Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Evaluation of the brown alga, *Sargassum muticum* extract as an antimicrobial and feeding additives

Avaliação da alga marrom, extrato de *Sargassum muticum*, como antimicrobiano e aditivo alimentar

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Abstract

Plant disease administration is difficult due to the nature of phytopathogens. Biological control is a safe method to avoid the problems related to fungal diseases affecting crop productivity and some human pathogenic bacteria. For that, the antimicrobial activity of the seaweed *Sargassum muticum* methanol and water extracts were investigated against human bacterial pathogens and fungal plant pathogens. By using 70 percent methanol, the seaweed powder was extracted, feeding additives assay, ultrastructure (TEM). Results revealed significant inhibition of *S. muticum* methanol extract against *Salmonella typhi* (25.66 mm), *Escherichia coli* (24.33 mm), *Staphylococcus aureus* (22.33 mm) and *Bacillus subtilis*. (19.66 mm), some fungal phytopathogens significantly inhibited *Fusarium moniliforme* (30.33mm), *Pythium ultimum* (26.33 mm), *Aspergillus flavus* (24.36mm), and *Macrophomina phaseolina* (22.66mm). Phytochemical investigation of *S. muticum* extract showed the presence of phenolic and flavonoid compounds. Results suggested that there is an appreciable level of antioxidant potential in *S. muticum* (79.86%) DPPH scavenging activity. Ultrastructural studies of *Fusarium moniliforme* hypha grown on a medium containing *S. muticum* extract at concentration 300mg/ml showed a thickening cell wall, disintegration of cytoplasm, large lipid bodies and vacuoles. In conclusion, our study revealed The antibacterial activity of *S. muticum* extract significantly against some Gram positive, Gram negative bacteria and antifungal activity against some phytopathogenic and some mycotoxin producer fungi. Flavonoids, phenolic play an important role as antioxidants and antimicrobial properties. Such study revealed that *S. muticum* methanol extract could be used as ecofriendly biocontrol for phytopathogenic fungi and feeding additives to protect livestock products.

Keywords: *Sargassum muticum*, mycotoxins, hyphae ultrastructure, feeding additives.

Resumo

A administração de doenças de plantas é difícil devido à natureza dos fitopatógenos. O controle biológico é um método seguro para evitar problemas relacionados a doenças fúngicas que afetam a produtividade das culturas e algumas bactérias patogênicas ao homem. Para isso, a atividade antimicrobiana da alga marinha *Sargassum muticum* metanol e de extratos aquosos foi investigada contra patógenos bacterianos humanos e fitopatógenos fúngicos. Usando metanol a 70%, o pó de algas marinhas foi extraído do ensaio de aditivos alimentares, a ultraestrutura (TEM). Os resultados revelaram inibição significativa do extrato metanólico de *S. muticum* contra *Salmonella typhi* (25,66 mm), *Escherichia coli* (24,33 mm), *Staphylococcus aureus* (22,33 mm) e *Bacillus subtilis* (19,66 mm). Alguns fitopatógenos fúngicos inibiram significativamente *Fusarium moniliforme* (30,33 mm), *Pythium ultimum* (26,33 mm), *Aspergillus flavus* (24,36 mm) e *Macrophomina phaseolina* (22,66 mm). A investigação fitoquímica do extrato de *S. muticum* mostrou a presença de compostos fenólicos e flavonoides. Os resultados sugeriram que há um nível apreciável de potencial antioxidante na atividade de eliminação de DPPH de *S. muticum* (79,86%). Estudos ultraestruturais da hifa de *Fusarium moniliforme* cultivada em meio contendo extrato de *S. muticum* na concentração de 300 mg/ml mostraram espessamento da parede celular, desintegração do citoplasma, grandes corpos lipídicos e vacúolos. Em conclusão, nosso estudo revelou a atividade antibacteriana do extrato de *S. muticum* significativamente contra algumas bactérias Gram-positivas, Gram-negativas e atividade antifúngica contra alguns fungos fitopatogênicos e alguns produtores de micotoxinas. Flavonoides e fenólicos desempenham papel importante como antioxidantes e propriedades antimicrobianas. Tal estudo revelou que o extrato metanólico de *S. muticum* pode ser usado como biocontrole ecologicamente correto para fungos fitopatogênicos e aditivos alimentares para proteger os produtos pecuários.

