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Anti-microbial and Anti-diabetic Activity of Six Seaweeds Collected from the Red Sea, Egypt

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ABSTRACT

Marine algae are a well-known source of active compounds with many biological activities. Many studies had point to the anti-microbial and anti-diabetic activity of seaweeds. Egyptian shores are rich with seaweeds, yet studies concerning their biological activity are inadequate. In the present work, the 80% methanolic extract of six seaweeds (*Actinotrichia fragilis*, *Cystoseira myrica*, *Hormophysa cuneiformis*, *Laurencia papillosa*, *Sargassum cinereum*, and *Turbinaria turbinate*) were tested for their antimicrobial activity using disc diffusion method and anti-diabetic activity using the Inhibition of α-glucosidase method. The six species were collected from Hurghada, Red Sea, Egypt during late December, 2012. The results should that the algal extracts were effective against both Gram-positive and Gram-negative bacteria when used at 2000µg/disc concentration. On *Candida albicuns*, both low and high concentrations showed activity. For anti-diabetic activity, *H. cuneiformis* was the most active species that reached to 53% inhibition of α-glucosidase at the highest concentration (1000 µg/ml) with IC_{50} 676.9 µg/ml. In conclusion, the tested seaweeds possess a good anti-microbial and anti-diabetic activity, especially the species *Hormophysa cuneiformis*.

Keywords: Seaweed, *Hormophysa cuneiformis*, *Actinotrichia fragilis*, α-glucosidase, anti-microbial, anti-diabetic

INTRODUCTION

Diabetes and antibiotic-resistance microbes are becoming a great problem that the world is facing nowadays (Palanisamy *et al.*,2014; WHO, 2015). According to the World Health Organization (WHO), many of the antimicrobial drugs that have been discovered could become ineffective due to anti-microbial resistant (WHO, 2015). Likewise, diabetes is becoming a world-wide disease as a result of im-balanced diet, obesity, and stress (WHO, 2017). Diabetes could be treated using drugs that inhibiting α-glucosidase and α-amylase enzymes, which capable of breaking the starch into glucose before its uptake into the bloodstream (Krentz and Bailey, 2005). Although acarbose is a well-known anti-diabetic drug, it could reason in many harmful effects such as abdominal disorder and diarrhea which reduce patient submission to the drug and treatment effectiveness (Kumar *et al*., 2011). There for, the searching for efficient therapeutic natural drugs with less side-effect and new mechanism of action is required. Marine algae, Known as seaweeds, are a well-known healthy food source for many Asian countries for centuries (Lee and Jeon, 2013). Seaweeds secondary metabolites may offer coverage for the need of novel bioactive compounds with many biological activities (Unnikrishnanand Jayasri, 2018). Seaweeds and seaweed-derived bio-active compounds have been exploited as functional foods with therapeutic uses (Akbarzadeh *et al*., 2018). Phyto-chemical investigations on seaweeds discovered rich varieties of biologically-active substances with antioxidant, antimicrobial, anti-diabetic, anti-inflammation, anticoagulant and antitumor activities (Lee and Jeon, 2013; Unnikrishnanand Jayasri, 2018). Red Sea is considered a valuable source of many types of seaweed that still need to be explored for their bio- activity. Therefore, the present study conducted to evaluate the anti-microbial and anti-diabetic activity for six Red Sea seaweeds that have a few studies upon their bioactivity.

MATERIALS AND METHODS

Sample collection and preparation

The six seaweeds (*Actinotrichia fragilis*, *Cystoseira myrica*, *Hormophysa cuneiformis*, *Laurencia papillosa*, *Sargassum cinereum*, and *Turbinaria turbinate*) were collected from the intertidal and subtidal zones from Hurghada reefs during late December, 2012. Fresh samples were washed in seawater to remove encrusting material, thoroughly washed with fresh water to remove excess salt and air dried in shade then grinded. Each grinded algal sample was mixed with 80% methanol, putted in a shaking incubator at 25°C overnight and then the extract was collected. The process was repeated three times or till the methanol extract became clear and it was combined together. The extract were then filtered through Whatman No.4 filter paper and evaporated to dryness under reduced pressure vacuum. The crude extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a 50 mg/ml stock solution.

