

Antibacterial Activity of Methanolic Extract of Dominant Marine Alga (*Padina pavonia*) of Tolmeta Coasts, Libya

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Abstract: This study mainly aimed to identify the marine algae of Tolmeta coasts and evaluate the antibacterial activity of the most dominant species (*Padina pavonia*) as compared with some famous antibiotics. During many sampling visits at 2009, Thirty four marine algal species (26 genera) were collected and identified at Tolmeta coasts (150 Km. eastern north Benghazi city). Two species (5.88%) of the collected algae (*Lyngbia* and *Rivularia*) were belonging to Cyanophyta, Six species (17.65%) belong to Chlorophyta, thirteen species (38.24%) belonging to Phaeophyta (with special reference to genera *Padina* and *Cystoseira*) and thirteen species (38.24%) belonging to Rhodophyta. The R/P ratio was 1.00 which indicated the rough weather of this area. *Padina pavonia* was the most dominant species at all samples, methanolic crude extract (at cold and 24 h.) were tested against *Escherichia coli* and *Staphylococcus aureus* bacteria and matched with some famous antibiotics. All of the treatments were affected *Escherichia coli*, they could statistically ranked dissentingly as Ci > E15 > Sxt at the first rank and Te30 > *Padina* extract at the second rank while P10 came at the third rank with significant values. Meanwhile, *Staphylococcus aureus* was affected only by E15 antibiotic.

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1. Introduction:

Edible seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition (Fayaz *et al.*, 2005). Most of the compounds of marine algae show antibacterial activities (Vairappan *et al.*, 2001), used as direct and indirect human food sources (Dawes, 1998 and Rajasulochana *et al.*, 2009), and used also in new pharmaceutical industries (Lima-Filho *et al.*: 2002: Ely *et al.*, 2004 and Tüney *et al.*, 2006) and recently showed antimicrobial activities (El-Gahmy, 2007, Venkateswarlu *et al.*, 2007, El-Fatemy, 2008, Ki-Bong Oh *et al.*, 2008 and Rajasulochana *et al.*, 2009). Said and Godeh, (2008) reported that Tolmeta coasts characterized by 32 marine algal species. Most of them could use as ecological quality indicators (Pinedo, *et al.*, 2007). *Padina* sps grow and dominated in the shore of Kanyakumari and Ramanthapuram Districts of Tamilnadu State, India giving significant effect when tested against *Escherichia coli* and *Staphylococcus aureus* bacteria (Rajasulochana *et al.*, 2009). Organic solvent always provides a higher efficiency in extracting compounds for antibacterial activities comparative water based methods (Tüney, *et al.*, 2006). El- Baghdadi (2000) evaluated that the extracts of *Dilophus spiralis* more effective more than those of *Padina pavonia* on some Bacterial species. El-Sal (2005) evaluated that some

algae of Musrata coasts could secret antibacterial substances. Recently, El-Fatemy (2008), El-Fatemy *et al.*, (2009) and El-Fatemy & Said (2011) tested some Dictyotales algae from Benghazi and Gheminis coasts on some pathogenic and dermatophytes isolated from some clinical departments of child and El-Gamaheria hospitals. So, this study tries to evaluate the antibacterial activities of some Libyan marine algae.

2. Material and Methods

The Study area:

The geographical location of the study area is illustrated in Fig. 1. Tolmeta coast lies, about 150 Km. northern east Benghazi at 32° 41' 45.68" N and 20° 57' 38.99" E. Their open rocky shores had little sandy parts and some small rocky islands very closed to their beaches. They are also had very small fishing ports without any pollution and human beings activities.

Sampling and sample preparations:

Specimens were harvested generally in the morning in ice tanks at nylon or polyethylene bags sprinkled with 4% formalin sea water solution for mounting on the herbarium sheets, glass bottles and some of them kept freshly at refrigerators for future use and subsequent taxonomic identification using

Pampanini (1931), Burrows (1991) and Aleem (1993). Epiphytes, impurities and salts were removed carefully and quickly at laboratory with tap and distilled sterilized waters. Samples were kept under sunshade for 7 days till complete drying then ground to powder form and packaged in paper for extractions (Rao and Parekh, 1981 and Vlachos *et al.*, 1996). The herbarium sheets have been deposited in the Herbarium, Department of Botany, Garyounis University, Benghazi {CHUG nos. FM. 650; 651}. Longitudinal and transverse sections of the axis at the apexes, midfronds and the bases were hand made and stained in 1% KI₂ or Anilin blue solution.



