Impact of The Microbial Suppression by Using The Brown Alga *Dictyota dichotoma* Extract

Ibraheem B.M. Ibraheem, N. Abdel-Raouf, H.M. Mohamed[*], R. Yehia[#] and S.M. Hamed[**]

Botany & Microbiology Department; [*]Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, 62514 and [**]Soil Microbiology Department, Soils, Water

IN THIS CONTEXT the brown alga *Dictyota dichotoma* isolated from Hurghada Sea-Shore, Red-Sea coast, Egypt (June 2015) was screened for its antimicrobial bioactivity. A laboratory experiment was conducted to test activity of ethanol extract of the brown alga *Dictyota dichotoma* against three types of Gram- positive and six of Gram-negative bacteria, and one type of fungi. Ethanol extract was selected for this study by using different concentrations (100, 200, 300, 400 mg) of alga extracts. Result revealed that the alga ethanolic extract exhibited high suppression activity against all tested pathogens. HPLC analysis of the chemical composition of algal extract was detect the presence of high amount of certain ingredients such as Flavonoids (Acacetin, Luteo.6-arabinose8-glucose, Naringin, Hespirtin, Rosmarinic and Naringenin by 3448.407, 453.209, 396.107, 387.429, 213.689 and 207.17 ppm, respectively) and Phenols (Benzoic, E-Vanillic, Pyrogallol, Catechein, Salycilic and Ellagic by 1075, 912, 785, 628 and 357 ppm, respectively). The present finding revealed that the tested alga can be used as possible natural source of active ingredients.

Key words: *Dictyota dichotoma*, Antimicrobial activity, HPLC analysis .

Introduction

The permanent uses of familiar antibiotics cause drug resistant pathogenic strains. Improvement of the resistant bacteria is globally deemed as main medical problem, thus initiate bighazard for human society (Neu, 1992). This has enhance a search for novel class of antibacterial materials from natural sources. Natural products are considered a significant source of therapeutic factors against bacterial and fungal diseases, cancer, lipid disturbances and immunomodulation (Haroun et al., 1995; Shridhar et al., 2009; Clardy & Walsh, 2004; Cragg et al., 1997; Voidarou et al., 2011; Elsayed et al., 2012 and Abdel-Raouf et al., 2015a).

Marine algae are widely distributed along the Egyptian and Red-Seashore (Haroun et al., 1995; Mohamed et al., 2006 and Ibraheem et al., 2014). Marine algae are potential sources of bioactive secondary metabolites with possibility for use in the development of recent pharmaceutical agents (Abdel-Raouf et al., 2008; Abedin &Taha., 2008; EL-Gamal, 2010 and AL–Saif et al., 2014) and various of these compounds have been confirmed to possess desirable biological activities (Faulkner, 2002; Ibraheem et al., 2008 and Abdel-Raouf et al., 2015b). Marine algae were announced to produce a wide diversity of bioactive secondary metabolites as antimicrobial, antifeedant, antiparasitic and cytotoxic agents and the bioactive substances inclusive alkaloids, polyketides, cyclicpeptide, quinines, glycerols, lipids, polysaccharide, phlorotannins, diterpenoids and sterols (Cabrita et al., 2010). Hence they have drawn major concern recently (Al-Haj et et al., 2009; Bazes et al., 2009; Vallinayagam et al., 2009; Cabrita et et al., 2010 and Ibraheem et al., 2012). Phaeophyceae have high amounts of polyphenols with a basic building block of 1,3,5-trihydroxybenzene (phloroglucinol) and were examined as a bioresource of polyphenols, biopolymers and bio-products.

