some major response and vital antifungal activity by the *C. schweinitzii* for the tested fungal species. Disc diffusion and agar well diffusion method was used to test the extract. The experiment was observed, where methanol extracts were inhibited the growth of the used bacteria. Completed tests of *C. schweinitzii* were carefully inspected against the resulted bacterial strains. The impregnated filter paper method process was used for investigation of the insecticidal potential of the algal extracts. The scope of our study is to understand the importance to natural resources (bioactive compounds) for pharmaceutical industry as well as our study is based on the premier findings in Nisatta region, district Charsadda.

Keywords: Antibacterial; Antifungal; *C. schweinitzii*; Insecticidal

Introduction

Algae are thought to be a vital character in our society by providing us various bioactive compounds. They create different active metabolites, which are important in pharmaceutical manufacturing **[1]**. The occurrence of calcium and magnesium carbonate in submerged muddy and watery bottom of the pools and ponds of Charsadda region is in galore. The presence of heavy water in the tested region is also in abundance, which is an excellent characteristic of the Charsadda region for collection of the said specie **[2]**. The aquatic creatures are also getting the diverse variety of food, shelter and other basic needs through algae **[3]**. Chara is identified by its macroscopic, multicellular, pro-fusely branched thalloid plant body, generally attains a height of about 20-30 cm (rarely about 1 meter) and is also differentiated into rhizoid and main axis **[4]**. Many vital food sources are provided by algae like; certain vitamins, some fatty acids, types of minerals and many polysaccharides. Algae is also important for its verse structure of poisonous and lively compounds for different other substances **[5-8]**. The plants of chara are of

great ecological value and highly important. As they are covered with calcium carbonate deposits, they deposit lot of calcium in the bottom of lake, etc., and after a considerable time the whole lake or pond is filled up with calcareous deposits [2]. Many aquatic structures are majorly based on algae, where algae provide them major food sources. *C. schweinitzii* is found in fresh water and belongs to the class; Charophyta. It is cosmopolitan specie and is found worldwide. Chara has some local names which are, "Sand grass, stonewort and musk Grass". Some of them grow in shallow water while most of them grow in deep water from, 4 cm to 20 m. The chara sticks to the bushy bottom of water, and are not found in oxygenated water or hard water. Their color and size ranges from green to light-gray and 1 mm - 5 cm in length, respectively. The branchlets make the whorl of musk grass, which are grouped at continuous linkages. They are easily been differentiated from other species of chara as cortex is absent and are monoecious. The above portion of the water surface covers it which makes the chara highly tolerable and protective into the environment. The need and focus of our study has been made that within the genus chara the condition of monoecism or dioecism is of limited taxonomic significance (especially in the tested region), and that our study reflects only minor variation, and is good sufficient to improve the literature for the genus chara [9]. The key objective of our study was to uncover the buried natural means, which are used in pharmaceutical industry for the benefit of the human beings.

Materials and methods

Collection sites

The experiment was performed at the Department of Botany, Bacha Khan University, Charsadda to screen out the biological activities of one of the algal specie "*C. schweinitzii* (A. Braun) Kützing". The test specie was collected from Nisatta, Charsadda region from fresh shallow water through gloved hands from various small and large freshwater bodies like; canals, small streams and ponds. Our study is based on the methods of Khalid *et al*. [8].

Algal material

The experiment was started in September 2014-15 and the algal material was initiated to collect. The material was then brought to the lab, where attached dust, animal dung, external parasitic organisms, aerophytes, litter, insects, algae, spirogyra and sand particles were removed by gloved hands and washed thoroughly with the clean tap water. The major mass of the material was shade dried under the laboratory conditions. After a day, the breakdown of bulky molecules of certain thermo labile natural products initiated. The dried mass was then cut into tiny pieces and weighed on the balance.

