SEASONAL VARIATION AND ANTIFUNGAL ACTIVITIES OF METHANOLIC ALGAL EXTRACTS OF SOME DICTYOTACEAE OF BENGHAZI COASTS, LIBYA

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Abstract

The results evaluated the efficiency of all used algal extracts with high significant differences and could be arranged dissentingly as *Dictyota linearis* > *Dictyota dichotoma var. dichotoma* > *Dictyopteris membranacea* > *Padina pavonica* (Dictyotaceae). The methanolic used algal extracts of spring and summer were more efficient without significant difference while the algal extractions of autumn came at the second rank with significant difference. The used fungi were *Aspergillus niger, Aspergillus flavus, Penicilium parasiticus, Fusarium oxysporum, Fusarium solani* and *Candida utilis*. All of them were affected by all used algal extracts without significant difference.

Key words: Dictyotaceae, Marine algae, Phaeophyta, pharmaceutical and pathogenic fungi.

Introduction

Benghazi coasts extended about 18 km at the eastern coast of Libya having approximately 25 marine macro brown algal species with many economical and pharmaceutical important marine algae some of them belonging to family Dictyotaceae (**Godeh, *et al.*, 1992**). The Libyan researches about their antibacterial and antifungal extracts and their effects were done by (**El-Baghdady**, **2001**; **El-Fergani**, **2001**; **Togan**, **2002**; **El-Sal**, **2005**; **El-Gahmy**, **2007** and **El-Fatemi**, 2008). **Harvey** (**2000**) reported that, about 10% of the world plants have been investigated for biological activity. **Vitor *et al***. (**2002**) could isolate many compounds from six marine algae providing valuable ideas for the development of new drugs against microbial infection and inflammation. Many recent bioactive compounds were discovered and isolated from marine organisms (**Donia and Hamann**, **2003**; **Hafez *et al.***, **2005** and **El-Gahmy**, **2007**) Marine macro algae contain high amounts of carbohydrates, protein, minerals (**Rupe'rez and Saura-Calxto**, **2001**) low fat contents, few calories (**Lahaye and Kae**, **1997**) and important source of minerals (**Nisizawa *et al.*, 1987**). This content varies greatly and demonstrates a dependence on such factors as season and

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environmental growth conditions **Abdallah** (**2007**) where, **Combaut *et al*.** (**1981**) evaluated that seasonal variation affected the concentration of algal compounds. So, **Aktan** (**2007**) knew which season and frequency could be encounter to some species to be economic. This work assesses the bioactive potentialities of seasonal methanolic brown algal extracts from Benghazi coasts on some pathogenic fungi growth isolated from clinical samples.

Materials and Methods

The Study area, Harvest and manipulation of algal material:

Benghazi city lies at 32° 03' 28.03" N and 20° 02' 29.18" E at the eastern Libyan coast. The algal species were usually harvested in the morning from some Benghazi coasts (Ishbelia and El-Sabry) during the period from April 2005 and October 2006 (Figure 1). They were collected and identified from a relatively closed coast of Ishbelia which received some sewage water and also from El-Sabry open coast. Four algal species were chosen for their dominancy and capability of secretion of some antifungal substances. The conditions of extracted were detected at previous paper so, the present work aimed to comparing the effect of seasonal methanolic algal extractions on growth of some pathogenic fungi. Algal species were packed in nylon bags and kept in iceboxes for subsequent manipulation in the laboratory, to remove the epiphytes; necrotic parts, salts and impurities and then rinsed with tap and distilled water, weighed and dried in dark at room temperature (25±3ºC.) for 7 days then grinding and kept in paper bags (**Rao and Parekh**, **1981** and **Vlachos *et al*.**, **1996**). The identification was according **Ardissone** Italian list (**1893**), **Pampanini** (**1931**), **Burrows** (**1991**), **Godeh *et al*.**, (**1992**) and **Aleem** (**1993**).

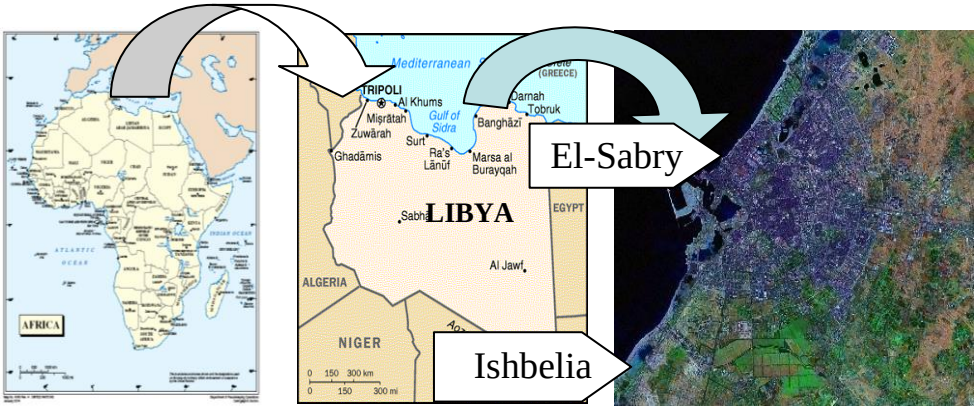

Figure (1): Maps of Africa, Libya and the study area

Algal Extracts:

The crude algal extractions obtained by socking 5g of grinded algae in 100 ml methanol for 24 hours at room temperature (25±3ºC) and Shaking at 100 rpm according to **Vlachos *et al***. (**1996**).

Microorganisms, Growth conditions and antimicrobial activities:

Under septic conditions, antimicrobial activities were tested against the used fungi which isolated from Benghazi children hospital (in addition to those given by staff member of Botany department). They sub-cultivated by scratching method on potato dextrose agar (PDA) media according to **Lustigman *et al*.** (**1992**) and **Crasta *et al***. (**1997**) for 24 h.; 48h.* or 72h.** (**Gonzalez del Van *et al*., 2001**). They were *Aspergillus niger*, Aspergillus flavus*, Penicilium parasiticus*, Fusarium oxysporum**, Fusarium solani** and *Candida utilis*. Hole-plate diffusion method used to evaluate the antimicrobial activities (**Vlachos *et al*., 1996**) at 27±3ºC for 48 hours (**Crasta *et al*., 1997**). Clear zones measured in millimeters carefully at least triplicate. The extracting agent (methanol) was tested as control.

Statistical analysis:

The data were statistically analyzed using **Statistical Analysis System** (**SAS**) (**1995**) according to general linear models:

Observation = Overall mean + Algal effect + Fungal effect + Seasonal effect + Error.

$$\mathbf{Y_{ijk} = \mu + S_i + A_j + F_k + (S\times A)_{ij} + (A\times F)_{jk} + e_{ijk}}$$

Where:

$\mathbf{Y_{ijk}}$ = Observation.

$\boldsymbol{\mu}$ = Overall mean.

$\mathbf{S_i}$ = The effect of the i^{th} season (1, 2 and 3).

$\mathbf{A_j}$ = The effect of the j^{th} algae (1, 2, 3 and 4).

$\mathbf{F_k}$ = The effect of the k^{th} fungi (1, 2, 3, 4, 5 and 6)

S×A = Interaction between i^{th} season and j^{th} algae.

A×F = Interaction between j^{th} algae and k^{th} fungi.

$\mathbf{e_{ijk}}$ = Error assumed to be NID $(0, \delta^2_e)$.

Results and Discussion

Unfortunately, the winter season not detected for the relatively shortage of marine algae and very bad weather. The results were illustrated in Table (1) showed significant seasonal effect on algal extracts. Spring and summer seasons

were the highest ones (24.40 and 23.07 mm, respectively) without any significant between them while autumn were the lowest season (19.94 mm) and significantly differ. Unfortunately, the winter season not detected for the relatively shortage of marine algae and pad weather. The results also evaluated the efficiency of all used algal extracts with highly significant differences and could be arranged dissentingly as *Dictyota linearis* > *Dictyota dichotoma var. dichotoma* > *Dictyopteris membranacea* > *Padina pavonica* with mean values 25.89, 24.28, 22.82 and 18.50 mm, respectively. More or less similar results were obtained at fertilization period by **Pesando and Caram** (**1984**). **Vidyavathi and Sridhar** (**1991**) also reported that spring season enhanced the algal extracts. Generally, the seasonal variations affected the activity of algal extractions (**Combaut *et al*.**, **1981**; **Tuney *et al*.**, **2006**).

All tested fungi were affected by all used algal extracts without significant difference between them but they could be also arranged dissentingly as *Fusarium oxysporum*, *Penicilium parasiticus*, *Fusarium solani*, *Aspergillus flavus*, *Aspergillus niger*, and logically *Candida utilis* came at the end with relatively similar mean values (24.64, 24.33, 23.15, 21.98, 21.73 and 21.08 mm, respectively). Due to the significant effect of both seasons and algae on fungi, the statistical analysis of the results showed non significance interaction between seasons and algae. Meanwhile, there is significant interaction between algae and fungi.