Antimicrobial disc diffusion assay Tested organisms and culture conditions

Standard isolates of *Bacillus subtiles* (B.s., ATCC 813106), *Staphylococcus aureus* (S.a., ATCC 25923) as gram positive bacteria, *Escherichia coli* (E.c., ATCC 25922) as gram negative bacteria, and *Candida albicans* (C.a., ATCC 16404) as fungus were acquired from the American type culture collection (ATCC). The bacterial organisms, the B.s, E.c and S.a, were stored on Muller Hinton agar (MHA) slants and were propagated in nutrient broth media. The fungal organism (C.a.) was stored on Sabouraud Dextrose Agar (SDA) medium and were propagated in Yeast Extract pepton Glucose medium (YPG).

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Antimicrobial test

Antimicrobial tests were carried out by disc diffusion method (Atlas and Unterman, 1999) using 100 µl of a suspension containing 10^8cfu/ml of the bacteria and 10^6 cfu/ml of the fungus; all were spread on MHA and SDA medium, respectively. The discs were impregnated with 200 and 2000µg/disc and located on the inoculated agar. Negative control was designed using the same solvents used in dissolving the algal extract (DMSO). Augmentin (AG), Chloramphenicol (C), and Streptomycin (S) were used as positive control at a concentration of 30µg/disc to determine the sensitivity of each tested microbial species. The inoculated plates were incubated at 37°C for 24h for clinical bacterial strains and 48h for the fungus. Antimicrobial activity was evaluated by measuring the inhibition zone of the extracts against the tested organisms.

Anti-diabetic activity: The effect of algal samples on the Inhibition of α-glucosidase

The chromatographic method described by Lee *et al*. (2010) was used to perform the α-glucosidase inhibitory assay. Briefly, yeast α-lucosidase (0.7 U, Sigma) was dissolved in 100mM DPBS (pH 7.0) as an enzyme solution. Five mM of p-Nitrophenyl-a-D-glucopyranoside in the same buffer (pH 7.0) was used as a substrate solution. In a 96-well plate, a 100µl of enzyme solution and 20µl of each algal extract in different concentrations were mixed. The absorbance was measured at 405nm at zero time. The mixture was incubated for 5min then the substrate solution (100µl) was added and incubated for another 5min at room temperature. Increasing the absorbance from zero time was measured and compared to a control that contains buffer solution in place of the extract. The blank was prepared via replacement of the enzyme solution with buffer solution and absorbance was recorded. Acarbose was used as a positive control. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as following:

$$\% \text{ Inhibition} = \frac{(C_A - C_B) - (S_A - S_B)}{(C_A - C_B)}$$

Where,

C_A = Control after enzyme treatment.

C_B = Control before enzyme treatment.

S_A= Sample after enzyme treatment.

S_B= Sample before enzyme treatment.

RESULTS

The antimicrobial activity study revealed that the algal extracts were effectively suppressed the growth of most of the tested microorganisms in a behavior comparable to the commercial antibiotics that were used as positive control. All the algal extracts were effective against both Gram-positive and Gram-negative bacteria when used at 2000µg/disc concentration, whereas the concentration of 200µg/disc was less effective (Table 1). For *Escherichia coli* the extracts of *A. fragilis* and *H. cuneiformis* were the only effective extracts at the low concentration. At the high concentration all the extracts showed activity keeping *A. fragilis* and *H. cuneiformis* to be the most effective (Fig. 1-A). *L. papillosa* was the only effective extract on *Staphylococcus aureus* at the low concentration. At the high concentration, all the extracts were effective against *S. aureus* showing *H. cuneiformis* and *T. turbinate* to be the highest among them (Fig.1-B). For *Bacillus subtiles*, the extract of *H. cuneiformis* was the most effective extract on both concentrations. At low concentration, *C. myrica*, *L. papillosa*, and *S. cinereum* did not show any activity on *B. subtiles*, while demonstrate a good activity at the high concentration (Fig. 1-C). On *Candida albicuns*, both low and high concentrations of the six extracts showed activity. The extract of *A. fragilis* was the most effective at the low concentration, while at the high concentration both *A. fragilis* and *C. myrica* were the most effective (Fig.1-D).