Antifungal activities

Our study included seven fungal species (Figures 1, 2 and 7) which were used to check the antifungal biological activities (*Trichophyton longifusus, T. harzianum, Trichoderma hamatum, Rhizoctonia solani, P. oedochilum, Pythium aphanidermatum, Microsporum canis, Fusarium moniliforme, F. oxysporum* and *Aspergillus flavipes*). The agar well diffusion method was used to test the antifungal activity for the above mentioned fungal species. The method for various tests and algal extraction process has been discussed earlier [8].

Antibacterial activities

The performed test was the *in vitro* anti-bacterial bioassay (Figures 3, 4, 5 and 8) which was on the various selected plant extracts against Xanthomonas and *Clavibacter michiganensis* (Phytopathogenic bacteria) and *E. coli* (human pathogenic bacteria) performing the disc diffusion method [10]. The numbers of concentrations were two. The Streptomycin sulphate (1mg ml^{-1}) was among the experiment and was

used as a positive control while methanol as a negative control. The incubation of the antibacterial assay plates was done at 37 °C for 24 hours and finally the diameters of the zone of inhibition were measured in mm.

Insecticidal activities

The impregnated filter paper process was used for investigating the insecticidal potential (Figures 6 and 9) of crude algal extracts **[11]**. The test was passed in petri plates (9 cm diameter) delivered with dual folds of the filter papers. To prepare the test sample, 200mg algal extract (from each extract) was dissolved in 3ml of methanol solvent. The sensitivity of the experiment was considered and the selection of the insect was done. The well insects (same size) were considered for the experiment.

The sizes of the petri plates were measured and the filter papers were cut of the same size. Double folds of the filter papers were kept inside the sterilized and clean petri plates. The tested sample was transferred on the filter paper. It was then freed so that the filter paper absorbs the solvent. The collected insects (healthy) were put into the petri plate with the help of the clean brush, which were further incubated at 27 °C into the incubator. The similar method was conducted for every selected insect species into the experiment. For the positive control and negative control, the permethrin and DMSO were used. The checking of the extracts petri plates were observed after a day of incubation and percent mortality for every used extract was find out by the formula:

Percent mortality = 100 - Number of alive insects in test / Number of alive insects in control × 100 **[8]**.

The insects which were used assess the insecticidal potential of the methanol extracts of algal species are:

Termite and

Tribolium castaneum.

Results

The crude ethanolic extract and various organic solvent fractions (ethanolic extracts) were brought into use. Different *in vitro* activities like; insecticidal activities, antibacterial activities and antifungal activities were conducted.

The crude extract revealed the highest potential (Table 1) against *T. harzianum* with the zone of inhibition (21 mm), following moderate activity against *Pythium sp*. with the zone of inhibition (21 mm), *Penicillium sp*. (19 mm), *Fusarium solani* (15 mm), *Aspergillus flavipes* (17 mm) and *Aspergillus niger* with the zone of inhibition (12 mm). While the extract showed least activity for *Microsporum canis* with zone of inhibition (11 mm) (Table 1). The percent inhibition of crude for *T. harzianum* revealed (34.38%), following *Pythium sp*. (65.62%), *Penicillium sp*. (59.25%), *Fusarium solani* (46.88%), *Microsporum canis* (53.12%), *Aspergillus flavipes* (34.38%), while the least percent inhibition of crude revealed for *Aspergillus niger* with (37.5%).

Table 1. Growth % inhibition of *C. schweinitzii* (A. Braun) Kützing against selected fungi strains

