# COMPARATIVE STUDY ON THE ANTIMICROBIAL ACTIVITY OF ORGANIC EXTRACTS OF TWO SEAWEEDS: *STYPOCAULON SCOPARIUM* AND *HALOPITYS INCURVUS*

KHELIL-RADJI, F.* – CHEMLAL-KHERRAZ, D. – MELIANI, A.

*Environmental Monitoring Network, Department of Biology, Faculty of Science of the Nature and Live, University of Oran1 Ahmed Ben Bella, Oran, Algeria*

*Mustapha Stambouli University, Mascara, Algeria*

**Corresponding author*
*e-mail: khelil_fz@yahoo.fr*

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**Abstract.** Six organic and aqueous extracts of two marine algae *Stypocaulon scoparium* and *Halopitys incurvus* were collected and studied from the Algerian west coast for their antimicrobial activities against Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*), Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and fungi (*Candida albicans, Fusarium oxysporum f. sp. albedinis*). The positive antibacterial activity with more than 10 mm diameter of inhibition zone was found by the ethanolic toluene extracts and the extract obtained by the Ethanol/Chloroform mixture of the two algae. Also, the results of the antifungal tests *against Fusarium oxysporoum f. sp. albedinis* have shown that the best results were obtained by the aqueous, methanol/dichloromethane and ethanolic extracts of the two algae. The methanolic and toluene extracts of *Halopitys incurvus* were very active with mycelial growth inhibition rates of 60% and 50%, respectively. The purification and the determination of chemical structure of active compounds from the two algae yet to be.
**Keywords:** *marine algae, antibacterial activity, antifungal activity, Algerian west coast, bioactive compounds*

## Introduction

Marine plants, macroalgae and halophilic plants living in coastal areas are a subject to many environmental stresses. Due to this stringent environmental constraint, some species developed a chemical defense. The secreted molecules are known to present high biological activities, acting at very concentrations (Stengel et al., 2011). In recent years, seaweeds or macroalgae have been exploited as potential sources of antimicrobial substances (Chiheb et al., 2009).

Furthermore, infectious diseases are a major cause of death and disability. Systematic exploration of these marine seaweeds as natural resources would give valuable antimicrobial lead compounds and drugs which can be exploited commercially in pharmaceutical industries (McGee, 2006).

Herein, the antibacterial activities of different solvent extracts of both brown and red alga, *Stypocaulon scoparium* and *Halopitys incurvus*, were evaluated in front of resistant bacteria and fungi.


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## Materials and methods

### *Study sites and time*

This study was conducted along the coastal areas of Oran (Mediterranean Sea, Algeria). The study was carried out in February and March 2016, when the conditions are most optimal for the growth of algae.

### *Collection of samples*

The samples of *Stypocaulon scoparium* and *Halopitys incurvus* were collected by handpicking at Oran coastal waters which is located in the west coast of Algeria. The collected samples were cleaned well with seawater to remove all extraneous matter such as epiphytes, sand particles, pebbles, and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with freshwater, blotted, and spread out at room temperature in the dark for drying. Finally the Shade-dried samples were grounded to fine powder and then stored in the refrigerator for further use (Ashwinikumar et al., 2014).

### *Preparing algal extracts*

About 20 g of dried algae were extracted consecutively with organic solvents with increasing polarity: toluene (T), dichloromethane (D), methanol (M), ethanol (E) water (A) and in a mixture of solvents mainly: ethanol/chloroform (E/C), and dichloromethane/methanol (D/M) (1:1 v/v). Each extraction was carried out three times by maceration for 24 h at room temperature. The extracts were pooled, filtered, and concentrated under reduced pressure in a rotary evaporator. Algal extracts were stored at −20°C until use.

### *Microbial strains*

The strains used to evaluate the antimicrobial activity were obtained from culture collection of the phytopathology laboratory (university of Oran 1, Algeria): *Fusarium albidinis, Candida albicans* ATCC 22019, *Pseudomonas aeruginosa* ATCC 24453, *Escherichia coli* ATCC 25921, *Bacillus subtilis* 6633 ATCC, *Staphylococcus aureus* ATCC 25923. The bacterial strains were maintained on the Nutrient agar media and yeast on the sabouraud's agar medium (El-Amraoui et al., 2010).

