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## Anti-MRSA Activity using Brown algae (*Padina pavonica* & *Sargassum vulgare*), *in vitro*

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### Abstract

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the most typical bacteria Infectious in Libyan hospitals. Therefore, the present study was conducted as an attempt to find an alternative treatment to reduce the infection MRSA, from crude acetone and ethanol extracts of brown algae (*Padina pavonica* and *Sargassum vulgare*) against three MRSA isolates: Wound (W), Skin (S) and diabetic foot ulcers (DFU), by comparing with three antibiotics (Imipenem, Gentamicin and Tetracycline), the drug susceptibility test was performed using a disc diffusion technique. The obtained results showed that all extracts of brown algae showed a clear inhibition effect toward the growth of two isolates of MRSA (W) and (S). On the other hand, *P. pavonica* extracts are more efficient than *S. vulgare* extracts, especially acetone, MRSA (DFU) was the most resistant isolates to antibiotics and extracts. Imipenem showed good activity against a tested MRSA species, which is a noteworthy results. Generally, it can be concluded that acetone extracts of *P. panovica* may be a good source of compounds effective for anti-MRSA.

**Keywords:** MRSA, *Padina pavonica* & *Sargassum vulgare*, Antibacterial.

### Introduction

*Staphylococcus aureus* is a gram-positive bacterium that produces round, golden-yellow colored colonies, which cause a wide variety of hospital diseases. Infections caused by this pathogen are common both in community-acquired and hospital-acquired settings, Its presence is associated with surgical site infections and open wounds and disease Infections of diabetic ulcers (1, 2). The treatment remains challenging due to the emergence of multi-drug resistant strains (3). Usually, *S. aureus* naturally presenting in a human body does not cause infection on normal skin, but entering the internal tissues or bloodstream may cause a variety of serious infections (4). It was first reported this Methicillin-resistant *Staphylococcus aureus* (MRSA) strain after methicillin using as a substitute for penicillin by patients in 1961(5), was classified by the World Health Organization (WHO) as one pathogen the most dangerous to human safety in hospitals and societies that endanger human health (6). For instance, a study reported a high prevalence of types several of MRSA in Libyan hospitals, especially in Benghazi (7). A new study ,the southern region of Libya (8). Due to strains resistant to  $\beta$ -lactam antibiotics, which Possess important virulence factors: biofilm, mechanisms for evasion of intra cellular immune cells and toxin production and clumping factors. Many attempts have been carried out all over the world to counteract MRSA ,bio-activity. In Korea, a study was able to reduce the risk of MRSA by using macro-algae(9), in Egypt, a study confirmed the possibility of developing an antibacterial agent for MRSA infection from Extracts of seaweed (10). *Padina pavonica* and *Sargassum vulgare* is a brown alga, belonging to order Dictyotales, distributed worldwide, especially in the Mediterranean coasts, a posses a multitude of biological activities such as the antioxidant, antimicrobial, and anticancer, anti-inflammatory ones (11), can be used for the management of phytopathogens, in the extracellular synthesis of bio-silver nanoparticles (12, 13). Therefore, the study aimed to use crude Extracts *Padina pavonica* and *Sargassum vulgare* against Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates from different diseases.

## Materials and Methods:

### Identification of Brown algae:

*Padina pavonica* & *Sargassum vulgare* Fresh samples were collected from Al-Hammahi North Al-Bayda City in the middle of August 2022. Both species were identified using morphologically characterize keys (14, 15), in the marine Resources Department laboratory - Tobruk. Algae washing with

distilled water and dried inside the laboratory at room temperature, grinded by an electric grinder and saved for use.

### **Bacterial Isolates:**

Two isolates Methicillin-Resistant *Staphylococcus aureus* from Al-Jalaa hospital -Benghazi, and one isolates from Alsaraya hospital-Egypt, samples were identified in the laboratory medical of Alsaraya hospital, -Egypt:

- MRSA (W): isolates from wound infection
- MRSA (S): Isolates from Skin
- MRSA (DFU): Isolates from diabetic foot ulcers.

### **Preparation of Extracts:**

20g of dry powder of brown algae was added to 200ml of different solvents (Acetone and Ethanol) in a glass flask, for three days with constant agitation in a vibratory shaker at 35 ° C, then filter and shaken in a centrifuge at 3500 rpm for 15 minutes. The next step was filtering with Whitman No.1 filter paper and drying in a Rotary evaporator to get dry powder (16).