Solvent	Test fungi	Antifungal activity of sample (mm)	Antifungal activity of control (mm)	% Inhibition
Chloroform	*T. harzianum*	21	32	65.62
	Pythium sp.	19	32	59.37
	Penicillium sp.	15	32	46.88
	Fusarium solani	19	32	59.38
	Microsporum canis	13	32	40.62
	Aspergillus flavipes	11	32	34.38
	Aspergillus niger	13	32	40.62
n-hexane	*T. harzianum*	17	32	53.5
	Pythium sp.	21	32	65.75
	Penicillium sp.	20	32	62.5
	Fusarium solani	13	32	40.62
	Microsporum canis	13	32	40.75
	Aspergillus flavipes	10	32	31.25
	Aspergillus niger	11	32	34.38
Crude methanol extract	*T. harzianum*	21	32	65.62
	Pythium sp.	19	32	59.25
	Penicillium sp.	15	32	46.88
	Fusarium solani	17	32	53.12
	Microsporum canis	11	32	34.38
	Aspergillus flavipes	12	32	37.5
	Aspergillus niger	12	32	37.5
Ethyl acetate	*T. harzianum*	20	32	62.5
	Pythium sp.	19	32	59.75
	Penicillium sp.	15	32	46.87
	Fusarium solani	11	32	34.62
	Microsporum canis	14	32	43.75
	Aspergillus flavipes	10	32	31.25
	Aspergillus niger	11	32	34.38
	T. harzianum	20	32	62.5

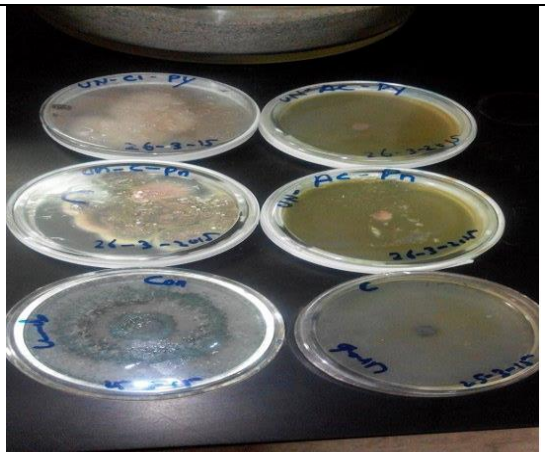

Figure 1. Anti-fungal % growth inhibition of *C. schweinitzii* (A. Braun) Kützing in control and crude extracts.

Figure 2. Anti-fungal % growth inhibition of *C. schweinitzii* (A. Braun) Kützing in control and crude extracts.

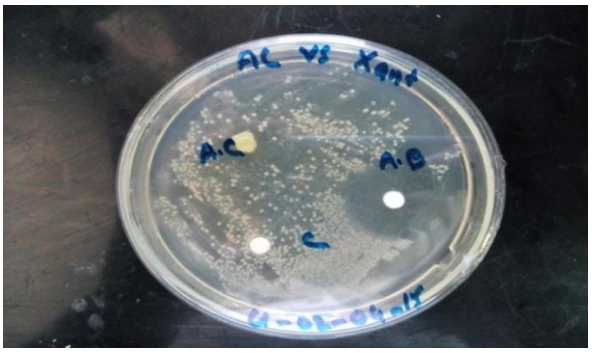

Figure 3. Zone of inhibition and % inhibition in *Xanthomonas campestral*.

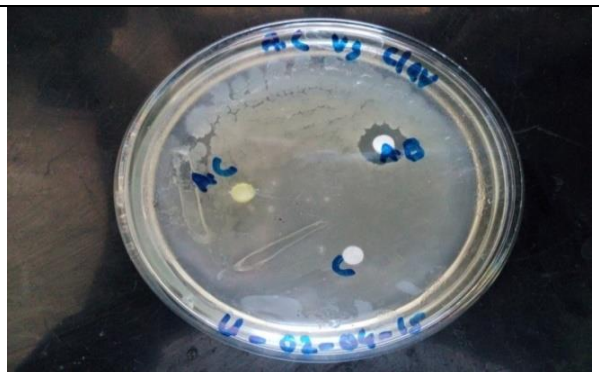

Figure 4. Zone of inhibition and % inhibition in Clavibacter.