### *Antimicrobial activity*

The antibacterial activity of the extract was carried out using the agar disk-diffusion assay (Boulekbache-Makhlouf et al., 2010). The Bacterial inocula were prepared by suspending it in 9 ml of sterile water colonies from 24 h culture on Nutrient agar media. The cultures were ready when they matched with the McFarland turbidity standard ($10^8$ CFU/ml). Bacterial lawn was prepared on Mueller Hinton agar media using cotton swabs separately for each kind of bacteria. 40 µl of each algae crude extract was loaded on sterile filter paper discs and air dried. Discs which contained extracting agents were tested as controls, and then incubated at 37°C. Inhibition zones were measured after 24 h of incubation. Standard disks of antibiotic: Clindamycine, Amoxicillin, Trimethoprim, Spiramycin, Cefoxitin, Gentamicin, Penicillin, Ceftazidim, Erythromycin, Chloramphenicol have served as positive antibacterial controls. All tests were performed in triplicate.


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The technique of Favel et al. (1994) was employed to screen the antifungal efficacy of seaweed extracts. 20 ml of Sabouraud agar amended with 40 µl seaweed extracts was poured into sterile Petri plates. Fungal discs of 6 mm diameter were cut with the help of a sterile cork borer from the periphery of 5 days old culture of *Fusarium oxysporium f. sp albidinis* and the discs were transferred aseptically on Sabouraud agar poisoned with seaweed extracts.

The inhibition percentage was calculated measuring the radial growth of the fungus grown on control and amended plates, using the following formula of Harlapur et al. (2007):

$$I\% = 100\ (C - T) / C$$

I%: inhibition percentage of pathogen growth; C: average radial growth in control plates; T: average radial growth in plates amended with seaweed extracts.

## Results and discussion

The results of antibacterial and antifungal activities of the marine algae extracts against pathogenic bacteria and fungi are summarized in *Tables 2* and *3*, respectively.

Ethanol/chloroform mixture presents the best yield (20.04% and 16.44%) (*Table 1*) for the two algal species (*Stypocaulon scoparium* and *Halopitys incurvus*) respectively. A yield of 15.02% and 11.35% was attributed to methanol mixture/dichloromethane. However, toluene reported a non-negligible rate with *Halopitys incurvus.*

***Table 1.*** *Yields of solvent extractions*

| Species | Extract | Yield (%) |
|---|---|---|
| *Halopitys incurvus* | Toluene | 11.32 |
| | Dichloromethane | 7.61 |
| | Methanol | 6.48 |
| | Ethanol | 5.4 |
| | Aqueous | 0.8 |
| | Methanol/dichloromethane | 11.35 |
| | Ethanol/chloroform | 16.44 |
| *Stypocaulon scoparium* | Toluene | 4.32 |
| | Dichloromethane | 3.27 |
| | Methanol | 6.92 |
| | Ethanol | 5.6 |
| | Aqueous | 0.5 |
| | Methanol/dichloromethane | 15.02 |
| | Ethanol/chloroform | 20.04 |

According to Michel et al. (2012) the yield of extractions by solvents of increasing polarity depends on the nature of the solvent used as well as the chemical properties of the molecules to be extracted. Similarly, the extraction method (maceration, decoction, infusion) also plays an important role in determining the yield as well as the chemical composition of the prepared extracts (Tefiani, 2015).


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As shown in *Table 2*, the ethanol/toluene and the ethanol/chloroform extracts mixture of the two algae presented a significant bacterial inhibition with diameters of 10.00 ± 0.93 and 35.00 ± 0.45 mm, respectively.