The interaction between seasons and algae summarized at table (2). Ignoring the effect of algae and fungi, there is high significant effect due to the seasons. The highest value (24.40mm) was observed for spring followed by summer (23.07mm) without any significant between them. Nevertheless, autumn season significantly differ and came at the second rank (19.94mm). **Lima-Filho *et al*.** (**2002**) and **Tüney *et al*.** (**2006**) pointed the seasonal variations as a first factor affected the production of secondary metabolites and antimicrobial activities of marine algae. **Tarig** (**1991**) and **Lustigman** (**1992**) produced antimicrobial substances by macroalgae with consideration of seasonal variations. **Bennamara, *et al*.** (**1999**) and **Horikawa *et al*.** (**1999**) reported significant anti-methicillin-resistant *Staphylococcus aureus* activity by crude methanol extracts from 11 species of Japanese marine algae, and they isolated four bromindoles from the red alga *Laurencia brongniartii* as antibacterial substances.

Table (1): The seasonal and algal effect (mm) on tested fungi (mm):

Overall mean	**22.81**
Standard deviation	**7.29**
Seasonal effect	
Spring	24.40a
Summer	23.07a
Autumn	19.94b
Algal effect	
Dictyota linearis	25.89a
Dictyota dichotoma var. dichotoma	24.28ab
Dictyopteris membranacea	22.82b
Padina pavonica	18.50c
Fungal effect	
Fusarium oxysporum	24.64
Penicilium parasiticus	24.33
Fusarium solani	23.15
Aspergillus flavus	21.98
Aspergillus niger	21.73
Candida utilis	21.08
Level of seasonal significant	**
Level of algal significant	***
Level of fungal significant	n. s.
S×A	n. s.
A×F	**

A, b and c = the means having the same letters not differ at level of significant p < 0.05.

*****= highly significant. Values of clear zones (mm) represent averages of, at least, six readings.**

At constant level of seasonal and fungal effect, there are highly significant differences between algae. *Dictyota linearis* came at the first rank (25.89mm) followed by *Dictyota dichotoma var. dichotoma* (24.28mm) and *Dictyopteris membranacea* (22.82mm) while *Padina pavonica* came at the last rank (18.50mm). Since, the fungi laboratory tested, had no significant response to the different algal extracts (p>0.05) and the mean values ranged between 21.08 and 24.64 mm so, we did not notice any interaction between fungi and seasons or

algae. **Gonzalez del Val *et al***. (**2001**) reported that there may be differences in the capability of the extraction protocols to recover the active metabolites result in different susceptibilities of the target strains. Further investigation on Libyan marine algal extracts and their crude and fractionated antimicrobial extractions must be done for evaluation all their pharmaceutical importance and antimicrobial effects from which season to be more effective and economic.

Table (2): Interaction between seasonal variations and used algal species generally on fungi (mm):

Seasons / Algae	Spring	Summer	Autumn	Mean
Dictyopteris membranacea	25.44	24.02	18.39	**22.82^{b}**
Dictyota dichotoma var. dichotoma	23.50	25.05	n. e.	**24.28^{ab}**
Dictyota linearis	27.55	26.13	23.86	**25.89^{a}**
Padina pavonica	21.57	17.08	17.58	**18.50^{c}**
Mean	**24.40**	**23.07**	**19.94**	

A, b and c = the means having the same letters not differ at level of significant p < 0.05.

n. e. = not estimated. Values of clear zones (mm) represent averages of, at least, six readings.

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التباين الموسمي والنشاط الضد فطرى لمستخلصات الميثانول لبعض طحالب عائلة الديكتيوتسى بشواطئ بنغازي، ليبيا

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أثبت البحث فاعلية ومقدرة جميع المستخلصات الطحلبية المستخدمة بفروق معنوية عالية وكان ترتيبها حسب قوة تأثيرها على نمو بعض الفطريات الممرضة كالتالي: *Dictyota linearis* < *Padina pavonica* < *Dictyopteris membranacea* < *Dictyota dichotoma var. dichotoma*. وكانت مستخلصات الطحالب المجمعة فى فصلى الربيع والصيف هي الأكثر فاعلية على نمو جميع الفطريات الممرضة المستخدمة دون فرق معنوي فيما بينهما وتلاهما مستخلصات فصل الخريف بفارق معنوي. ولم يتم التجميع فى فصل الشتاء للصعوبة الشديدة فى تجميع الطحالب القليلة نسبيا نظرا لسوء الأحوال الجوية. أثرت جميع مستخلصات الطحالب بدون معنوية على كل الفطريات المستخدمة وهى *Fusarium oxysporum* و *Fusarium solani* ، *Penicilium parasiticus*، *Aspergillus flavus*، *Aspergillus niger*، *Candida utilis*. هذا ويوصى البحث بمواصلة الدراسات الحقلية والمعملية على الطحالب البحرية الليبية ومستخلصاتها الضد ميكروبية على أن يتم فصل وتعريف المواد الفعالة بتلك الطحالب إبرازاً لأهميتها الاقتصادية والدوائية الكبيرة.