



Antibacterial activity of *Ulva intestinalis*, *U. fasciata* and *U. lactuca* against biofilm associated bacteria

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ABSTRACT

The contemporary investigation was conducted to study the antimicrobial activity of Ulvales found in the coastal area of Karachi. The examined seaweeds (*Ulva intestinalis*, *U. fasciata* and *U. lactuca*. *U. intestinalis*) were collected from Sandspit and Kakkapir, whereas, *U. fasciata* and *U. lactuca* were gathered from Buleji. The selected species were examined to detect the antimicrobial activity against gram-positive *Staphylococcus aureus*, *Staphylococcus epidermidis* and gram-negative pathogen *Shigella* sp. Accumulated bacteria- biofilms were found in the rocks of Buleji, Hawks Bay and Manora Island. It is remarkable to note that gram-positive and gram-negative bacteria are responsible for producing infectious diseases in humans. Seaweeds extracts were successively prepared in five solvents; namely, chloroform, ethanol, methanol, n-Hexane and distilled water. The present experiment was performed using the method of measuring the zone of inhibition followed by disc diffusion. Findings showed that *U. fasciata* and *U. lactuca* exhibited a scanty zone of inhibition against gram-negative and gram-positive strains. Although ethanol and methanol extract of *U. intestinalis* showed neither more nor less zone of inhibition against *Shigella* sp., yet aqueous solvent of seaweeds produced no activity against any bacteria. Furthermore, recording the greatest zone of inhibition, the extract with ethanol showed the highest against the *S. aureus*. Notably, chloroform displayed a high limit of inhibition against *S. epidermidis*. It is worth mentioning that Sandspit collected *U. intestinalis* showed high bactericidal activity.

INTRODUCTION

In bioactive entities, marine algae is considered one of the renowned sources. Many seaweeds secrete certain bioactive components that possess detrimental properties on the growth of gram-positive and gram-negative bacteria (Schwartzman *et al.*, 2001; Kolanjinathan *et al.*, 2009). The seaweeds produce different metabolites that serve as the antimicrobial compounds (Lustigman & Brown, 1991; Chiheb *et al.*, 2009; Manilal

et al., 2010). Moreover, different coloured seaweeds (green, red and brown) possess cytostatic, antiviral, anthelmintic, anti-fungal and anti-bacterial compounds. (Lindequist & Schweder, 2001; Newman *et al.*, 2003; Chakraborty *et al.*, 2010).

Benthic algae play a vital role in antibacterial, antifungal, phytotoxic and insecticidal (Ara *et al.*, 1998, 1999, 2002a, 2002b; Rizvi & Shameel, 2004). Due to the innovation in analytical chemistry and requirement of new antimicrobial agents, researchers investigated thoroughly in seaweeds chemistry and antimicrobial agents (Troell *et al.*, 2006; Leary *et al.*, 2009; Yaich *et al.*, 2011; Prabha *et al.*, 2013; Papenfus *et al.*, 2013). Antagonistic bacterial efficacy of seaweed extracts on gram-positive and gram-negative bacteria has won the interest of many researchers all over the world (Kumar & Rengasamy, 2000; Salvador *et al.*, 2007; Patra *et al.*, 2009; Vallinayagam *et al.*, 2009). Marine products have been processed using a variety of seaweeds (Ulvaes) that were examined to detect pathogenic bacteria. (Henrikson & Pawlik, 1995; Wahl, 2008; Hellio *et al.*, 2004).

Latest improvements showed that compounds extracted from seaweeds are more efficient antimicrobial agents that prevent infectious diseases and microbial contaminations (Troell *et al.*, 2006; Leary *et al.*, 2009; Yaich *et al.*, 2011; Papenfus *et al.*, 2013; Prabha *et al.*, 2013). Phytochemical analyses of aqueous, ethanolic, chloroform, petroleum ether and hexane extracts of seven green seaweeds exposed the occurrence of a high-quality of secondary metabolites have been profoundly discussed (Babu *et al.*, 2014).