Palavras-chave: *Sargassum muticum*, micotoxinas, hifas ultraestrutura, aditivos alimentares.

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Received: January 3, 2022 – Accepted: July 4, 2022

1. Introduction

Seaweeds are abundant sources of bioactive compounds. Special interest has been paid in recent years to these secondary metabolites that have beneficial effects on human health. The algal seaweeds are considered as antioxidants, anticancer, antidiabetic, and antimicrobial (Shahidi and Rahman, 2018). Seaweed extracts have shown a strong antibacterial and antifungal efficacy (Vimala and Poonghuzhali, 2017). One of the marine macroalgae belonging to the Phaeophyceae family is *sargassum* sp., which is widely distributed in tropical and temperate oceans. *Sargassum glaucescensas*, *Sargassum polycystum* and *Sargassum tenerrimum*. While Kausalya and Rao (2015) demonstrated a promising antibacterial and antifungal activity alongside, it belongs to the Family Sargassaceae and order Fucales. A wide variety of bioactive properties was identified for *Sargassum* (Devi et al., 2013).

Brown algae are known to be the source of bioactive compounds, which contain a broad range of secondary metabolites such as antioxidants, antibacterial and antifungal (Kang et al., 2003). In addition, seaweeds are naturally renewable sources and are used in many parts of the world such as feed and fertilizer. Seaweeds have been widely screened around the world to isolate life-saving medications or biologically active compounds (Kang et al., 2003). Therefore, antimicrobial effects of seaweeds against distinct pathogens and their extracts display important immune stimulatory properties (Caipang et al., 2011). The ethanol extract of *Sargassum swartzii* a marine brown alga contains bioactive constituents with the highest antibacterial activity against bacterial and fungal pathogenic (Sujatha et al., 2019). The chemical compounds produced by some *Aspergillus* species are aflatoxins, which are widely found in a number of agricultural and livestock products. Moreover, the high feed prices, mycotoxin contamination of poultry and livestock feed is the most important problem (Naseem et al., 2018). In the food industry, the contamination of food by these toxic metabolites has been a big problem. Therefore, *Aspergillus flavus* is the most widespread source of feed spoilage among the aflatoxin-producing fungi (Saleemi et al., 2017). Taxonomic analysis and toxigenic detection of *A. flavus* are necessary for approach needed (Martinez-Miranda et al., 2019). Therefore, the control of this problem by seaweed extract was used in this study.

Because of the emergence of modern and advanced approaches, phytochemical studies have drawn the interest of researchers. These methods have played an important role in the research for additional supplies, agricultural and medicinal (phytochemical) raw materials (Mungole et al., 2010). Seaweeds create a natural supply of a number of medicines, including carotenoids, for prescription, nutritional, and cosmetic applications. Terpenoids, steroids, amino acids, phlorotannins, phenolic substances, halogenated ketones, alkanes and cyclic polysulphides which are capable of increasing tolerance and immune response to several infectious agents and have long been used in conventional medicine (Taskin et al., 2007; Guedes et al., 2011, Fitton, 2006). To our knowledge, there is little study about the mechanism of ultra-structure study by transmission electron microscope (TEM) explaining the mode of action of *Sargassum muticum* extract on fungal infections. The goals of this study are to, (1) assess the antimicrobial activity of *S. muticum* extract against some human pathogenic bacteria and som phytopathogenic fungi, (2) examine the extract phytochemically, (3) clarify the mode of action of the extract on fungal cells by means of the TEM, and (4) determine the effectiveness of feed additives as a potential mycotoxin binder.