Table 1: Antimicrobial activity test of 80% methanol algal extracts at two concentrations (1 and 2) in comparison with negative control (DMSO) and 30 µg/disc of three commercial controls (AG, C and S).

Sample	Treat.	*E. coli*	*S. aureus*	*B. subtiles*	*C. albicans*
Negative Control	DMSO	-ve	-ve	-ve	-ve
	AG	12 ±0.23	10 ±0.12	10 ±0.25	-ve
Positive control	C	11 ±0.12	17 ±0.17	15 ±0.06	18 ±0.12
	S	10 ±0.17	20 ±0.07	19 ±0.06	-ve
A. fragilis	1	6^{a} ±0.12	-ve	6^{c} ±0.06	8.5^{a} ±0.01
	2	8^{a} ±0.17	7.5^{b} ±0.01	7^{d} ±0.12	11^{a} ±0.06
C. myrica	1	-ve	-ve	-ve	7^{c} ±0.06
	2	7^{c} ±0.06	7.5^{b} ±0.6	7.5^{c} ±0.1	11^{a} ±0.12
H. cuneiformis	1	6^{a} ±0.12	-ve	8.5^{a} ±0.01	6^{e} ±0.06
	2	8^{a} ±0.6	9^{a} ±0.12	10.5^{a}±0.2	6.5^{e} ±0.12
L. papillosa	1	-ve	6.5 ±0.06	-ve	6.5^{d} ±0.01
	2	7.5^{b} ±0.1	7^{c} ±0.1	7.5^{e} ±0.2	8^{c} ±0.12
S. cinereum	1	-ve	-ve	-ve	7.5^{b} ±0.01
	2	6.5^{d} ±0.1	6^{d} ±0.12	6^{f} ±0.12	9^{b} ±0.06
T. turbinata	1	-ve	-ve	8^{b} ±0.12	6^{e} ±0.06
	2	7.5^{b} ±0.1	9^{a} ±0.12	8.5^{b} ±0.1	7^{d} ±0.6

For treatment (Treat.): AG refers to Augmentin antibiotic; C to Chloramphenicol; S to Streptomycin; 1 to 200 µg/disc as algal extract concentration, and 2 to 2000 µg/disc. Each value below the microbial name is the mean of the inhibition zone (mm) of 3 replica ±SD. Within the same column, means carrying different superscripts are significantly different from each other at $P \leq 0.05$ or less.

