ANTIMICROBIAL POTENTIAL OF *JANIA RUBENS* EXTRACTS AND EPIPHYTIC BACTERIA

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Abstract
Red alga *Jania rubens* and its epiphytic bacteria were investigated for their antimicrobial potential. Organic crude extracts of the alga collected seasonally were tested against 18 indicators bacteria and the yeast *Candida albicans*. Else, culturable epiphytic bacteria were isolated and tested for activity against the same indicators microorganisms. Pronounced antimicrobial activities were obtained for alga extracts with seasonally variation. The summer period seems to be the appropriate period for secondary metabolites production especially against *Streptococcus* sp. and *Staphylococcus aureus*. Besides a collection of nineteen culturable epiphytic bacteria were isolated from the alga surface and seven of them showed antimicrobial activities. The whole alga isolates were resistant to their host extracts.

Keywords: Algae, Antibiotics, Bacteria, Mediterranean Sea

Introduction
In response to environmental pressures most epiphytic micro and macro-organisms in marine ecosystem produce secondary metabolites (Mayer et al., 2013). These compounds are with useful bio-applications mainly in pharmaceutical industry (Armstrong et al., 2001).

Materials and methods
Samples of *J. rubens* were sampled seasonally from the northern coast in Tunisia. For each sample, organic crude extracts were prepared by dichloromethane (D) and dichloromethane/methanol (D/M) solvents and antimicrobial activities were tested against 18 bacteria and the yeast C. albicans using disc diffusion method (Casida, 1986). Epiphytic bacteria were isolated from the alga using Marine agar medium and tested for their antimicrobial activity by drop method (Rao et al., 2005). For active isolates the 16S rDNA sequences were identified and submitted to the DNA GenBank and had the following accession numbers: J2 (JN391161), J9 (JN391168), J11 (JN391170), J13 (JN391172), J16 (JN391175), J17 (JN391176) and J18 (JN391177) (Ismail-Ben Ali et al., 2012).

Results and discussion
J. rubens showed a pronounced antimicrobial potential with large inhibitory spectrum especially during summer followed by spring, winter and it is almost inactive during autumn as shown in figure 1.

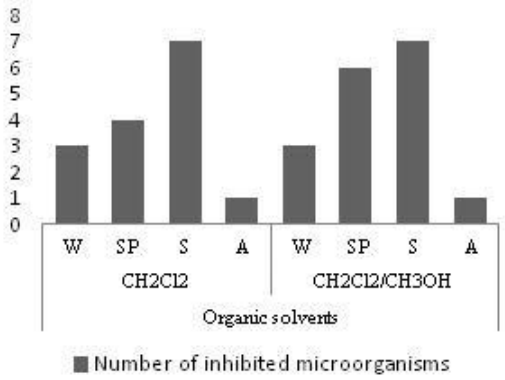

Fig. 1. Figure 1: Variation of inhibition rate with season. Dichloromethane and dichloromethane/methanol. Extract of Jania rubens were used. W: winter, SP: spring, S: Summer, A: autumn.

Extracts with moderate polarity solvents (D/M) were more effective. Previous studies on alga antimicrobial activities also noted a clear variation with season and geographic location (Salvador et al., 2007; Ismail-Ben Ali et al., 2010). *J. rubens* was active only against Gram-positive bacteria while no activity was recorded against Gram-negative one. Nineteen culturable bacteria (J1-J19) were isolated from *J. rubens* surface. Seven amongst the 19 isolates (J2, J9, J11, J13, J16, J17 and J18) were active especially against *S. aureus*, *Micrococcus sp. Pseudomonas cepacia*, *Streptococcus sp.*, and *Enterococcus feacalis*. These active isolates were identified as closely related to genus *Bacillus* (J2), *Aquimarina* (J9), *Pseudomonas* (J11 and J13), *Paracoccus* (J16) and *Pseudoalteromonas* (J17 and J18) (Ismail-Ben Ali et al. 2012). Our results agree with previous research in which *Pseudomonas*, *Pseudoalteromonas* and *B. pumilus* species isolated from *Laminaria saccharina* were found to produce antibacterial substances (Wiese et al. 2009).The whole isolates were resistant to their host extracts. This suggests that *J. rubens* epiphytic bacteria were adapted to local environment and to antibacterial substances produced by their host. Else, alga inhibitory effect is selective, since all epiphytic isolates can live in close association with the host while it prevents pathogenic bacteria from colonizing the alga.

Conclusion
Summer seem to be the best period to collect *Jania rubens* for antimicrobial secondary metabolites production. Isolates from *J. rubens* which are closely related to genus *Bacillus*, *Aquimarina* and *Pseudomonas* were significantly active against indicator bacteria. Since bacteria cultivation is easy, these bacteria may be of considerable interest by producing bioactive compounds. This would allow the exploitation of seaweed as a natural source of potential antimicrobial substances while preserving its natural stock.

References
1 - Armstrong E, Yan L, Boyd KG, Wright PC, Burgess JG. 2001. The symbiotic role of marine microbes on living surfaces. Hydrobiologia 461: 37-40.
2 - Casida L.E. 1986. Industrial Microbiology, Wiley Eastern Limited, New Delhi. 106-107.
3 - Ismail-Ben Ali A, El Bour M, Ktari L, Bolhuis H, Ahmed M, Boudabbous A and Stal LJ. 2012. Jania rubens associated bacteria: molecular identification and antimicrobial activity. J. Appl. Phycol 24: 525–534.
4 - Ismail-Ben Ali A, Ktari L, Boudabbous A, El Bour M (2010). Seasonal variation of antibacterial activity of the brown alga Padina pavonica (L) Thivy collected from northern coast of Tunisia. Bulletin de L'Institut National Des Sciences et Technology de la Mer de Salammbô 37: 127-132.
5 - Mayer AMS, Rodríguez AD, Taglialatela-Scafati O, Fusetani N (2013). Marine pharmacology in 2009-2011: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. Mar Drugs 11(7): 2510-2573.
6 - Rao D, Webb JS, Kjelleberg S. 2005. Competitive interactions in mixed-species biofilms containing the marine bacterium Pseudoalteromonas tunicata. Appl Environ Microbiol 71:1729-1736.
7 - Salvador N, Garreta AG, Lavelli L, Ribera MA. 2007. Antimicrobial activity of Iberian macroalgae. Sci Mar 71(1): 101-113.
8 - Wiese J, Thiel V, Nagel K, Staufenberger T, Imhoff JF. 2009. Diversity of antibiotic-active bacteria associated with the brown alga Laminaria saccharina from the Baltic Sea. Mar Biotechnol. 11:287-300.