2. Materials and Methods

2.1. Collection of Sargassum muticum

Sargassum muticum was collected from Marsa Alam, Red Sea, Egypt, and immediately transported in plastic bags containing seawater to the laboratory to stop the degradation of the alga. The identified alga by Jha et al. (2009) and Azzazy et al. (2019) (Figure 1), were extensively washed with sterilized seawater to remove foreign materials. The specimen was dried in the shade until constant weight and was blended into a powder. The powdered samples were placed in airtight containers and kept in the refrigerator.

2.1.1. Preparation of algal extracts

In the polar solvents such as 70 percent methanol, seaweed powder was soaked in a 1:3 w/v ratio and held for 48 hours, and then the methanol extract was prepared. With Whatman No.1 filter paper, the extract was filtered through a Buchner funnel. Water extracts were prepared using the same process then purified using a rotary vacuum evaporator at 50°C, and then evaporated until dryness under pressure. Crude extracts 7.5 g/100 g of methanol solvent and 5.6 g/100 g of aqueous extracts were measured then screened for antibacterial and antifungal activity (Yuvaraj et al., 2011).

2.2. Pathogens used for the assay

2.2.1. Bacterial pathogens

The bacterial isolates (*Staphylococcus aureus* (DSM 799), *Escherichia coli* (CN 6455), *Salmonella typhi* (trpE2), and *Bacillus subtilis* (NBRC13169)) have been obtained from Bacteriology Lab, Faculty of Science, University of Mansoura, Egypt.

Figure 1. *Sargassum muticum.*

2.2.2. Fungal pathogens

Microorganisms associated with bean root rots were isolated from rotted samples collected from different localities. The isolates were purified and identification based on colony characteristics, conidia, phialides, conidiophores and mycelium structure (Kubicek and Harman, 2002). Mycology Center, Assiut University, Egypt, validated identified as, *Phthium ultimum* 4413 AUMC. However, *Fusarium moniliforme*, *Aspergillus flavus* and *Macrophomina phaseolina* were obtained from the Plant Pathology Lab, Faculty of Agriculture, University of Mansoura, Egypt, and the Fungi were identified according to their morphological characteristics according to Booth (1985).

2.3. Antibacterial activity assay

The procedure of agar well diffusion method accompanied by *S. muticum* was used by nutrient agar medium (Murray et al., 1995).

On the surface of solid media, bacterial colonies inoculated and wells were prepared by the aid of a sterilized cork borer, and then filled with 300 mg/ml of algal extracts. Dimethyl sulfoxide (DMSO) was used as a negative control, while antibiotic ampicillin was used as a positive comparative efficacy control, replicated three times. The plates were incubated for 24 hours at 37°C, and then the inhibition zone around the well was measured and registered on each plate.

2.3.1. Antifungal activity assay

Antifungal activity was measured by agar well diffusion (Suay et al., 2000). The wells were filled with algal extracts of 300 mg/ml, prepared with the aid of a sterilized cork borer. Both plates were observed for growth inhibition zones after incubation at 28°C±0.1 for 48 h, and the diameters of these zones were measured in millimeters. Both experiments were administrated in triplicates under sterile conditions. For positive control, Nystatin was used, while DMSO was used as a negative control.

2.4. Phytochemical analysis

The molecular structure of *S. muticum* extract was performed for phenolic, flavonoid and other active compounds identified by high-performance liquid chromatography (HPLC-MS) at Central Labs Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Analyses were performed using a Dionex Ultimate 300(Bremen, Germany) composed of a pump with an online degasser, a thermostatic column compartment, a photodiode array detector (DAD), an autosampler, and Chromelon software. HPLC separation was performed on the Zobrax SB-C18 column (150 mm×4.6 mm, 1.8 µm, Agilent Company, USA). (Bellah et al., 2013)

2.4.1. Feeding additive assay

Corn grains were used and relatively clean corn was used as the control grains. The target levels of mycotoxins in the feed were done for aflatoxin (AF) to be approximate, 150 potentially affecting bird performance. Samples of the grains, and resulting treatment feeds were sent to the Quality Control Laboratory Accredited according to ISO 17025/2005 Faculty of Agriculture Mansoura University, Egypt for analysis of the following mycotoxins: Aflatoxin, six feed treatments were formulated for turkey hen poults to 6 weeks of age.