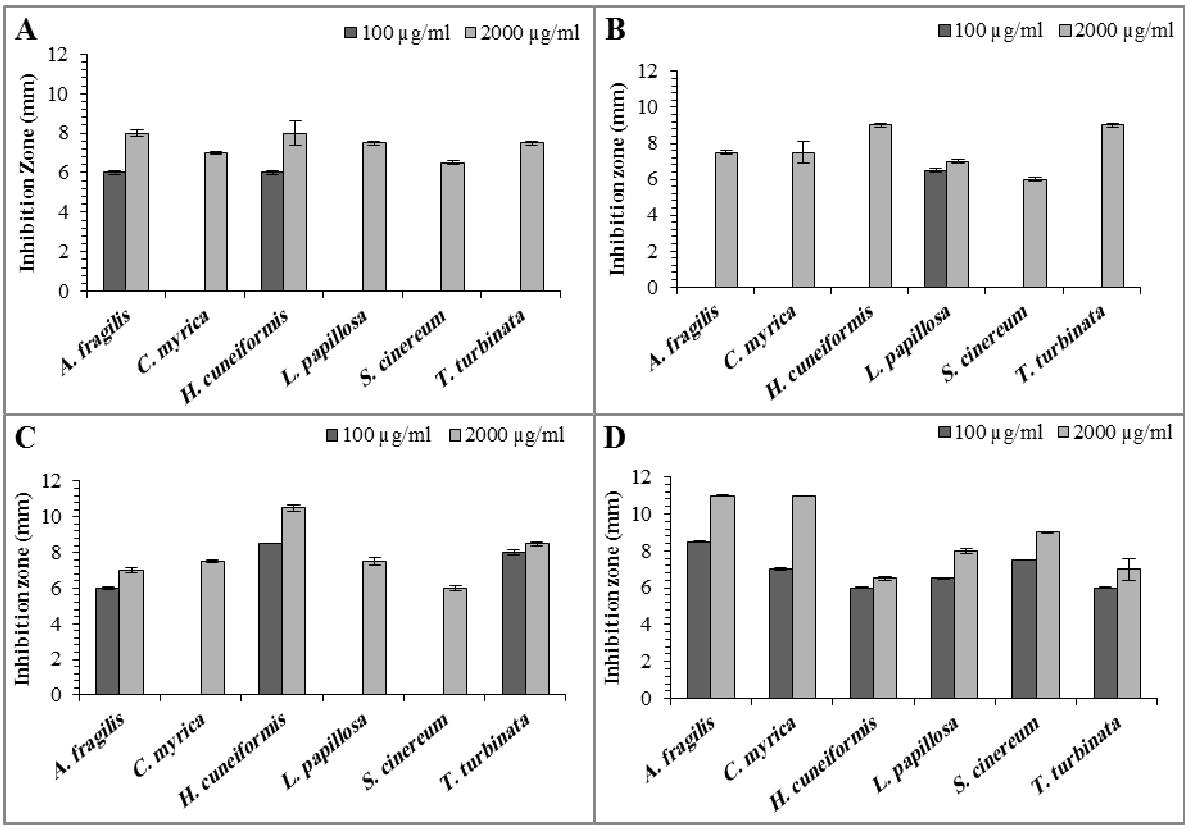

Figure 1: Comparison of the inhibition zone for the six studied 80% methanol seaweed extracts at two concentrations (100 and 2000µg/disc) against different microorganisms, where A= (*E. coli*), B = (*S. aureus*), C = (*B. subtiles*), D = (*C. albicans*). The values expressed as mean of triplicate ± SD.

The results for the α-glucosidase inhibitory activity showed that *H. cuneiformis* was the most active species that reached to 53 % ±2.3 inhibition at the highest concentration (1000 µg/ml) followed by *A. fragilis* with 51.27 % ±2.2 inhibition at the same concentration (Fig. 2).

Furthermore, the inhibitory effectiveness was compared on the basis of IC_{50}. The results in Table 2 showed that the extract of *H. cuneiformis* had the lowest IC_{50} (676.9 ±2.5 µg/ml) among the studied species. *A. fragilis* showed higher IC_{50} (920 ±1.3 µg/ml), while the rest of the species did not reach to 50% inhibition. For comparison, the Acarbose, the commercial positive control used in this study, inhibited the activity of α-glucosidase to 85.6% at the 1000 µg/ml concentration with IC_{50} 417.9 ±0.5 µg/ml.