Fig. 1: Map of Libya and the study area (Tolmeta coast).

Species richness:

Species richness index calculated according to Wilhm, (1975) by direct count of different algal species (taxa) at every sampling site where, the decrease in number of species and increase in number of individuals is a characteristic feature of polluted water.

Algal Extracts:

The crude algal active methanolic extractions obtained according to Crasta, *et al.*, (1997) by soaking 5g. of cleaned, washed, dried and grinded algal tissues in 100 ml. of methanol solvent 96% (Kaufman, *et al.*, 1999) by Soxhlet for 2 hours at 80 °C and concentrated at 2.5 ml. in dark at room temperature (25±3°C) according to Vlachos *et al.*, (1996). The crude methanolic extracts and some antibiotics were tested against the bacterial growth. The crude algal active extractions were tested to be easily for many people to introduce fresh algae in their food and pharmaceutical uses.

Bacterial Growth conditions and antibacterial activities:

Under septic conditions, antibacterial activities were tested against the Bacteria (100 µm of

10⁸ conc.) which isolated from Benghazi children hospital, sub-cultured and routinely maintained by three dimension streaking method on both Nutrient agar and Muller Hinton agar media according to Cheesbrough, (1984) for 18 - 24 hours at 37±2°C.

Hole-plate and disc diffusion methods used to evaluate the antibacterial activities of algal extract and some antibiotics, respectively (Bauer, *et al.*, 1996). Clear zones around holes were measured in millimeters (mm) carefully at least six replicate with crude algal extracts and different antibiotics. The extracting agent (methanol) was tested as control (Tüney *et al.*, 2006). Bacterial suspensions were kept at 4°C for further treatments. The stock cultures were maintained in sterilized soil (3 successive days) at 4°C and sub-cultured in agar slants whenever required. The standard disc diffusion method was used with five specific antibiotics.

Statistical analysis:

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

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where: Y_{ij} = The J^{th} clear zone of i^{th} algal extract and antibiotics.

μ = Overall mean.

A_i = Fixed effect of the i^{th} algal extract and antibiotics (1, 2, ..., 6).

e_{ij} = Error assumed to be NID (0, σ^2_e).

3. Results and Discussion

Tolmeta coasts were characterized by only 34 species and 26 genera of marine algae. Cyanophyta represented only 2 species (5.88%) and 2 genera (7.69%) of the recorded algae (Table 1). There are just 6 species (17.65%) and 6 genera (23.08%) belonging to Chlorophyta (Table 2), Phaeophyta (Table 3) represented by 13 species (38.24%) and 6 genera (23.08%) and Rhodophyta (Table 4) represented by 13 species (38.24%) and 12 genera (46.15%). At relatively similar area and conditions, Godeh *et al.*, (2008) reported that, Tobruk coasts characterized by thirty six species of different marine algae.

According to the species richness indication of Wilhm, (1975), one could conclude that, Tobruk coast is more or less pure and sustained than Tolmeta coast. Said *et al.*, (2005) used the species richness parameters carefully to evaluate the purity and pollution state of different four Egyptian water bodies. According to the finding of Diaz-vades *et al.*, (2007) and Pinedo, *et al.*, (2007) many of the identified marine algal taxa considered as indicators to the good and very good ecological quality waters

like *Cystoseira*, *Corallina*, *Hypnea*, *Jania* and *Laurencia*.

Contrarily, Rhodes Island was relatively richer where 155 macroalgal taxa (Tsiamis, *et al.*, 2007) had. Diaz-Valdes, *et al.*, (2007) identified 65 Littoral macroalgae using them to assess the environmental quality of Valencian rocky coasts (SE Spain). Diapoulis and Tsiamis (2007) also found 88

marine benthic macroalgal taxa at the upper infralittoral zone of South Aegean Sea (Greece).

Cyanophyta represented only 2 species (5.88%) and 2 genera (7.69%) of the recorded algae (Table 1), they were *Lyngbia* and *Rivularia*. Both of them were present as very small batches on the much closed rocky parts of the shores.