2.5. Ultrastructural studies (TEM)

The Ultra structural studies (Transmission Electron Microscope TEM) According to Karnovsky (1965), sample treated with 2.5% buffered glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4, leave tissue overnight at 4 ° C, then washed 3 x 15 minutes (min.) in 0.1 M sodium phosphate buffer and 0.1 M Sucrose, post-fix 90 min. in 2% sodium phosphate-buffered osmium tetroxide pH 7.4, wash 3 x 15 minutes (min.) in 0.1 M sodium phosphate buffer and 0.1 M Ultra-thin sections were observed at 160 kV at the Electron Microscopy Unit, Mansoura University, Egypt, using JEOL JEM -2100.

2.6. Antioxidant activity of S. muticum extract

Detection of antioxidant activity approach was performed according to the method of Elslimani et al. (2013). The extracts affect free radical scavenging by the DPPH radical 0.2 ethanol solution.

2.7. Statistical analysis

The average of triplicate determinations is both values. Data is analyzed statistically using one direction variance analysis (ANOVA) in conjunction with the SPSS (1999). LSD is abbreviated as the least important difference and calculated at P 0.05

3. Results

3.1. Antibacterial activity

Data in (Table 1) showed that *S. muticum* methanol extract was efficient for suppressing bacterial growth. Methanol extract of *S. muticum* significantly inhibited *Salmonella typhi*. *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus Subtilis*, (25.66, 24.33, 22.33, and 19.66 mm, respectively) when we compared to normal Ampicillin (control). Bacterial growth subjected to methanol extract was greater than that of aqueous extract.

3.2. Antifungal activity

Data in (Table 2) showed that the methanol extract of *S muticum* exhibited a maximum antifungal activity against *F. moniliforme*. These algae's methanol extracts were substantially successful against fungal pathogens, *F. moniliforme*, and *Pythium ultimum*, *Aspergillus flavus* and *Macrophomina phaseolina* (30.33, 26.33, 24.36 and 22.66, respectively). The methanol extract of *S. muticum* was able to inhibit the growth of all tested pathogenic fungi rather than aquatic extract, and growth zones compared to normal Nystatin as a control.

Table 1. Effect of *Sargassum muticum* extract on bacterial growth.

Seaweed	Item	Zone of Inhibition (mm)			
		Salmonella typhi	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus subtilis***
Sargassum muticum (300mg/ml)	Methanol	25.66[b]	24.33[b]	22.33[b]	19.66[b]
	Water	6.66[c]	11.33[c]	9.67[c]	8.67[c]
	Ampicillin	30.66[a]	35.33[a]	29.33[a]	22.33[a]
	L.S.D.	2.33	2.65	2.82	3.24

Different superscript letters within a column indicate significant differences between samples at the level of P<0.05. The least significant difference (LSD) test is used in the context of the analysis of variance, when the F-ratio suggests rejection of the null hypothesis

Table 2. Effect of *Sargassum muticum* extracts on fungal growth.

Seaweed	Item	*Zone of Inhibition (mm)			
		Fusarium moniliforme	***Pythium ultimum***	***Aspergillus flavus***	***Macrophomina phaseolina***
Sargassum muticum (300mg/ml)	Methanol	30.33[b]	26.33[b]	24.36[b]	22.66[b]
	Water	10.35[c]	8.33[c]	8.66[c]	6.33[c]
	Nystatin	35.33[a]	28.66[a]	26.33[a]	24.66[a]
	L.S.D.	3.50	3.15	2.33	2.74

Different superscript letters within a column indicate significant differences between samples at the level of P<0.05. *Means comparing control fed group to mycotoxin fed group are significantly different.

3.3. Phytochemical analysis by HPLC

To detect the phytochemical constituents such as phenolic, and flavonoids in methanol extract of *S. muticum* seaweeds, HPLC was used. The phytochemical structure of the chosen seaweed studied was summarized in (Figure 2). These results revealed that the amounts of flavonoids were higher than phenolic compounds.