Dictyota species are the optimal studied sources of secondary metabolites and the ecological purpose and biological activities of these compounds are deeply studied (Viano et al.,

[#]Corresponding author Rehab Yehia Fessehy, Botany and Microbiology Department, Faculty of Science, Beni-Suef University. email: rehab.yahia55@gmail.com,
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2009; Rasher & Hay., 2010; Rasher et al., 2011 and Othmani et al., 2014). *Dictyota dichotoma* become increasingly common due to its rising economic significance as a source of diterpenes (Gupta & Abu-Ghannam., 2011) and polyunsaturated fatty acids (Pereira et al,. 2012) and have possibility in biosorption of heavy metals (Laib & Leghouchi, 2012 and Hannachi et al., 2015). *Dictyota* sp. is particularly rich in bioactive terpenes (Viano et al., 2009; Othmani et al., 2014 and Da Gama et al., 2014) of which some have an established potential as anti-fouling coatings, algicidal (Kim et al., 2006), anti-bacterial (Enoki et al., 1983), anti-fungal (Tringali et al., 1986) and molluscicidal activity (Tringali et al., 1986 and Barbosa et al., 2007). Antibacterial compounds have been isolated from marine resources but some of these do not show activity against Gram-negative bacteria (Morton et al., 1998 and Correa et al., 2011). Therefore, it is worthwhile to search for such compounds from marine resources showing potent antibacterial activity against human pathogenic bacteria. Subsequently, in present study we have screened the ethanolic extract of *Dictyota dichotoma* as antagonistic agent against some Gram positive and Gram negative bacteria, as well as HPLC analysis of the ethanolic extract has been characterized in order to find alternative drugs and promising source of pharmaceutical agents.

Materials and Methods

Studied area

The investigated area extends along Hurghada coast on Red-Sea, Egypt (Fig. 1). Safaga city located in the west coastal area of Red Sea shore between longitude 34° 17`E and latitude 26° 06`N..

Algal collection and preparation

Dictyota dichotoma was collected on April (2015) from Safaga coastal along the Red Sea, Egypt, and identified according to Aleem (1993) and Coppejans et al. (2009). Samples were cleaned with seawater to remove impurities and transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder until a fine powder was obtained (Chiheb et al., 2009).

Ethanolic extraction of Dictyota dichotoma

The dried material of *Dictyota dichotoma* was mixed with ethanol (1:50, w/v) and placed into the Soxhlet apparatus (Fig. 2). Extraction solvent was evaporated under vacuum and stored in -20°C until used.

Fig. 1. Location of study area.

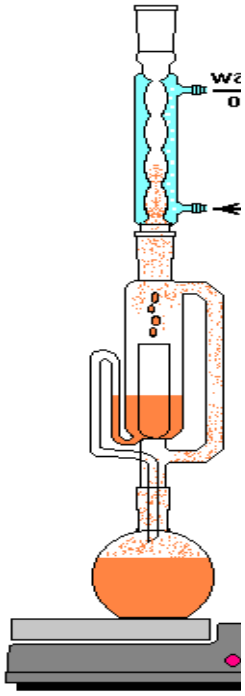

Fig. 2. Soxhlet extractor of marine alga *Dictyota dichotoma.*

Antimicrobial activity of the studied algae

Gram positive

For testing the anti-Gram positive activity, the following isolates including *Bacillus subtilis* (RCMB 01001 69-3), *Staphylococcus aureus* (RCMB 010027), *Micrococcus luteus* (RCMB 01001 76-9) were selected.

Gram negative

For testing the anti-Gram negative activity, the following isolates including *Escherichia coli (RCMB 01002 52-6), Pseudomonas aeruginosa (RCMB 01002 43-5), Serratia marcescens (RCMB 01002 75b-8), Salmonella typhi (RCMB 01002 15-4), Vibrio* sp. and *Aeromonas hydrophila* were selected.

Fungi

For antifungal activity, the unicellular *Candida albicans* (RCMB 05036), was used for this purpose.