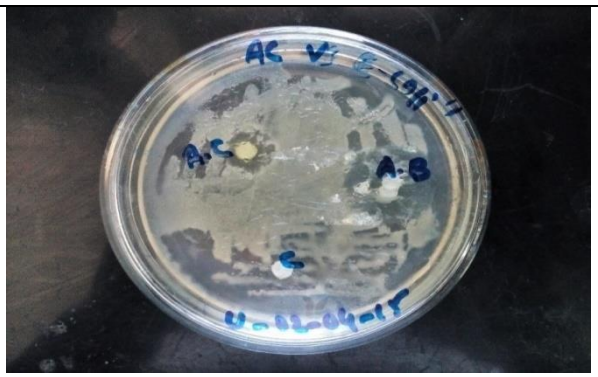

Figure 5. Zone of inhibition and % inhibition in *E. coli*.

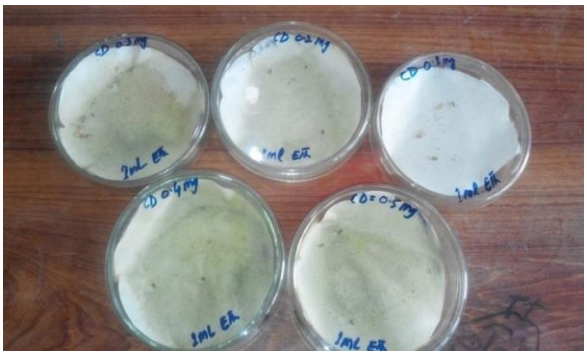

Figure 6. Insecticidal activity of the aqueous solution against *Tribolium castaneum*.

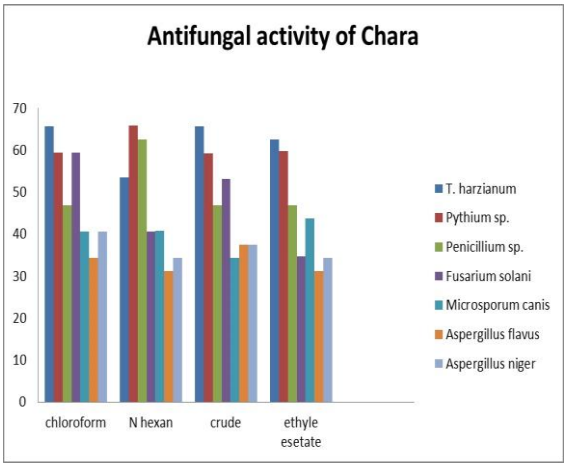

Figure 7. Antifungal activity of chloroform, n-hexane, crude and ethyl acetate against the selected strains of chara.

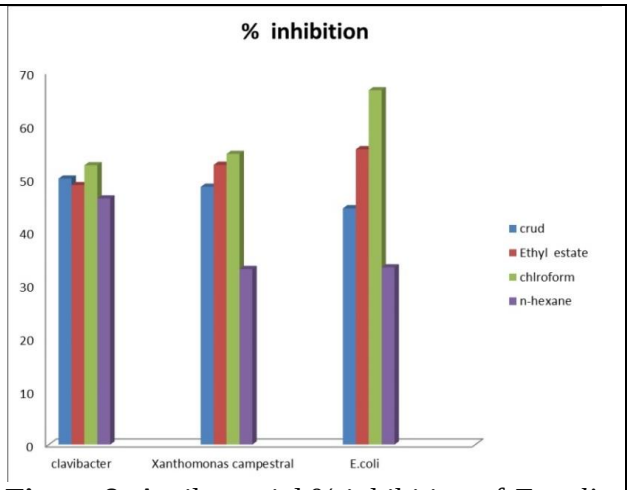

Figure 8. Antibacterial % inhibition of *E. coli*, *X. campestral* and clavibacter against the selected strains of chara.