3.4. Feed additive

Data obtained in (Table 3) showed performance of turkey hens reared to six weeks' age as affected by mycotoxins and *S. muticum* feed additives. Comparing the control fed group to the mycotoxin fed group was significantly different, where the mycotoxin contaminated feed showed decrease in the weight of turkey hens but the addition of *S. muticum* as feed additives showed an increase in turkey hen's weight. Data showed that, in control (without additive) week no1 (Wk1 (g/bd) 140 and 235,422, 536, 608g/bd and 2.26kg for first, second, third, fourth, fifth, and six weeks respectively. On the other hand, in the case of the addition of *S. muticum* extract as feed additives and the presence of mycotoxins the data were, 147 and 253,451, 525, 672g/bd, and 2.33kg for first, second, third, fourth, fifth, and six weeks respectively. While, in case of treatment with mixing of mycotoxin with additive (M x A) the data revealed that, P were NS,0.1, NS, NS, NS, NS, and 0.07 for first, second, third, fourth, fifth, and six weeks respectively.

Means comparing control fed group to mycotoxin fed group are significantly different. *

a,b Means within control and mycotoxin treatment groups with superscripts are different.

3.5. Ultrastructural studies

Ultrastructural studies using TEM demonstrate the influence of the extract of *S. muticum* on *Fusarium monliforme* cells (Figure 3A-3D).

3.6. Antioxidant activity of S. muticum (DPPH radical scavenging activity)

To assess the ability of the antioxidant compounds, present in the algal extracts, this assay is used. In the methanol samples extracted, DPPH activity was more than 79 percent in case of 300 mg/ml extract of *S. muticum* (Table 4 and Figure 4)

4. Discussion

Seaweeds play an important role in the ecosystem. Brown algae are economically valuable seaweeds as a source of polysaccharides (e.g. alginate, laminaran, cellulose and fucoidan (Kadam et al., 2015). The findings in (Table 1) show that *S. muticum* methanol and water extracts are effective in suppressing some human pathogenic bacterial growth. A methanol extract from *S. muticum* significantly inhibited the growth of *Salmonella typhi, Escherichia, coli. Staphylococcus aureus* and *Bacillus Subtilis,* (25.66, 24.33, 22.33, and 19.66 mm, respectively) are human pathogenic bacteria, similarly the antibacterial reaction in the methanol extract of *Sargassum wightii* against *E. coli* was observed (Rao et al., 1986). Ethanol extract from *S. muticum* is the best effective against *E. coli*, *Salmonella* sp. and *Klebsiella sp*. (Rebecca et al., 2012). Several scientists have identified the antibacterial action of various algae against Gram-positive