Antimicrobial activity by disc diffusion method

In the present study, antibacterial activity was determined against the above bacteria using the paper disc assay method (El-Masry et al., 2000). Whatman No. 3 filter paper disc of 6-mm diameter was sterilized by autoclaving for 15 min at 121°C. The sterile discs were saturated by 20µl with different concentrations (100, 200, 300 and 400 mg/ml) to become loaded by 2, 4, 6 and 8 mg for each disc, respectively. Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2 X 10^8 CFU/ml. The impregnated discs were placed on the Muller Hinton medium suitably spaced apart and the plates were incubated at 37°C for 24 h. Ethanol was used as a negative controlwhile commercial antibiotic discs (Ampcillin 10 mg/disc) were used as a positive control. The above procedure is allowed for fungal assays but expects the potato dextrose agar media instead of nutrient agar media. The plates were incubated at 25^0C for 48 h in the case of investigated fungus species. The diameter of inhibition halos measured in millimeter. All the assays were carried out in triplicate (Fig. 3).

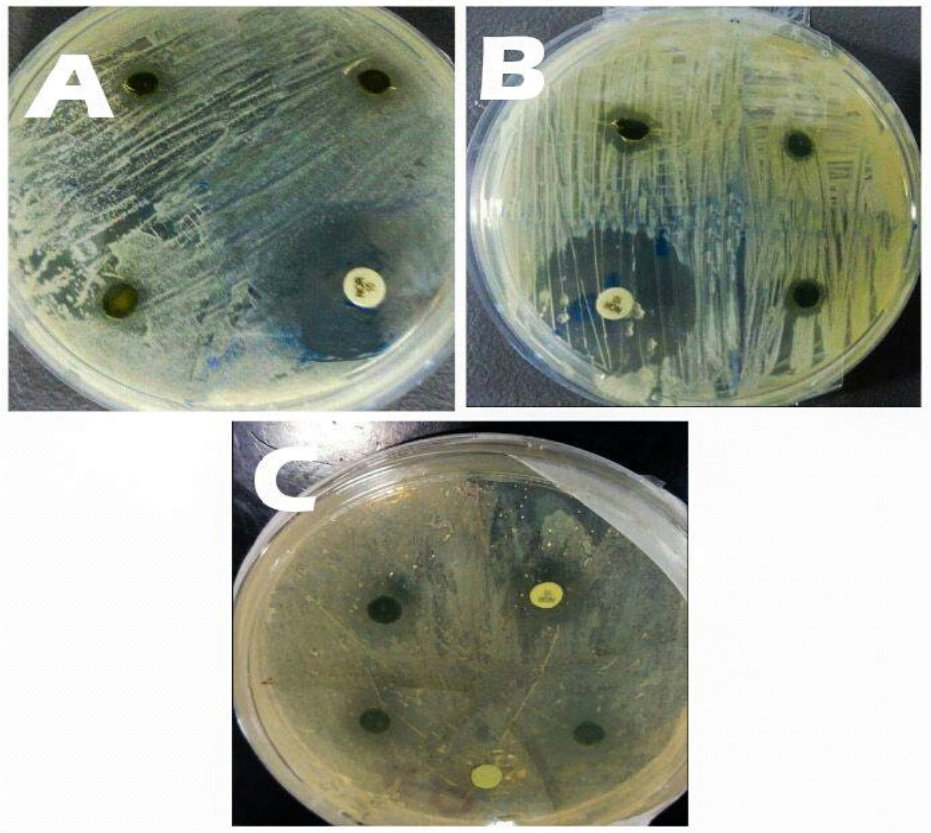

Fig. 3. Inhibition zones obtained by the ethanolic extract of *Dictyota dichotoma* against *Staphylococcus aureus* (A), *Pseudomonas aeuroginosa* (B), *Escherichia coli* (C).

Chemical analysis of Dictyota dichotoma HPLC analyses of flavonoid, phenolic and polysaccharides compounds

Flavonoid, phenolic and polysaccharides in the ethanolic extract of *Dictyota dichotoma* were determined according to method described by Goupy et al. (1999) and Zielinski et al. (2014). The detection were conducted using high performance liquid chromatography (HPLC Agilent 1200 series) equipped with Quaternary pump, Auto sampler, column compartment set at 35°C, multi wavelength detector set at 330 nm, 280 nm.