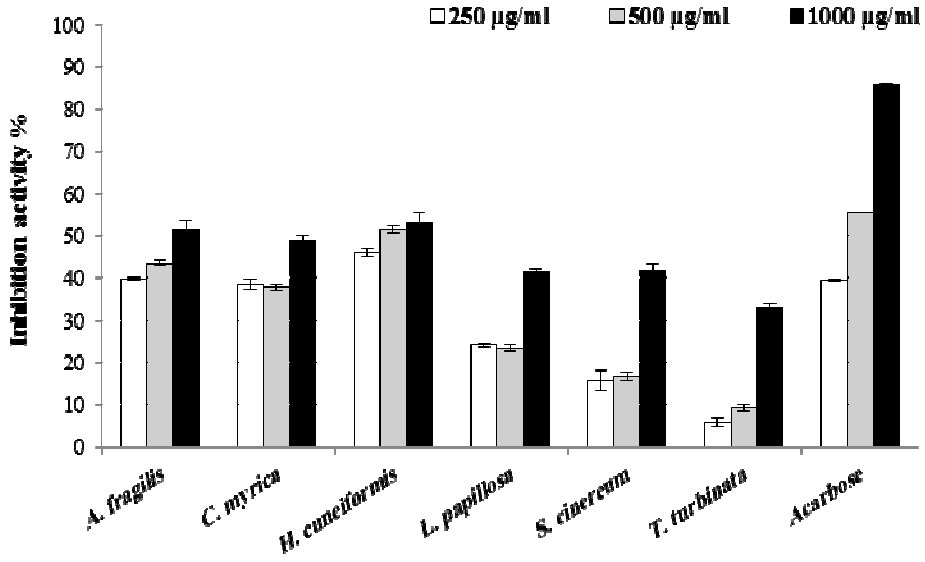

Figure 2: Inhibitory effect of 80% methanol extract of the six studied seaweeds on α-glucosidase. Acarbose was selected as a positive control for inhibition. Inhibition is expressed as mean of triplicate ± SD.

Table 2: Representing the IC_{50} values for the 80% methanol extract of the six studied seaweeds inhibitory effect and acarbose on α-glucosidase. The values of IC_{50} were determined in triplicate.

IC_{50} in µg/ml						
A. fragilis	***C. myrica***	***H. cuneiformis***	***L. papillosa***	***S. cinereum***	***T. turbinata***	**Acarbose**
920[a]±1.3	> 1000	**676.9**[b] ±2.5	> 1000	> 1000	> 1000	**417.9**[c]±0.5

Within the same column, means carrying different superscripts are significantly different from each other. $P \leq 0.05$ was accepted as an indication of statistical significance difference.

DISCUSION

Oxidative stress, Diabetes, resistant-microbes had become serious public problems for health worldwide in developing and advanced nations. Most of these diseases are formed from bad nutritional habits and go along with the increased production of the free radicals and/or insufficient antioxidant defense systems (Pitozzi *et al*., 2003). Seaweeds are proved to stand as a good source of natural bioactive compounds with many therapeutic activities (Ambreen *et al*., 2012).

In the present study, the 80% methanol extracts of six studied algal species were investigated for *in vitro* antimicrobial activity. The antimicrobial activity test showed that the extracts had broad spectrum effect against most species under investigation at the higher concentration (2000µg/ml). The effect against Gram-positive bacteria (*S. aureus* and *B. subtiles*) and *C. albicans* were more relatively conspicuous than Gram-negative (*E. coli*) and that effect was dose dependent, which increased with concentration. The result was agreed with Gonzalez del Val *et al*. (2001), Demirel *et al*. (2009) Taskin *et al*. (2007), Kandhasamy and Arunachalam (2008). The present results indicated that *H. cuneiformis* was the most effective species against the studied microorganisms except for *C. albicans*, *Actinotrichia fragilis* found to be the most effective species. The current results could be different from other results obtained in previous studies due to several factors. This may be owing to the method of extraction, the operated solvents, and season at which species were collected that would give rise to different susceptibilities of the target strains (Kandhasamy and Arunachalam, 2008). Many bioactive compounds from marine macro-algae were verified to possess antimicrobial activity including phenolic compounds hydrocarbons, terpenes, acids, phenols, sulfur-containing compound, aldehydes, naphthalene skeleton and alcohols (Bansemir *et al*., 2006). Fatty acids and sterols considered to be one of the most bioactive compounds with antimicrobial activity (Demirel *et al*., 2009).