Table (1): Distribution of Blue-green marine algae at Tolmeta coasts:

Cyanophyta
<i>Lyngbia</i> C. Agardh ex Gomont 1895
<i>Lyngbia sordida</i> (Zanardini) Gomont
<i>Rivularia</i> Bullata
<i>Rivularia bullata</i> (Poiret) Berkeley
Number of genus = 2 Number of species = 2

Table (2): Distribution of green marine algae at Tolmeta coasts:

Chlorophyta
<i>Acetabularia</i> Lamouroux 1817
<i>Acetabularia acetabulum</i> (lamx.) Silva
<i>Anadyomene</i> Lamouroux 1812
<i>Anadyomene stellata</i> (Wulf.) C. Agardh
<i>Caulerpa</i> Lamouroux 1809
<i>Caulerpa prolifera</i> (Forsskål) Lamouroux
<i>Dasycladus</i> C. Agardh 1828
<i>Dasycladus vermicularis</i> (Scopoli) Krasser
<i>Flabellia</i> Reichenbach (<i>Udtea</i> Lamouroux)
<i>Flabellia petiolata</i> (Turva) Nizamuddin
<i>Halimeda</i> Lamouroux 1816
<i>Halimeda tuna</i> (Ellis ét Solander) Lamouroux
Number of genus = 6 Number of species = 6

Table (3): Distribution of brown marine algae at Tolmeta coasts:

Phaeophyta
<i>Cystoseira</i> C. Agardh 1820
<i>Cystoseira barbata</i> (Goodenough ét Woodward) J. Agardh
<i>Cystoseira cinitophylla</i> Ercegovic
<i>Cystoseira compressa</i> Gerloffi ét Nizamuddin
<i>Cystoseira elegans</i> Sauvageau ét Feldmann
<i>Cystoseira discors</i> (Linn.) C. Agardh emend. Sauvageau
<i>Cystoseira gerloffi</i> Nizamuddin
<i>Cystoseira stricta</i> (Montagne) Sauvageau
<i>Dictyopteris</i> Lamouroux 1809
<i>Dictyopteris membranacea</i> (Skackhouse) Batters
<i>Dictyopteris tripolitana</i> Nizamuddin
<i>Dictyota</i> Lamouroux 1809
<i>Dictyota dichotoma</i> (Hudson) lamouroux
<i>Padina</i> Adanson 1763
<i>Padina pavonia</i> (Linnaeus) Lamouroux
<i>Sargassum</i> C. Agardh 1820
<i>Sargassum hornscuchii</i> C. Agardh
<i>Scytosiphon</i> C. Agardh 1820
<i>Scytosiphon lomentaria</i> (Lyngbye) Lamouroux
Number of genus = 6 Number of species = 13

Chlorophyta were represented by Just 6 species (17.65%), 6 genera (23.08%) of the total recorded algae (Table 2), The reduction of green species may be due to the presence of *Caulerpales* which considered strong competitors and its production of toxic substances, which inhibit their grazing (David *et al.*, 2004 and Piazzi *et al.*, 2005).

Thirteen species (38.24%), six genera (23.08%) of them were belonging to Phaeophyta (Table 3) with special reference to genera *Padina* and *Cystoseira*. Contrarily, Mubina and Nausheba, (1992)

identified 48 brown species at Karachi coast in India. *Cystoseira* species could used as an additional important argument for securing a more wise and sustainable use of the coastal ecosystem that they indeed play a critical role in the conservation of species and habitat diversity (Turk, *et al.*, 2007).

Rhodophyta (Table 4) also represented by thirteen species (38.24%), twelve genera (46.15%). The result was completely different with South Aegean Sea (Greece) which dominated by 60 red algal taxa (Diapoulis and Tsiamis, 2007).

Table (4): Distribution of red marine algae at Tolmeta coasts:

Rhodophyta
<i>Acrosorium Zanardini 1869</i>
<i>Acrosorium uncinatum</i> (J. Agardh) kylin
<i>Amphiroa Lamouroux</i>
<i>Amphiroa rigida</i> Lamouroux
<i>Botryocladia Kylin 1931</i>
<i>Botryocladia botryoides</i> (Wulf.) Feldmann
<i>Chondriopsis J. Agardh 1863</i>
<i>Chondriopsis mediterranea</i> (Kütz.) J. Agardh
<i>Chrysmenia J. Agardh 1842</i>
<i>Chrysmenia ventricosa</i> (Lamour.) J. Agardh
<i>Dermatolithon Forslie</i>
<i>Dermatolithon pustulatum</i> (Lamouroux) Foslie
<i>Hypnea Lamouroux 1813</i>
<i>Hypnea musciformis</i> (Wulfen) Lamouroux
<i>Jania Lamouroux 1812</i>
<i>Jania adhaerens</i> Lamouroux
<i>Jania rubens</i> (Linnaeus) Lamouroux
<i>Laurencia Lamouroux 1813</i>
<i>Laurencia papillosa</i> (Forsskål) C. Agardh
<i>Mesophyllum Lemoine</i>
<i>Mesophyllum lichenoides</i> (Ellis ét Solmander) Lemoine
<i>Peyssonnelia Decaisne 1842</i>
<i>Peyssonnelia elegella</i> Harvey
<i>Rytiphlaea C. Agardh 1824</i>
<i>Rytiphlaea tinctoria</i> (Clemente) C. Agardh
Number of genus = 12 Number of species = 13

The R/P ratio is equal one at Tolmeta due to the balance of both Rhodophyta and Phaeophyta (13 species of each). Nizamuddin (1985) evaluated that eastern Libyan coasts were generally poor in algal growth and continuously exposed to rough conditions and fluctuating cold to mild weather because they belong to Pleistocene deposits. Nevertheless, R/P ratio of Rhodes Island, Greece was 3.5; this suggests a warm-temperate aspect of macroalgal flora (Tsiamis *et al.*, 2007).

Padina pavonia was the most dominant species at all samples, methanolic crude extract (at cold and 24 hours method) were tested against

Escherichia coli and *Staphylococcus aureus* bacteria and matched with some antibiotics by measuring the clear zones (Table 5). All of them were affected on *Escherichia coli* with overall mean 21.5, they could statistically ranked dissentingly as $C_i > E_{15} > S_{xt}$ at the first rank and $Te_{30} > Padina$ extract came at the second rank while P10 came at the third rank with significant values. Meanwhile, *Staphylococcus aureus* was affected only by E15 antibiotic. These may be due to the lower concentrations or the sampling program, time, method, drying, extraction and nature of organisms (Brooks, *et al.*, 2007). These results were more or less similar to those of some

green algae reported by El-Sal (2005) and El-Gahmy (2007). An ideal antimicrobial agent exhibits selective toxicity, which means that the drug is harmful to the host (Brooks, *et al.*, 2007). So, Crude extract used to be easy in addition to the main aim of this work to change the culture of many people to eat and treat with marine edible seaweeds (at least 500 Species) for their indefinite usefulness (Bilgrami and Saha, 1996; Dawes, 1998 and Nybakken, 2001).

Gonzalez del Val *et al.*, (2001) tested methanolic extracts of 44 Italian marine algal species as antifungal substances. Souhaili, *et al.*, (2004) used the ethanolic and water extracts of some marine algae of Morocco as antimicrobial agents meanwhile, the methanolic, chloroform and hexane extract were less

effective. Hafez, *et al.*, (2005) tested many extract of *Ulva lactuca* of Sweze canal of Egypt to prevent the growth of some gram positive and negative Bacteria and Fungi. Tüney, *et al.*, (2006) tested 11 Turkish marine algae against some pathogenic Bacteria which showed highly sensitivity. *Padina sps* grow and dominated in the shore of Kanyakumari and Ramanthapuram Districts of Tamilnadu State, India giving significant effect when tested against *Escherichia coli* and *Staphylococcus aureus* bacteria with special reference to methanolic extract and chloroform: methanol (2:1 v/v) where, methanolic extracts of *Padina sp.* were able to exhibit only 25-30% maximum activity against test organisms (Rajasulochana *et al.*, 2009).

Table (5): Effect of crude methanolic extracts of *Padina Pavonia* and some antibiotics on *Escherichia coli* and *Staphylococcus aureus* growth (mm):

Treatments		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Overall mean		21.5	-
<i>Padina</i> extract		15.7b	-
Antibiotics	Sxt	26.2a	-
	E15	29.6a	13.6
	Cip	30.3a	-
	P10	8.7c	-
	Te30	18.5b	-

sxt: sulphatame thoxazole e15: erythromycin
cip: ciprofloxacin p10: penicillin te30: tetracycline

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