Statistical analysis

All results are presented as the mean ± SD. Statistical analysis was carried out by using one-way ANOVA (Duncan, 1957). Statistical significance was considered at $p < 0.05$.

Results

Antimicrobial essay

Ethanolic extract of the brown alga *Dictyota dichotoma* was assayed for antibacterial & antifungal activity by using agar diffusion method (Table1). Data revealed that, *Dictyota dichotoma* extracts demonstrated a good antimicrobial activity against all gram-positive and gram-negative pathogenic bacteria. It can be seen that the ethanolic extract in concentration of 10 mg/disc gave the highest inhibition zone (16 mm) against *Bacillus subtilis*. Moreover, it gives a moderate inhibition activity (9 to 12 mm) against the other test organisms.

HPLC analysis of Dictyota dichotoma ethanolic extract

Ethanolic extraction of *Dictyota dichotoma* was subjected to HPLC for the identification of constituents present in the alga extract which suggest being the cause of antimicrobial activity in *Dictyota dichotoma*. The quantitative HPLC analysis of ethanol extract of *Dictyota dichotoma* displayed three major compounds flavonoids, phenols and saccharide which were estimated. HPLC analysis of the flavonoid constituents of the alga ethanolic extract exhibited a presence of 19 compounds (Table 2). Acacetin (3448.407 ppm), followed by Luteo.6-arabinose8-glucose, Naringin, Hespirtin, Rosmarinic and Naringenin by 453.209, 396.10, 387.429, 213.689 and 207.173 ppm, respectively. On the other hand, the remain flavonoid contents were represented by moderately or less amounts.

TABLE 1. Antimicrobial activities of the *Dictyota dichotoma* ethanolic extract (inhibition of growth expressed as mean ± SD).

Pathogen	Diameter of inhibition zone, mean ± SD					
	*Dictyotadichotoma*concentrations mg/disc (Tested sample)				Ampcillin(positive) control	Ethanol (negative) control
	2 mg/ disc	4 mg/ disc	6 mg/ disc	8 mg/ disc	10 mg/disc	20 µl/disc
Gram positive bacteria						
Staphylococcus aureus	7.50±0.5	8.00±1.00	9.00±1.00	12.40±0.57	30.00±01.00	00
Micrococcus luteus	9.00±1.00	10.00±1.00	10.00±1.00	12.30±0.57	35.00±01.00	00
Bacillus subtilis	11.00±1.00	12.00±1.00	15.00±1.00	**16.00±1.30**	25.00±12.66	00
Gram negative bacteria						
Serratiamarcescens	06.33±0.57	8.00±1.00	10.00±1.00	11.30±0.57	30.00±01.00	00
Salmonella sp.	08.80±0.76	10.00±1.00	10.60±1.15	09.66±2.50	25.00±05.00	00
Vibrio sp.	08.00±1.00	08.50±0.50	9.00±1.00	10.50±0.50	28.00±15.66	00
Aeromonashydrophila	07.50±1.00	08.00±1.00	08.00±1.00	08.00±1.00	25.00±1.50	00
Pseudomonas aeuroginosa	07.00±1.00	10.00±1.00	11.30±0.57	11.60±1.50	35.00±02.8	00
Escherichia coli	ND	ND	ND	08.60±1.15	12.00±01.00	00
Fungi						
Candida albicans	10.00±1.00	11.00±1.00	11.00±1.00	12.00±1.00	27.00±01.15	00

Data are expressed as the mean ± standard deviation (SD) of three replicates. Represent the statistical comparisons between alkaloid extract and positive control by using ANOVA ($p<0.05$). ND: not detectable.

TABLE 2. HPLC analysis for flavonoid contents ofe thanolic extract of *Dictyota di-chotoma* isolated from Safaga Sea-shore during April to June 2015.