***Table 2.*** *Antibacterial activity of seaweeds*

| Name of the seaweeds | Extracts | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | ***Pseudomonas aeruginosa*** | ***Escherichia coli*** | ***Bacillus subtilis*** | ***Staphylococcus aureus*** | ***Candida albicans*** |
| *Stypocaulon scoparium* | 1 | 9.55 ± 0.72 | 21.00 ± 1.02 | 19.00 ± 0.07 | 20.00 ± 0.45 | 18.00 ± 0.13 |
| | 2 | 10.00 ± 0.93 | 9.50 ± 0.07 | 06.00 ± 0.17 | 12.00 ± 0.09 | 16.00 ± 0.05 |
| | 3 | 12.00 ± 0.41 | 12.00 ± 0.98 | 14.00 ± 0.69 | 12.00 ± 1.15 | 14.00 ± 1.25 |
| | 4 | 13.00 ± 0.20 | 10.00 ± 1.37 | 18.00 ± 0.09 | 12.50 ± 0.13 | 15.00 ± 0.03 |
| | 5 | 11.00 ± 0.03 | 10.00 ± 0.47 | 13.50 ± 0.63 | 12.00 ± 0.05 | 10.00 ± 0.77 |
| | 6 | R | R | 13.00 ± 0.37 | 10.00 ± 1.03 | 10.00 ± 0.15 |
| *Halopitys incurvus* | 1 | R | 35.00 ± 0.45 | 20.00 ± 1.05 | 20.00 ± 0.93 | 13.50 ± 0.13 |
| | 2 | 13.00 ± 0.61 | 13.50 ± 0.25 | R | 07 ± 0.27 mm | R |
| | 3 | 22.00 ± 0.73 | 10.00 ± 0.75 | 10.00 ± 0.15 | 18.00 ± 1.10 | R |
| | 4 | 10.00 ± 0.21 | 13.50 ± 0.41 | 16.00 ± 0.93 | R | R |
| | 5 | 14.00 ± 0.65 | 15.00 ± 0.83 | 20.00 ± 0.75 | 10.00 ± 1.03 | 10.00 ± 0.17 |
| | 6 | R | R | R | R | 30.00 ± 0.57 |

Antifungal activities of the extracts were tested based on the measurement of the zone of the inhibition of mycelial growth. Of the many solvents tested, aqueous, methanol/dichloromethane and ethanolic extracts of the two algae showed the best result inhibition of *Fusarium oxysporoum f. sp. albedinis* (*Table 3*). Moreover, we noted that the methanolic and toluene extracts of *Halopitys incurvus* showed inhibition of mycelial growth (60% and 50%).

***Table 3.*** *Mycelial growth inhibition rate (%)*

| Algae | Extract | ***Fusarium oxysporum* f. sp. *albedinis*** |
|---|---|---|
| *Halopitys incurvus* | To | 50% |
| | DC | 28.66% |
| | Eth | 48.71% |
| | Me | 60% |
| | Eth/Ch | 14% |
| | Me/DC | 44% |
| | Aq | 61.53% |
| *Stypocaulon scoparium* | To | 6.66% |
| | DC | 4% |
| | Eth | 42.3% |
| | Me | 13.33% |
| | Eth/Ch | 27% |
| | Me/DC | 54% |
| | Aq | 60.25% |

To, toluene; DC, dichloromethane; Eth, ethanol; Me, methanol; Eth/Ch, ethanol/chloroform; Me/DC, methanol/dichloromethane; Aq, aqueous


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The inhibitory effect of antibiotics against the tested bacteria is given in (*Table 4*). Chloramphenicol showed the higher inhibitory activity as inhibition zone diameters against the four bacteria reached 27–37 mm, while inhibitory activity of spiramycin was rather low against *S. aureus* as reached up to 12 and 32 mm.

***Table 4.*** *Antibiotic susceptibility and resistance*

| Bacteria / Antibiotic | ***E. Coli*** | ***S. aureus*** | ***P. aeruginosa*** | ***Bacillus*** |
|---|---|---|---|---|
| Chloramphénicol | 35.00 ± 0.12 | 37.00 ± 0.54 | 32.00 ± 0.15 | 27.00 ± 0.31 |
| Amoxicillin | R | R | 15.00 ± 0.25 | R |
| Céfoxitin | 17.00 ± 0.17 | R | R | 15.00 ± 0.14 |
| Erythromucin | R | R | 17.00 ± 1.02 | 24.00 ± 0.15 |
| Céftazodime | 12.00 ± 0.37 | R | 12.00 ± 0.75 | R |
| Pénicillin (G) | R | R | R | 40.00 ± 0.39 |
| Trimethoprim | 26.00 ± 0.19 | R | 26.00 ± 1.03 | 15.00 ± 0.77 |
| Gentamicin | 20.00 ± 0.95 | R | 12.00 ± 0.39 | 20.00 ± 0.19 |
| Clindamycin | R | R | 10.00 ± 1.03 | 20.00 ± 0.93 |
| Spiramycin | 12.00 ± 0.53 | R | 32.00 ± 0.19 | 22.00 ± 0.39 |

## Discussion

Seaweeds present a very important role in the marine environment. They are an integral component of marine ecosystem provide life to such as habit.