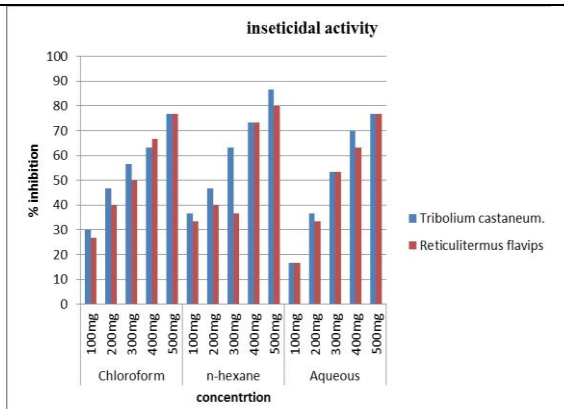

Figure 9. Insecticidal activity of n-hexane, chloroform and aqueous solution against *T. castaneum* and *R. flavipes.*

The greatest activity was revealed by the chloroform extract for *T. harzianum*, with the zone of inhibition (21 mm). The moderate activity was shown by *Pythium sp*. with (19 mm), followed by *Penicillium sp*. (15 mm), *Fusarium solani* (19 mm), *Microsporum canis* (13 mm) and *Aspergillus niger* (13 mm). While the least activity was revealed by *Aspergillus flavipes* with the zone of inhibition (11 mm) (Table 1).

The n-hexane extract revealed maximum activity for *Pythium sp*. with the zone of inhibition (21 mm) while the moderate activity was revealed by *T. harzianum* (17 mm), *Penicillium sp*. (21 mm), *Fusarium solani* (20 mm), *Microsporum canis* (13 mm) and *Aspergillus niger* (13 mm), while the least potential was revealed by *Aspergillus flavipes* with the zone of inhibition (10 mm).

The ethyl acetate extract demonstrated greatest activity for *T. harzianum* with the zone of inhibition (20 mm), while moderate potential for *Pythium sp.* (19 mm), *Penicillium sp.* (15 mm), *Fusarium solani* (11 mm), *Microsporus canis* (14 mm) and *Aspergillus niger* with the zone of inhibition (11 mm), while the least potential was showed against *Aspergillus flavipes* with the

zone of inhibition (10 mm). Our study is in line with the experiment of Mahadevi *et al*. **[9]**.

The antibacterial examinations to find out the extracts of *C. schweinitzii* were tested against various bacterial strains (Table 2). The zone of inhibition for clavibacter revealed (17 mm), followed by ethyl acetate (21 mm), chloroform (22 mm), while the least was revealed against n-hexane (20 mm). The zone of inhibition for *Xanthomonas campestral* revealed (0.47 mm), followed by ethyl acetate (0.51 mm), chloroform (0.53 mm), while the least was showed against n-hexane (0.32 mm). The zone of inhibition for *E. coli* crude was revealed (0.40 mm), followed by ethyl acetate (0.50 mm), chloroform (0.60 mm), while the least was revealed against n-hexane with the zone of inhibition (0.30 mm).

The percent inhibition in crude for clavibactor was recorded (50%), followed by ethyl acetate (48.75%), chloroform (52.5%), while the least percent inhibition was recorded for n-hexane (46.25%). The percent inhibition for *Xanthomonas campestral* in crude extract revealed (48.45%), followed by ethyl acetate (52.57%), chloroform (54.63%), while for n-hexane it revealed (32.98%). The percent inhibition of *E. coli* for crude extract revealed (44.4%), followed by ethyl acetate (55.5%), chloroform (66.6%) while in n-hexane it revealed (33.3%).