Diabetes type II is a metabolic disorder syndrome diagnosed by hyperglycemia, owing to the lessened insulin secretion, insulin activity or both cases (Akbarzadeh *et al*., 2018). The major goal of the anti-diabetic drugs is to lower sugar blood level by suppressing the gastrointestinal absorption of glucose through the inhibition of either α-glucosidase or α-amylase (Krentz and Bailey, 2005). The presently available treatments for diabetes-type II include insulin and several oral drugs. Though, these treatments have either imperfect efficiency or side effects. Therefore, recently, there has been increasing demand of using alternative natural products for diabetes therapy, especially those derived from plants due to their less toxic with fewer side effects than synthetic ones (Lee and Jeon, 2013). Many researches have been reported seaweeds for its anti-diabetic activity (ex. Kim *et al*., 2012; Jensen *et al*., 2013; Akbarzadeh *et al*., 2018; Unnikrishnan and Jayasri, 2018). Therefore, the six studied species was screened for their anti-diabetic effect via the inhibition of α-glucosidase in the presence of acarbose as positive control. The result indicated that *H. cuneiformis* is the most active species in inhibiting α-glucosidase with. The rest of the species did not reach to 50% inhibition at the studied concentrations. The result is considered lower than those obtained by Lee and Jeon (2013), and Akbarzadeh *et al*., 2018 that maybe due to the difference in environmental and geographical conditions. Many compounds from seaweeds proved to have anti- diabetic activity such as polysaccharides, fucoxanthin, Polyphenols as well as some minerals including, zinc, magnesium, potassium and calcium (Lakshmanasenthil *et al*., 2014; Unnikrishnan and Jayasri, 2018).

CONCLUSION

In conclusion, the seaweeds under investigation could be a source for developing anti-microbial and anti-diabetic drugs especially *Hormophysa cuneiformis* and *Actinotrichia fragilis*. More studies are required in order to isolate, purify and identify the bioactive compounds responsible for their bioactivities.

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النشاط المضاد للبكتيريا ومضاد للسكري لستة أعشاب بحرية تم جمعها من البحر الأحمر ، مصر

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الملخص العربـــــي

يهدف العمل الحالي لتقييم الخصائص العلاجية لمستخلصات ستة أنواع من الطحالب البحرية والتي جمعت من مناطق الشعاب المرجانية بالغردقة الواقعة على البحر الأحمر وهي سيستوزيرا ميريكا، هورموفيزا كيونيفورمز، سراجسيوم سينيريوم، تربيناريا تربيناتا ، اكتينوتريشيا فراجيليس و لورانسيا بابيلوزا. وقد تم تقييم هذه الطحالب عن طريق قياس النشاط المحتمل لهذه المستخلصات كمضاد للميكروبات و مرض السكري. وقد اتبعت فى تقدير ذلك عدد من الطرق والمراجع العلمية المستخدمة فى تعريف الطحالب وتقدير القيم السابق ذكرها. وقد اسفرت الدراسه على ما يلى:-

النشاط المضاد للميكروبات : اظهرت مستخلصات الطحالب فاعليتها في تثبيط نمو معظم الميكروبات قيد الدراسة في سلوك مماثل للمضادات الحيوية التجارية. وقد كان هذا التاثير بصورة اوضح في التركيز الاعلي (2000 ميكروجرام) وكانت جميع مستخلصات الطحالب فعالة ضد كل من البكتيريا إيجابية وسالبة الجرام.بمقارنة النشاط المضاد للميكروبات لمستخلاصات الطحالب قيد الدراسة والمضادات الحيوية التجارية وجد ان مستخلصات الطحالب في التركيز الاعلي قد تكون مماثله في الفاعلية ضد الميكروبات.

النشاط المضاد لمرض السكري : وفقا للدراسة، كان طحلب الهورموفيزا الأكثر نشاطا من بين الطحالب قيد الدراسة في تثبيط انزيم الالفاجلوكوسيداز. وجاء طحلب الاكتينوتريشيا في المركز الثاني من حيث تثبيط الانزيم. بينما لم تظهر باقي الطحالب تاثيرا ذو اهمية. ومن جهة أخرى، أظهر الدواء التجاري لمكافحة السكري (الاكربوس) اعلي تاثير في تثبيط الانزيم.