Flavonoids contents	ppm
Luteo.6-arabinose8-glucose	**453.209**
Apig.6-rhamnose8-glucose	119.520
Apig.6-glucose8-rhamnose	74.300
Naringin	**396.107**
Luteo.7-glucose	52.094
Hespirdin	51.514
Rutin	20.226
Quercetin-3-o-glucoside	16.206
Rosmarinic	**213.689**
Apig.7-o-neohespiroside	29.945
Kamp3,7-dirhamoside	120.396
Quercetrin	35.493
Quercitin	95.927
Naringenin	**207.173**
Hespirtin	**387.429**
Kampferol	176.258
Rhamnetin	113.216
Apigenin	15.101
Acacetin	**3448.407**

With respect to the HPLC analysis of the phenolic contents of the ethanolic extract of *Dictyota dichotoma* (Table 3), the recorded data indicated the presence of twenty two phenolic compounds in different concentrations. From these compounds, Benzoic, E-Vanillic, Pyrogallol, Catechein and Salycilic in high amount reached 1323, 1075, 912, 785 and 628 ppm, respectively.

Additionally, HPLC analysis of the saccharides contents of the ethanolic extract of *Dictyota dichotoma* (Table 4) revealed the presence of relatively low percentage of mono, di- and poly saccharides in the crude extract.

Discussion

According to the present data, both Gram (+) and Gram (-) bacteria exhibited a sensitive effect to the alga crude extract, it means that *Dictyota dichotoma* contains a specific natural compounds display strong potential to penetrate the complex structure of the Gram (+) and Gram (-) bacterial cell wall. However, the Gram (+) strains were exhibited more sensitivity to the alga extract than Gram (-) bacteria. This was agree with reports of Strik et al. (2007) and Salem et al. (2011) who suggest that algal extract were generally more effective against Gram (+) than Gram (-) bacteria. The high sensitivity in Gram-positive bacteria may be attributed to the lack of the outer membrane (lipopolysaccharide layer) which is present in Gram-negative bacteria (Nikaido, 2003). The limited susceptibility in Gram-negative bacteria could be attributed to the limited outer membrane permeability and presence of porins in the membrane which narrows penetration of the extract (Delcour, 2009 and Nikaido., 2003).

TABLE 3. HPLC analysis for phenolic contents of ethanolic extract of *Dictyota dichotoma* isolated from Safaga Sea-shore during April to June 2015.

Phenolic contents	Ppm
Pyrogallol	**912**
Gallic	89.9
4-Amino-benzoic	155
Protocatchuic	16.2
Catechein	**785**
Catechol	14.9
Chlorogenic	185
Epicatechein	33
P-OH-benzoic	130
Caffeine	97.1
Vanillic	195
Caffeic	4.11
P-coumaric	52.8
Ferulic	124
Iso-Ferulic	100
E-Vanillic	**1075**
Benzoic	**1323**
Ellagic	**357**
Coumarin	37.9
3,4,5-methoxy-cinnamic	167
Salycilic	**628**
Cinnamic	8.2

TABLE 4. HPLC analysis for saccharides contents of ethanolic extract of *Dictyota dichotoma* isolated from Safaga Sea-shore during April to June 2015.

Saccharides contents	%
• **Free saccharides:**	
Glucuronic	0.05
Stachyose	0.26
Sucrose	0.07
Maltose	0.04
Glucose	0.04
Xylose	0.05
Galactose	0.04
Rhaminose	0.06
Fructose	0.05
Manitol	0.01
Sorbitol	0.03
• **Saccharides after hydrolysis :**	
Glucuronic	0.20
Galacturonic	0.44
Sucrose	0.19
Glucose	0.21
Xylose	0.35
Galactose	0.20
Mannose	0.17
Fructose	0.09
Manitol	0.09
Sorbitol	0.05

It is clear that this anti-bacterial activity is not as effective as their 'gold' standard (ampicillin) counterparts. This could be due to the fact that antibiotics such as ampicillin, penetrate the outer membrane of (primarily Gram-negative) bacteria via porins (James et al., 2009) and then act as a competitive inhibitor of transpeptidase needed by the bacterium to make a cell wall, an effect that ultimately leads to cell lysis and an overall reduced ability of the pathogens to successfully replicate. In contrast, *Dictyota dichotoma* extracts is thought to impart anti-microbial effects possibly via the induction of changes in the cell membranes of the targeted pathogen organisms, with alterations in the cell envelope causing impaired regulation of osmolality and ultimately cell death. Notwithstanding, this is a preliminary study, hence detailed investigations to identify the compositions of each extract is necessary for the recognition of major marine algae constituents that could serve as strong antimicrobial compounds (Sakineh et al., 2016).