In this regard, microalgae have been studied being that they are abundant and easily accessible.

Algae are less evolved organisms without a physical defense, living in severe environmental and ecological conditions (pressure, salinity, lack of light, inter-and intra-specific skills….).

Nowadays, macro algae are increasingly viewed as potential sources of secondary metabolites to be used in biotechnology and in the bioformulation of new drugs (Lamia et al., 2012).

Furthermore, these organisms produce their own chemical defenses via an important metabolic pathway. Their secondary metabolites such as phlorotannins (Imbs et al., 2018), halogenated compounds, fucoxanthin, and fucosterol are biologically active molecules being involved in in vivo investigations and many clinical trials, identifying the research opportunities, to valorize these molecules (Rosa et al., 2020).

Most of them have antimicrobial properties. Among the important bioactive compounds produced in algae tissues, we have phenolic compounds, halogenated compounds, sterols, terpenes, and other small peptides (Kayalvizhi et al., 2012; Pereira, 2018).

On this basis, all the mixtures displayed significant antibacterial activity. Dichloromethanolic extract of *Stypocaulon scoparium* has antimicrobial activity against *S. aureus* and *Candida albican* while that of *Halopitys incurvus* inhibits the growth of *Pseudomonas aeruginosa* and *Escherichia coli.*

All the methanolic extracts of *Stypocaulon scoparium* prevent the growth of the germs tested which is manifested by diameters varied between 10.00 ± 1.37 and 18.00 ± 0.09 mm, while that of *Halopitys incurvus* reacted positively on *Escherichia coli* and *Bacillus subtilis* (13.50 ± 0.41 and 16.00 ± 0.93 mm) respectively.


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The extract obtained by the Methanol/Dichloromethane mixture of *Stypocaulon scoparium* has prevented the growth of *Bacillus subtilis* (13.00 ± 0.37 mm), while that of *Halopitys incurvus* has shown single inhibitory effect against *Candida albicans*. This suggests that constituents of this prepared extract may exert synergistic effects.

Therefore, the antibacterial activity of algae could be considered as moderate, as the extract obtained by the Methanol/Dichloromethane mixture of *Stypocaulon scoparium* has prevented the growth of Bacillus subtilis (13.00 ± 0.37 mm).

However, the *Halopitys incurvus* extract afforded a single inhibitory effect against *Candida albicans*.

Antimicrobial activity induced by extracts of brown seaweed *Stypocaulon scoparium* and red one *Halopitys incurvus* indicates that the extraction process carried out has allowed us to have natural substances having a significant inhibitory effect on the growth of all the microorganisms tested.

Moujahid et al. (2004) argue that red algae (Rhodophyceae) produce terpenes, acetogenins and halogenated substances which are compounds produced by the polymerization of acetates. These Rhodophyceae possess antimicrobial activity by cytotoxic effect. The *Candida albicans* strain is sensitive to most of the extracts tested and this sensitivity is dose dependent. This study allowed us to show that the extracts tested have a more or less accentuated anti candidal activity in vitro growth of *Candida albicans*. These data agree with Kporou et al. (2009) results.

However, the lack of effect may be due to the choice of solvent, since the experimental methods and the type of solvent used are also very important factors in determining the bioactivity of seaweed (Hellio et al., 2004; Kim and Lee, 2008).

By comparing the results in *Tables 3* and *4,* we note that the extracts of the two algae prepared in toluene and the mixture of ethanol/chloroform solvents indicates a much more effective than the orthodox medicines without fewer side-effects.

Indeed, this differences between our results and those of other studies may be caused by several factors, namely the algal species, the climatic factors, the factors geological conditions, period of sampling, intraspecific variability in the production of secondary metabolites sometimes linked to seasonal variations, the method for evaluating antimicrobial activity, differences in the extraction protocols used to recover the active metabolites as well as the differences between the pathogenic strains tested (Farid et al., 2009).

## Conclusion

When regarding these results, it appears that these algal species *Stypocaulon scoparium* and *Halopitys incurvus* have good antimicrobial activity against Gram-negative and Gram-positive bacteria, yeast and fungus. To the best of our knowledge, it is necessary to identify the best bioactive constituents that displayed a significant antimicrobial activity. Furthermore, it is clear that there is a wide variety of very bioactives molecules, with many of them in dire need of further testing. In order to use them for therapeutic purposes and strengthen antibiotic therapy.

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