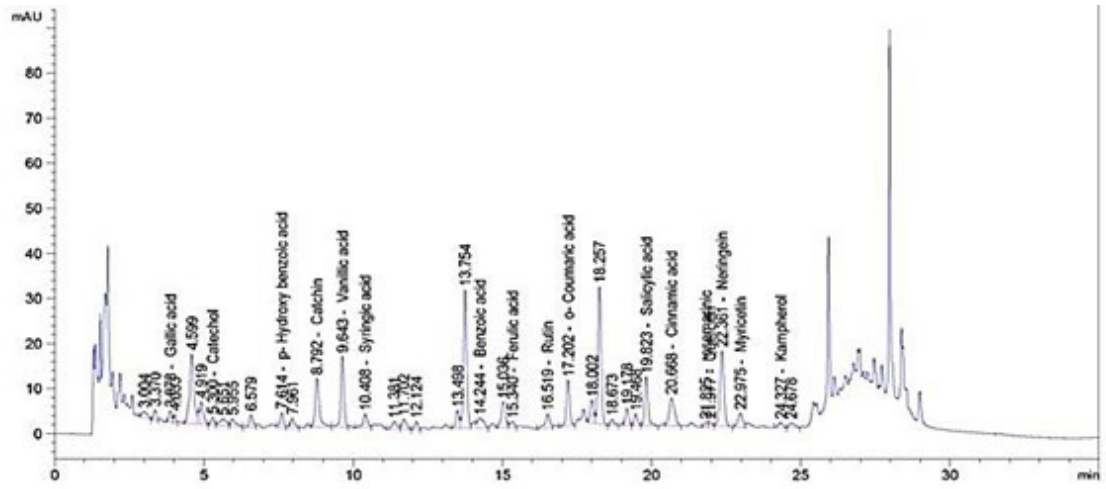

Figure 2. Shows phytochemical analysis by HPLC-MS.

Table 3. The performance of turkey hens reared to six weeks' age as affected by mycotoxins and *Sargassum muticum* powdered feed additives.

Wk6 (kg/bd)	Wk5 (g/bd)	Wk4 (g/bd)	Wk3 (g/bd)	Wk2 (g/bd)	Wk1 (g/bd)	Place (g/bd)	Treatment
Control							
2.26	608	536	422	235[b]	141	42	No additive
2.33	630	555	415	247[a, b]	137	42	BioFix
2.22	628	559	413	253[a]	132	42	Kallsil
2.10*	622*	550*	416*	245*	136*	42	Mean
Mycotoxin							
2.04	688	506	463	245b	135	42	No additive
2.33	672	525	451	253a,b	147	42	BioFix
2.10	665	503	440	245[a, b]	126	42	Kallsil
2.09*	675*	511*	451*	247*	126*	42	Mean
P							
0.001	0.004	0.0001	NS	0.0001	0.0001	NS	Mycotoxin
NS	NS	NS	NS	NS	NS	NS	Additive
0.07	NS	NS	NS	NS	0.1	NS	M x A

P = Probability; NS = Not Significantly. *Means comparing control fed group to mycotoxin fed group are significantly different.

Table 4. Antioxidant activity of *S. muticum* (DPPH radical scavenging activity).

Sample I.D	Sample Concentration (mg/ml)	DPPH % "RSA"
Sargassum muticum extract	100	54.56
	150	62.32
	200	63.74
	250	75.35
	300	79.86

RSA: DPPH free radical scavenging activity.

and Gram-negative bacteria (Kolanjinathan et al., 2009). However, the difference in antimicrobial activity may be due to the prevalence among these organisms of multiple antibacterial substances as indicated by Lustigman and Brown (1991) and due time and position of collection of samples, the ability of the extraction protocol to recover the active metabolites and the methods of assay. In this connection, *Sargassum gracilis* methanol extract has greatly inhibited the growth of *Bacillus mesentericus* (Prieto et al., 1999). A strong antibacterial activity against *S aureus* was reported of *Sargassum* sp. in methanol extracts against some human pathogenic bacteria. (Agbaje-Daniels et al.