HPLC analysis of the chemical composition of algal extract was detect the presence of high amount of certain ingredients such as Flavonoids (Acacetin, Luteo.6-arabinose8-glucose, Naringin, Hespirtin, Rosmarinic and Naringenin by 3448.407, 453.209, 396.107, 387.429, 213.689 and 207.17 ppm, respectively) and Phenols (Benzoic, E-Vanillic, Pyrogallol, Catechein, Salycilic and Ellagic by 1075, 912, 785, 628 and 357 ppm, respectively). Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health in fighting disease (Deyab et al., 2016). In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions (Aliyu, et al., 2009). In this respect (AL–Saif et al., 2014) reported that macroalgae produce a wide variety of chemically active metabolites including alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities. Also (Priyadharshini et al., 2011) have reported that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids have exhibited different biological activities.

Conclusion

In conclusion, the results of the present investigation indicated that the brown alga *Dictyota dichotoma* has biologically active compounds which are effective in inhibiting the growth of the pathogenic bacteria and fungi. Further the Red-Sea marine environment has potential to return pharmaceutically useful marine algae which can be harnessed for the development of drugs for use in management of human pathogens, and many human degenerative diseases.

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التأثير المثبط لمستخلص الطحلب البني ديكتيوتا ديكوتوما للنمو الميكروبي

إبراهيم برعى محمد إبراهيم، نيفين عبدالرؤف، هناء محمود محمد*، رحاب يحيي فصيحي و سهام موسي حامد**

قسم النبات والميكروبيولوجي- كلية العلوم - جامعة بني سويف ، *قسم الحيوان - شعبة الوراثة - كلية العلوم - جامعة بني سويف - بني سويف و **قسم ميكروبيولوجيا التربة - معهد التربة والمياه والبيئة - مركز البحوث الزراعية –الجيزة - مصر.

في هذا البحث تم جمع الطحلب البني ديكتيوتا ديكوتوما من ساحل البحر الأحمر بمدينة سفاجا، مصر (أبريل 2015). أجريت تجربة مختبرية لاختبار نشاط المستخلص الإيثانول لهذا الطحلب ضد ثلاثة أنواع من البكتيريا الموجة لصبغ جرام وستة من البكتيريا السالبة لصبغ جرام، ونوع واحد من الفطريات أحادية الخلية. تم اختيار مستخلص الايثانول لهذه الدراسة باستخدام تركيزات مختلفة (100، 200، 300، 400 ملي جرام) من مستخلص الطحلب. كشفت النتائج أن مستخلص الطحلب الإيثانولى أظهر نشاط عالى ضد جميع مسببات الأمراض المختبرة. كما تمتد دراسة التحليل التركيب الكيميائى لمستخلص الطحلب حيث كشف التحليل عن وجود كمية عالية من بعض المكونات مثل الفلافونويد (أكاسيتين، Luteo.6-arabinose8-غلوكوس، نارينجين و هيسبرتين و روزمارينيك و نارينجينين بنسبة 3448،407 و 453،209 و 396،107 و 387،429 و 213،689 و 207،17 جزء في المليون على التوالي) و الفينولز (البنزويك و E-فانيليك و بيروجالول و كينتشين و ساليسيليك و إلاجيك بمقدار 1075 و 912 و 785 و 628 و 357 جزء في المليون، على التوالي) .وقد بينت النتائج الحالية أن طحلب ديكتيوتا ديكوتوما يستحق تعميق الدراسة عليه حيث يمكن أن يُستخدم كمصدر طبيعي للمواد النشطة بيولوجياً.