### **Antibacterial Susceptibility Testing:**

The mediums were sterilized for 15 minutes in an autoclave at 121°C bacteria were grown on Mueller- Hinton agar medium. For screening, sterile filter paper disks (6 mm) impregnated with the extract were placed on the surface of inoculated bacteria mediums. The dishes were incubated for 20-24 hours at 37°C with three replications per dish, then a measure of diameters of inhibitory zones minus the diameter of the disc (17). Putting for antibiotics disks for comparison (Imipenem 10µg, Gentamicin 30µg and Tetracycline 30µg).

### **Statistical Analysis:**

The study's experiment was designed according to the Completely Randomized Design (CRD). The statistical analysis was performed using (Minitab17) program and ANOVA test was carried out means were compared using Turkey's test at  $P < 0.05$ .

## **Results**

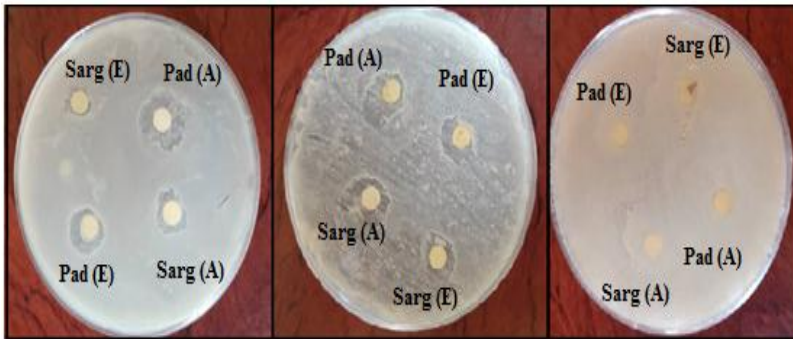
### **Sensitivity and resistance of (MRSA) isolates to algal extracts:**

Table 1 and fig. 1 show the results of resistance and sensitivity of three strains of (MRSA) isolated from different diseases to two brown algae extracts. MRSA (d) was the most resistant isolates to (Aceton and ethanol) extracts for both algae. While result showing weakened sensitivity to an acetone extract of *P.pavonica* with an inhibition diameter (1.0 mm). The

MRSA isolates from wounds (W) and skin (S) recorded middle sensitivity to extracts (acetone and ethanol) of *S.vulgare* algae, with diameters between (1.0-3.0mm), also, the two isolates had a high sensitivity to extracts (acetone and ethanol) of *Padina pavonica*, with diameters between (2.4-7.3mm).

**Table 1: Effect of organic solvent extracts of *Padina pavonica*, *Saragassum vulgare* on the growth of bacteria.**

Pathogens bacteria		MRSA		
		Wound (W)	Skin (S)	Diabetic foot ulcers (DFU)
<i>Padina pavonica</i>	Acetone	5.0±0.5 a	7.3±1.0 a	1.1±0.0 b
	Ethanol	2.4±0.2 c	4.9±0.5 b	0.0±0.0 c
<i>Saragassum vulgare</i>	Acetone	3.0±0.6 b	2.8±0.4 c	0.0±0.0 c
	Ethanol	1.0±0.0 d	2.0±0.2 d	0.0±0.0 c



**Fig. 1: Antibacterial activity of extracts of *P.pavonica* & *S.vulgare*.**

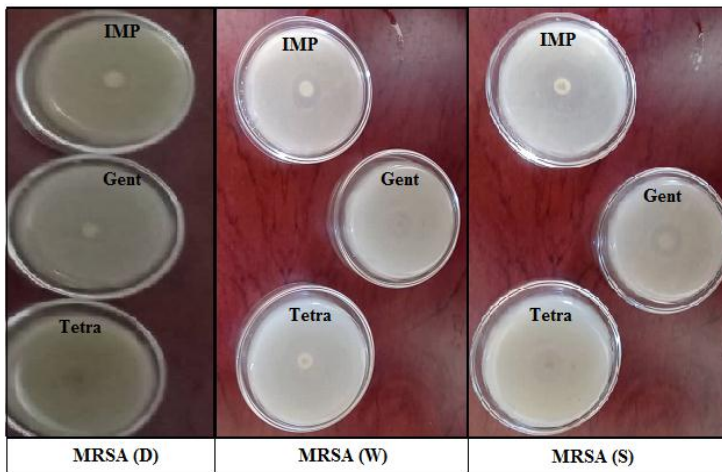
**Sensitivity and resistance of (MRSA) isolates to antibiotics:**