Table 2. Zone of inhibition and % inhibition of antibacterial bioassay of the test insects

Treatments	**Test species**					
	Clavibacter		***Xanthomonas campestral***		***E. coli***	
	Zone of inhibition (cm)	**% Inhibition**	**Zone of inhibition (cm)**	**% Inhibition**	**Zone of inhibition (cm)**	**% Inhibition**
-ve control	0	-	0	-	0	-
+ve control	0.8±0.10	-	0.97 ± 0.25	-	0.90 ± 0.01	-
Crude	0.40±0.10	50	0.47 ± 0.06	48.45	0.40 ± 0.01	44.4
Ethyl acetate	0.39±0.01	48.75	0.51± 0.08	52.57	0.50±0.01	55.5
Chloroform	0.42±0.12	52.5	0.53±0.07	54.63	0.60±0.02	66.6
n-hexane	0.37±0.10	46.25	0.32 ± 0.01	32.98	0.30±0.04	33.3

The insecticidal activities of crude methanolic extracts in chloroform, n-hexane and aqueous were conducted by screening technique against *Tribolium castaneum* and *Reticulitermus flavipes* (Table 3). The *T. castaneum* and *R. flavipes* were selected as the test insects. The crude extracts and the fractions revealed noteworthy insecticidal potential for both the tested insects. The crude extracts and fraction revealed dose dependent potential.

Results revealed (Table 3) important percent lethality for the tested insect (*T. castaneum*). Our data showed the highest percent lethality in chloroform (76.67%) at (500mg) while the least percent lethality revealed (30.00%) at (100mg). The percent lethality revealed at (200mg), (300mg), (400mg) were (46.67%), (56.67%) and (63.33%), respectively. Our data showed the highest percent lethality for n-hexane (86.67%) at (500mg) while the least percent lethality was revealed (36.67%) at (100mg). The percent lethality revealed at (200mg), (300mg), (400mg) revealed (46.67%), (63.33%) and 7(3.33%), respectively.

Our results declared important percent lethality for the test insect (*T. castaneum*). Our data revealed the highest percent lethality in aqueous (76.67%) (500mg) while the least was revealed at (100mg) which is (16.67%). The percent lethality revealed at

(200mg), (300mg) and (400mg) was (36.67%), (53.33%) and (70.00%), respectively. Results (Table 4) revealed the important percent lethality for the test insect (*R. flavipes*). Our data revealed the greatest percent lethality for chloroform (76.67%) at (500mg) while the least was revealed at (100mg) which is (26.67%). The percent insecticidal lethality against *T. castaneum* revealed (40.00%) at (200mg), followed by (50.00%) at (300mg) and (66.67%) at (400mg). Results (Table 4) revealed the significant percent lethality against the test insect (*R. flavipes*). Our data revealed the greatest percent lethality for n-hexane (80.00%) at (500mg) while the least percent lethality revealed (33.33%) at (100mg). The percent lethality at (200mg) revealed (40.00%), followed by (36.67%) at (300mg) and (73.33%) at (400mg).

Table 3. Insecticidal activity against *Tribolium castaneum*

S. No	Extracts	Conc. (mg)	Total no. of insects	No. of dead insects	No. of alive insects	% Lethality	LCL	UCL	LD_{50}
1	Chloroform	100	30	9	21	30.00	144.53	295.72	219.97
		200	30	14	16	46.67			
		300	30	17	13	56.67			
		400	30	19	11	63.33			
		500	30	23	7	76.67			
2	n-hexane	100	30	11	19	36.67	115.35	231.16	178.58
		200	30	14	16	46.67			
		300	30	19	11	63.33			
		400	30	22	8	73.33			
		500	30	26	4	86.67			
3	Aqueous solution	100	30	5	25	16.67	260.48	320.82	260.48
		200	30	11	19	36.67			
		300	30	16	14	53.33			
		400	30	21	9	70.00			
		500	30	23	7	76.67			

Table 4. Insecticidal activity against *Reticulitermus flavipes*

S. No	Extracts	Conc. (mg)	Total no. of insects	No. of dead insects	No. of alive insects	% Lethality	LCL	UCL	LD_{50}
1	Chloroform	100	30	8	22	26.67	179.72	321.74	245.51
		200	30	12	18	40.00			
		300	30	15	15	50.00			
		400	30	20	10	66.67			
		500	30	23	7	76.67			
2	n-hexane	100	30	10	20	33.33	112.11	502.59	237.37
		200	30	12	18	40.00			
		300	30	11	19	36.67			
		400	30	22	8	73.33			
		500	30	24	6	80.00			
3	Aqueous solution	100	30	5	25	16.67	220.50	343.32	274.99
		200	30	10	20	33.33			
		300	30	16	14	53.33			
		400	30	19	11	63.33			
		500	30	23	7	76.67			