Brown seaweeds antimicrobial activity was observed against *E.coli* causative agent of diarrhoea. (Rizvi & Shameel, 2003; Khan & Qari, 2012). It also showed the antibacterial activity against gram-positive and gram-negative bacteria. The aimed to assay the antagonistic bacterial activity of two species of Ulvaes lying on biofilm forming bacteria occurred at the Karachi coast.

MATERIALS AND METHODS

Collection of seaweeds

U. fasciata and *U. lactuca* were collected from intertidal zone of Buleji, whereas *U. intestinalis* was collected from two different sites: Sandspit and Kakapir, unpolluted and polluted areas, respectively.

Preparation of seaweed extracts

The collected seaweeds of *U. fasciata*, *U. lactuca* and *U. intestinalis* were washed; epiphytes and other associated debris were removed. Hundred grams of seaweeds were extracted and dispersed in 200 ml of solvent (chloroform, ethanol (95.5%), methanol (95.5%) and n-hexane (95.5%) till the solvent passes through for the extraction of antimicrobial compounds. (Jin *et al.*, 1997). The extracts were vaporized to dry by using a rotary vacuum evaporator, and past form extracts were stored in a refrigerator at 4°C for future processes.

Collection of test microbial cultures

The biofilm was taken under the aseptic condition from Buleji, Hawks Bay at Manora Channel of Karachi coast. These biofilms were brought into the laboratory. To acquire pure cultures of gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) mannitol salt agar. Whereas, gram-negative bacteria (*Shigella* sp.) was cultured in MacConkey agar.

Assay of Antibacterial activity of *Ulva* species

In vitro cultivation of test microbes

Test microbial cultures were isolated from biofilm inoculated in zobell agar (exclusive for marine bacteria) and incubated at 37°C for 24 hours.

Screening of antimicrobial activity

Agar disk-diffusion method

Inoculums of bacterial species were inoculated in agar plates. Filter paper discs (~5mm in diameter), containing 2 drops (100 µL) of seaweed extracts of selected seaweeds were inoculated on the agar surface. The Petri dishes were incubated at 37°C for 24 hours. The antimicrobial agent was diffused into the agar to suppress or increase growth mass of the analysed microorganism, and then, the diameters of inhibition growth zones were calculated in millimetre.

RESULTS

The antibacterial activity with different concentrations of solvents was examined against three bacteria. Ethanol was eminent against *S. aureus* with a greatest zone of inhibition (8.8 ±1.3 mm), while chloroform showed a high zone of inhibition against *S. epidermidis* (8.3±1.8 mm). On the other hand, both chloroform and methanol showed a maximum zone of inhibition against *shigella* sp.. Ethanol and methanol recorded a slight limit (0.2±0.2 and 0.3±0.2mm) of antibacterial activity against *S. epidermididis* as compared to *S. aureus* and *Shigella* sp. whereas, distilled water showed no activity against any of these bacteria (Table 1-Fig. 1).

Table 1. Antibacterial activity of Controls against bacterial pathogens

S. No.	Pathogen	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	n-Hexane (mm)	Distilled water (mm)
1.	<i>S. aureus</i>	7.1±3.2	8.8 ±1.3	7.8±2.4	7±3.3	-
2.	<i>S. epidermididis</i>	8.3±1.8	0.2±0.2	0.3±0.2	7±3.3	-
3.	<i>Shigella</i> sp.	8.6±1.5	8.5±1.7	8.6±1.5	8.5±1.7	-