Table 2 and Fig. 2 show the results of resistance and sensitivity of isolates (MRSA) to antibiotics (Imipenem 10µg, Gentamicin 30µg and tetracycline 30µg), Where all strains were affected by the antibiotic Imipenem with inhibition diameters between (2.5-6.8mm), the inhibition highest was for MRSA (S), and the inhibition lowest was for MRSA (DFU). As well as, The

MRSA isolates (W) and (S) showed low-middle sensitivity to Gentamicin with an inhibition diameter (1.6mm) and (2.5mm), respectively. Were found to be ineffective this antibiotic against MRSA (DFU). On the other hand, all MRSA isolates showed severe resistance against tetracycline (30ug).

**Table 2: Antibiotic Sensitivity testing**

Pathogens bacteria		MRSA		
		Wound (W)	Skin (S)	Diabetic foot ulcers (DFU)
<b>Imipenem</b>	<b>10 µg</b>	3.5±0.5 a	6.8±1.2 a	2.5±0.5 a
<b>Gentamicin</b>	<b>30 µg</b>	1.6±0.1 b	2.5±0.3 b	0.0±0.0 b
<b>Tetracycline</b>	<b>30 µg</b>	0.0±0.0 c	0.0±0.0 c	0.0±0.0 b



**Fig. 2: Antibacterial activity of Antibiotic.**

**Discussion**

MRSA has developed special resistance to different, commonly used antibiotics, particularly in developing countries (18). The current study focuses on the application of *P. pavonica* & *S. vulgare* extracts against MRSA, which showed that brown algae can be an activist alternative to antibiotics. The result agrees with other studies that have reported on the

activity compounds of brown algae against MRSA (10, 19). The results from the study showed that *P. pavonica* extracts are more efficient than *S. vulgare* extracts, especially acetone extracts. This result was in agreement with (20, 21). MRSA (DFU) was the most resistant isolates to antibiotics and extracts, the result agrees with study that confirms the should use of antibiotics narrow-spectrum antibiotics for patients infected with diabetic foot ulcers (22). However, this result was disagreeing with (16), a bout effectiveness to alga over the other. As well as, the results indicate Imipenem maybe is effective in inhibiting bacteria, which is similar to what was found by researchers (23). While tetracycline is non-affecting on all MRSA isolates, due to inhibitors of multidrug resistance efflux pumps (24). The results of this study may be helpful to pharmacologists and medical drugs in the production of anti-MRSA from brown algae, which can edge the spread of infection, especially in Libyan hospitals, which has been reported recently.

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## النشاط المضاد للمارسا MRSA باستخدام الطحالب البنية (*Padina Pavonica* و *Sargassum vulgare*) , معملياً.

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### المستخلص العربي

تعد المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) واحدة من أكثر أنواع البكتيريا المعدية شيوعاً في المستشفيات الليبية. لذا، أجريت الدراسة الحالية كمحاولة لإيجاد علاج بديل لتقليل الإصابة بجرثومة MRSA، باستخدام المستخلصات الخام الأسيونوية والإيثانولية للطحالب البنية *Padina Pavonica* و *Sargassum vulgare* ضد ثلاث عزلات من المارسا: الجروح (W) , والجد (S), قرحة القدم السكري (DFU), ومقارنتها مع ثلاثة أنواع من المضادات الحيوية (Imipenem, Gentamicin, Tetracycline), تم إجراء اختبار الحساسية باستخدام تقنية نشر القرص. أظهرت النتائج أن جميع مستخلصات الطحالب البنية أظهرت تأثيراً واضحاً في تثبيط نمو عزلي المارسا (W) و(S). من ناحية أخرى، تعتبر مستخلصات *P. Pavonica* أكثر فاعلية من مستخلصات *S. vulgare* وخاصة المستخلصات الأسيونوية، وكانت بكتيريا المارسا المعزولة من قرحة القدم السكري (DFU) أكثر العزلات مقاومة للمضادات الحيوية والمستخلصات. أظهر المضاد الحيوي Imipenem نشاطاً جيداً ضد أنواع البكتيريا المختبرة، وهي نتيجة جيدة بالأهتمام. يمكن استنتاج أن المستخلصات الأسيونوية لطحلب *P. panovica* قد تكون مصدراً جيداً للمركبات الفعالة في مكافحة المارسا.

الكلمات المفتاحية: المارسا, *Padina Pavonica* و *Sargassum vulgare* , مضاد بكتيري.