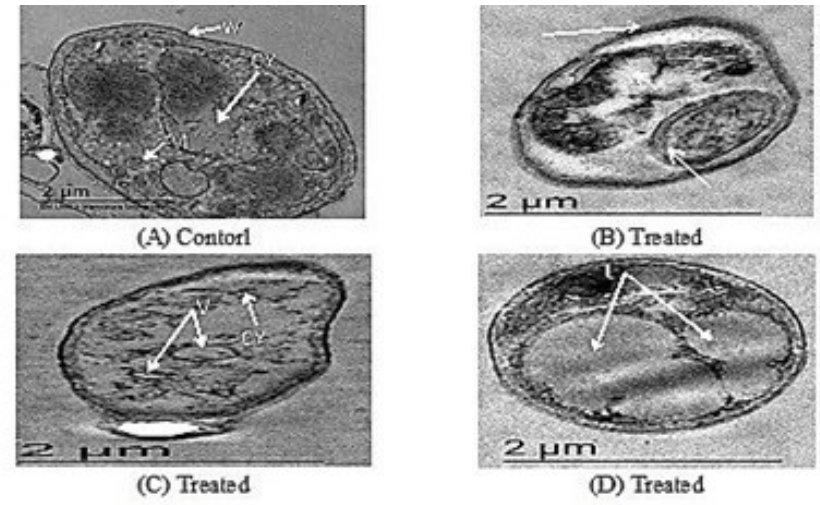

Figure 3. (A, B, C and D) shows the effect of *S. muticum* methanol extract *on Fusarium moniliforme.* (A-control) Cell irregular surface and thickening of the wall, (B treated) irregular surface and thickening of the cell (C treated) disintegration of cytoplasm, (D treated) organelles collected in clumps. Scale Bar = 2µm.

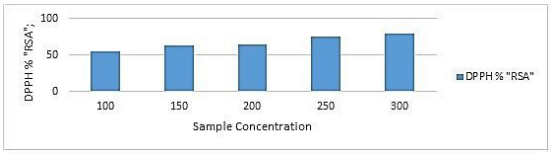

Figure 4. Shows the antioxidant activity of *S. muticum*, (DPPH Radical Scavenging Activity with different concentrations).

2020) demonstrated that extracts from West African Coast macroalgae species had antibacterial compounds.

Our observations in (Table 2) indicate that the methanol extract of *S. muticum* has been greatly successful against fungal pathogens studied such as *F moniliforme*, *Pythium ultimum*. *Aspergillus flavus* and *Macrophomina phaseolina*. (30.33, 26.33, 24.36 and 22.66), respectively. *Sargassum muticum* significantly inhibited fungal pathogens such as *Colletotrichum lagenarium* growth, according to (Ruch et al. 1989). *S. muticum* has demonstrated an important efficacy against fungal pathogens, including the antimicrobial activity of brown seaweed (Kausalya and Rao, 2015), however, it confirms the results of current investigation. Seaweed antifungal activity depends on the organisms from various divisions (Saidani et al., 2012). The antimicrobial function of *S. muticum* in this sample extracts has been stabilized and was found to be more active compared with aqueous extract, supporting the previous findings (Jeyanthi et al., 2012). Crude extracts of *Sargassum* sp. showed an important antimicrobial activity against fungi. The highest number of active constituents in the methanol extracts is found in seaweeds. (Rajasekar et al. 2019) assess the antioxidant ability, and antimicrobial properties of the algae *Ascophyllum nodosum* against one of the key enteric swine pathogen *Escherichia coli*.

The present study showed that active constituents were present in *Sargassum* extracts. The phytochemical constituents of the examined selected seaweed are summarized in (Figure 2). *S. muticum* methanol extract revealed the presence of phytochemical constituents such as flavonoids and phenolics that may responsible for the antimicrobial property (Battu et al., 2011). Our findings reported that flavonoid levels were higher than phenolic compounds, since flavonoids are bioactive compounds exited the Sargassaceae family, these flavonoids indicate that seaweed can be used in medicine and agriculture as an alternative source of natural antimicrobial, human pathogenic bacteria and plant pathogenic fungi. Flavonoids are effective antioxidants and have lately been of great importance in treating diseases because of their possible beneficial effects on human health (Subathraa and Poonguzhali, 2013). The antimicrobial function of algal extracts is responsible for flavonoids and phenolic compounds (Selvan et al., 2014). Brown algae are a valuable source of phytochemicals such as phenolic compounds (Mekinić et al., 2019).

The latest research has revealed that *S. muticum* methanol extract includes several types of phenolic compounds that are essential for invasive bacteria and other types of environmental stress in plant defense mechanisms (Hellio et al., 2001). In addition, aromatic rings and hydroxyl groups include phenolic compounds and their derivatives, including basic phenols, flavonoids, phenyl proponoids, tannins, lignins and many other substances, which decide the radical scavenging ability of the compound (Dziedzic and Hudson, 1983. Seaweeds are a great source of ingredients such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and there are numerous biological activities of carotenoids (Bhakuni and Rawat, 2005; Priyadharshini et al., 2011). The brown seaweeds possess a high amount of flavonoid and the cause for antifungal activity may be due to phenolic compounds (Cowan, 1999), these bioactive compounds may go to and bind to the microbe's cell wall, contributing

to growth inhibition. *Sargassum wightii* exhibits strong antibacterial and anti-fungal activity. The studied macro-algae are a good source of bioactive compounds (Cyril et al., 2017). These phenolic compounds and natural antioxidants are the origins of *Sargassum oyglocystum* extracts (Sanger et al., 2019).