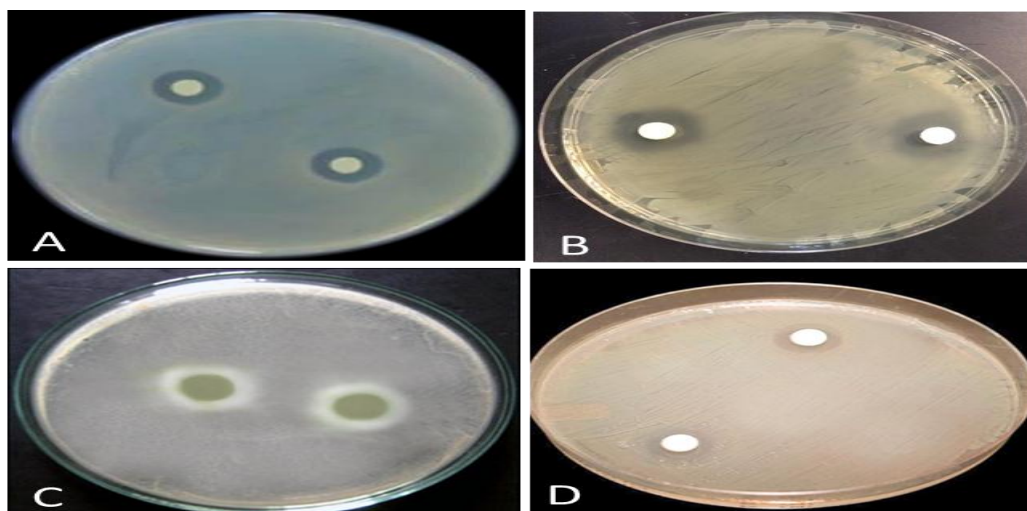


Fig. 1. Unpolluted *U. intestinalis* ethanolic extract on *Shigella* sp. cultured medium showed inhibition, B- Methanolic extract of unpolluted *U. intestinalis* on *Shigella* sp. Produced the clean area around the bacterial culture, C- Ethanolic extract of polluted *U. intestinalis* on *Shigella* sp. Exhibited zone of inhibition, D- Methanolic extract of polluted *U. intestinalis* on *Shigella* sp. showed zone of inhibition.

Assay of Antibacterial activity of *Ulva* species

Kakapir collected *U. intestinalis* showed 5.67 ± 1.63 mm zone of inhibition against *Shigella* sp. In contrast, Sandspit collected *U. intestinalis* range of inhibition zone was 8.66 ± 1.6 mm. No antibacterial activity was recorded against *S. aureus* and *S. epidermididis* (Table 2).

Table 2. Antibacterial activity of *U. intestinalis* against *Shigella* sp.

S. No.	Site	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	n-Hexane (mm)	Distilled water (mm)
1	Kakapir	-	5.67 ± 1.03	5.67 ± 1.63	-	-
2	Sandspit	0.78 ± 0.25	8.83 ± 1.3	8.67 ± 1.6	0.88 ± 0.15	-

DISCUSSION

The extracts of the green seaweeds *U. intestinalis* collected from Sandspit and Kakapir demonstrated the considerate results in solvents (ethanol and methanol) against *Shigella* sp. Whereas, *U. fasciata* showed no resistant bacterial activity. In their study, **Thirumaran and Anantharaman (2006)** stated that ethanolic and methanolic extracts of

similar species showed high adverse activity against *Shigella* sp. Conversely, the extracts of *U. intestinalis* in 70% ethanol showed activity against *S. typhimurium*, *S. aureus*, and *B. subtilis* (Soltani *et al.*, 2012).

In the present study, both n-hexane and chloroform were ineffective against all tested bacterial strains. The extracts were not effective against *U. fasciata* collected from Buleji prepared in n-hexane. Moreover, chloroform indicated no inhibition against either gram-positive (*Staphylococcus* sp.) or gram-negative (*Shigella* sp.) bacteria (Valeem *et al.*, 2011). The current study offsprings concluded that the antibacterial activity of seaweeds may be due to the different physicochemical factors, extraction and nature of solvents (Thirumaran & Anantharaman, 2006; Karthikaidevi *et al.*, 2009) although the same species of seaweeds have different antibacterial effects due to altered study sites. Scientists have hypothesized that organisms nurture in unfavorable environments might develop compounds that could provide shield adjacent to pathogenic microorganisms showing elevated antimicrobial activity (Stix, 2006; Wright & Sutherland, 2007; Bennett, 2008; Li & Nikadio, 2009; Fischbach & Walsh, 2009; Gootz, 2010; Lee *et al.*, 2010; Kiran, 2013; Kiran *et al.*, 2014). This may be applied to *U. intestinalis* procured from Kakapir and Sandspit backwaters (Shoib *et al.*, 2017) has been growing in a polluted environments (Shoib *et al.*, 2017) comparatively to Buleji.

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