Discussion

Our study is in line with the experiment of Hadia *et al*. **[12]**, who assessed antibacterial (Enterobacter, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *E. coli*) and antifungal activities in various solvents benzene, chloroform and ethanol extract. *Datura stramonium* of chloroform extract produced maximum zone of inhibition and ethanol extract of *D. stramonium* gave maximum zone of inhibition against *K. pneumonia* while minimum against *E. coli*. Results further revealed that chloroform extract was very active against *S. aureus*, *P. aeruginosa* and *M. luteus*. All the extracts of *D. stramonium* have shown significant antifungal activity against *Saccharomyces cerevisiae*, *Aspergillus fumigatus* and *Aspergillus niger* with maximum antifungal activity against *S. cerevisiae* and zone of inhibition was about 16±0.2 mm by ethanol extract, 15±0.3 mm by chloroform and 14±1.6 mm by benzene extract while minimum antifungal activity was observed against *A. niger*.

Our study is also parallel to the experiments of Daljit K **[13]**, who screened antimicrobial activities of the aqueous solution of the *Moringa oleifera* against two yeast strains, three Gram positive and seven Gram negative bacteria by agar well diffusion assay. Results concluded that the MICs of seed coat, stem bark and pod's husks ranged from 0.5-1.25mg ml^{-1}, 3.0-4.2mg ml^{-1} and 4.0-5.6mg ml^{-1}, respectively. Both seeds' coat and pods' husks extracts exhibited microbicidal properties which were totally inhibited for 12 hours, respectively. Seeds' coat extract was the most effective against *E. coli*, while some pathogens, treated with stem bark extract, exhibited regrowth again after 24 hours apparently.

Our study was involved in antifungal activity, which is in line with the study of Paola *et al*. **[14]** who evaluated the extracts of antifungal activities of 10 plant species against the phyto pathogenic fungus *Alternaria sp*. Their study determined minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Results revealed that the MIC values ranged between 1.25 and 25µg mL^{-1}. The MFC values of the extracts ranged between 1.25µg mL^{-1} (*Rosmarinus officinalis* L.) and 10µg mL^{-1} (*Cynara scolymus* L.). MICs and MFCs values obtained from leaves (*Salvia officinalis* and *R. officinalis*) and seeds extracts (*Salvia sclarea* L.) were quite comparable to values obtained with the conventional fungicide captan (2.5µg mL^{-1}). The extracts of *Salvia sclarea*, *S. officinalis* and *R. officinalis* could be considered as potential sources of antifungal compounds for treating diseases in plants. These extracts showed maximum activity, even at very low concentrations, and the same fungicide effects as chemical fungicide.

Conclusion

Our experiment exposed the biological activities and nutraceutical profile of the algae "*C. schweinitzii* (A. Braun) Kützing". The physiochemical study of the said algae into our experiment showed the occurrence of some important elements. Our results of biological potential of *C. schweinitzii* (A. Braun) Kützing crude methanol extract and various plant extracts (methanol extracts) revealed the *C. schweinitzii* (A. Braun) Kützing includes some vital insecticidal activities, antibacterial, phytotoxic, cytotoxic and antifungal and is resulted that *C. schweinitzii* (A. Braun) Kützing has a greater amount of nutrition as well as offers strong biological activities.

Authors' contributions

Conceived and designed the experiments: U Begum, Performed the experiments: U Begum, Contributed reagents/materials and analysis tools: U Begum, Analysis of data: U Ahmad & I Ahmad, Wrote the paper: U Ahmad, Data collection: U Ahmad

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