The study of feeding additives revealed that, Chronic low levels of dietary mycotoxins can reduce animal performance (Tilley et al., 2017). Our findings, (Table 3) revealed that, the difference in body weight for birds fed on feed additives to be heavier when fed the control feed versus those feed additives and mycotoxin feed. It is possible that the action between the feed additive and the mycotoxins resulted in this eating behavior. Fed crude AF, collected from a natural outbreak of *Aspergillus flavus* in corn, at 0, 100, 200, 400, or 800 ppb to either 14-d old turkey poults or broiler chickens for 35 d. For turkey poults, AF at or greater than 400 ppb was toxic where high morbidity and mortality were observed as well as decreased weight gain and increased feed conversion (Giambrone et al., 1985).

Data obtained (Figure 3A-3D) revealed that Transmission Electron Microscopy (TEM) found that *S. muticum* methanol extract treated hyphae showed intracellular differences and changes rather than untreated. Ultrastructural of the treated cells revealed abnormal shapes of cells treated with date extract, cell membranes that lost their integrity, cytoplasmic material accumulation and broad separation of plasma lemma from the cell wall (Shraideh et al., 1998). Transmission electron microscopy shows damage in *F. oxysporium* conidia treated with chitosan, this ultrastructural damage in conidia is similar to that seen for the hyphae of this fungus, including plasma membrane alterations, cell wall thickening, and cytoplasm aggregation (Laflamme et al., 1999). This ultrastructural damage in conidia is comparable to that seen for the hyphae of this fungus. The effect on cell morphology and ultrastructure might be correlated with the action of crude extract *Smilacina japonica* (Liu et al., 2019).

Our findings (Table 4 and Figure 4) Showed that DPPH activity in the methanol-extracted samples was more than 79 percent in *S. muticum*. DPPH scavenging operation in methanolic extract of many species of *Sargassum* (*S. coreanum, S. fulvellum, S. piluliferum, S. siliquastrum* and *S. thunbergii*). Ethanolic and aqueous *Sargassum* spp. extracts, more than 60 per cent of DPPH radical scavenging activity was developed by (*S. horneri, S. macrocarpum and S. siliquastrum*). The ethanol extract of the brown algae *Sargassum wightii* had a strong reduction capacity (Silva et al., 2005; Thoudam et al., 2011). It was observed, and involvement of reluctant is responsible for capability reduction and is involved in the prevention of chain initiation, binding of metal ions and peroxide decomposition, according to Yuvaraj et al. (2011). Seaweeds have antioxidant potential as natural sources of bioactive compounds (Gomez-Zavaglia et al., 2019). Seaweed contains many antioxidant compounds (Jacobsen et al., 2019).

5. Conclusion

Our study revealed that antibacterial activity of *S. muticum* extract was significantly high against some Gram-positive and Gram-negative bacteria. This extract exhibited also the antifungal activity against phytopathogenic fungi. The phytochemical analysis of the methanol extract of *S. muticum* showed the presence of different groups of secondary metabolites such as flavonoids and phenolic, which are important indicators of the antimicrobial properties of seaweeds. In conclusion, the feed additives used in this study did alleviate the effect of dietary mycotoxins to some degree, especially with respect to feeding conversion. This study revealed that methanol extract of *S. muticum* could be used an eco-friendly antimicrobial agent. Further studies of longer duration are wanted.

Acknowledgements

The authors would like to express their deep thanks to all participants in this study for their valuable advice, and guidance throughout the whole work. This study was supported by a grant from the Research support system of the University of Sadat City, Egypt as a part of the Research Program

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