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Optimization of Seaweed Harvesting for Maximum Antimicrobial Activity – Impact of Seasonal Variation, Temperature, and Salinity

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ABSTRACT

Algae are highly adaptable and can thrive in different environments, making them ideal for synthesizing effective and affordable antibacterial compounds. To optimize the harvesting of marine algae and determine the period during which the algae display maximum activity, the effect of seasonal variation (during a closed year) as well as temperature and salinity on the antimicrobial activity of fifteen species of algae (four species belonging to the Chlorophyceae class, two species belonging to the Phaeophyceae class and four species belonging to the Rhodophyceae class) was studied. These algae were tested for their antimicrobial activity against *S. aureus, B. cereus, E. coli, and C. neoformens*. These observations revealed that the optimal harvesting season for phaeophyceae and rhodophyceae extracts is spring, whereas that for chlorophyceae is during the summer period, when temperature and salinity are at their highest. In this study, it was found that the largest inhibition diameter of algae extracts occurs during summer.

Keywords: algae, antibacterial activity, seasonal variation, lagoon, Moroccan Atlantic coast.

INTRODUCTION

The synthesis of secondary metabolites by marine algae is influenced by changes in environmental parameters, such as light, temperature, nutrients, and salinity, as well as biotic interactions. These new molecules, which may be biologically active, represent a promising source for scientific research. (Xu et al., 2018). Numerous studies have revealed that these molecules fluctuate mainly according to seasonal cycles (Heavisides et al., 2018; Kumar et al., 2021; Padmakumar & Ayyakkannu, 1997; Stengel et al., 2011). In recent years, several studies have been conducted to investigate the antimicrobial activity of marine algae from the Moroccan coast. However, only a few species have been examined in depth (Aamiri et al., 2023; Bahammou et al., 2021; Boujaber et al., 2016; Chibi et al., 2019; Ibtissam et al., 2009).

The aim of this study was to improve the harvesting period to study the antimicrobial activity along the Atlantic coast of Morocco. The seasonal variation in temperature and salinity was monitored in 14 species belonging to the three classes of algae: Chlorophyceae, Phaeophyceae, and Rhodophyceae.

MATERIALS AND METHODS

Sampling and preparation of extracts

Seaweed harvesting in the Oualidia lagoon occurs during four seasons (spring, summer, autumn, and winter). After harvesting, distilled water was used to rinse the algae several times to remove any foreign bodies that might interfere with the examination of biological activity. The algae were then sorted and identified. Following the extraction methodology of Caccamese (Caccamese & Azzolina, 1979), the algal powder produced was extracted for each species at a rate of 5 g of algal powder/ml of solvent in a mixture of organic solvents (methanol/ethanol, 50:50). After filtering through Whatman paper, the extracts were evaporated in a rotary evaporator. The dried extracts were stored at 4°C until use in biological experiments. For this study, the algal species belonging to three classes of macroalgae were used, including: Chlorophyceae (*Codium elongatum* (Turner) C.Agardh, *Codium tomentosum* Stackhouse, *Enteromorpha linza* (Linnaeus) J. Agardh, and *Ulva lactuca* C. Agardh,, Pheophyceae (*Bifurcaria bifurcata* Ross, *Cystoseira humilis* Kutzing, *Cystoseira tamariscifolia* (Hudson) Papenfuss, *Fucus spiralis* Linnaeus, *Fucus vesiculosus* Linnaeus, *Saccorhiza polyschides* (Lightfoot) Batters) and Rhodophyceae (*Asparagopsis armata* Harvey, *Corallina officinalis* Ellis and Solander, *Gelidium sesquipedale*, (Turner), *Gigartina acicularis* (Roth) Lamouroux).

Search for biological activities

Antimicrobial activity

The Gram-positive bacterial species tested included: *Bacillus cereus* CIP 783, *Staphylococcus aureus ssp*. ATCC 6538, and *Escherichia coli* ATCC 10536 as Gram-negative bacteria and *Cryptococcus coliforme* as fungi.

Measuring antimicrobial activity

Cellulose disks were used to measure the antibacterial activity of the organic extracts (Bauer et al., 1966). A Petri dish that had already been inoculated with the test strain was directly topped with cellulose paper disks that had been coated with quantities of 100 μg to 500 μg of the extract to be examined. This was done after the solvent had evaporated. The diameter (in mm) of the zone of inhibition that developed around the pellets after 16 h of incubation at 37°C was used to calculate the antibacterial activity. Similarly, following incubation for 24 h at 27°C, antifungal activity was evaluated. In studies of antibacterial activity, disks impregnated with common antibiotics such as tetracycline, streptomycin, or chloramphenicol (at 50 or 100 μg/ml) were used as controls.

Seasonal variation in algal extract, temperature, and salinity

To study the relationship between antibacterial activity, harvesting season (spring, summer, autumn, and winter), and certain physicochemical parameters such as temperature and salinity, the antibacterial activity toward *B. cereus, S.aureus, E. coli* and antifungal activity toward *C. coliforme* were evaluated throughout the year for the species that showed inhibitory activity using the method of Bauer et al., 1966.

RESULTS AND DISCUSSION

The algae were evaluated for their antimicrobial activity against *S. aureus, B. cereus, E. coli, and C. neoformens*. The results are shown in Figures (1, 2, 3). These data show that some species exhibit positive activity against at least one bacterial strain throughout the year. The results of the analysis of the seasonal variation in antibacterial activity within the chlorophyceae class (Figure 1) reveal a particularly marked interest during the summer. Sixty-seven percent of the extracts showed antimicrobial activity, compared with only 33% in spring. However, this activity was significantly reduced during winter and autumn. (Elshouny et al., 2017) found that antibacterial activity was observed to be high in both winter and spring. A study of the biological properties of *U. rigida* fractions collected in spring (April) revealed strong antimicrobial activities (Trigui et al., 2013).

The optimal harvesting period for the brown algae (Figure 2) that were evaluated was spring and autumn, with antimicrobial activity of 70% and 54%, respectively. However, antimicrobial activity is less pronounced during summer and winter. In particular, the extracts of *C. humilis* algae harvested in summer exhibited the highest inhibitory diameter against *S. aureus* and *B. cereus*. Other studies have reported that the brown seaweed extracts harvested in winter are more biologically active than those harvested in other seasons (Karkhaneh yousefi et al., 2020). Cagalj et al., (2022) showed the best antibacterial activity for Cystoseira in summer, when sea temperatures were highest, but rockweed showed significant activity in autumn. The summer *Padina pavonica* brown seaweed extracts showed the broadest spectrum of activity against the panel of pathogenic bacteria tested (Grozdanić et al., 2019).

During spring, the extracts from the Rhodophyceae class (Figure 3) showed marked activity, with 87% of the samples tested revealing efficacy against microbial strains. However, this antimicrobial activity decreases significantly during summer, which is consistent with the following results: (García-Bueno et al., 2015). Researchers have discovered that harvesting red algae is an interesting activity (Elshouny et al., 2017).

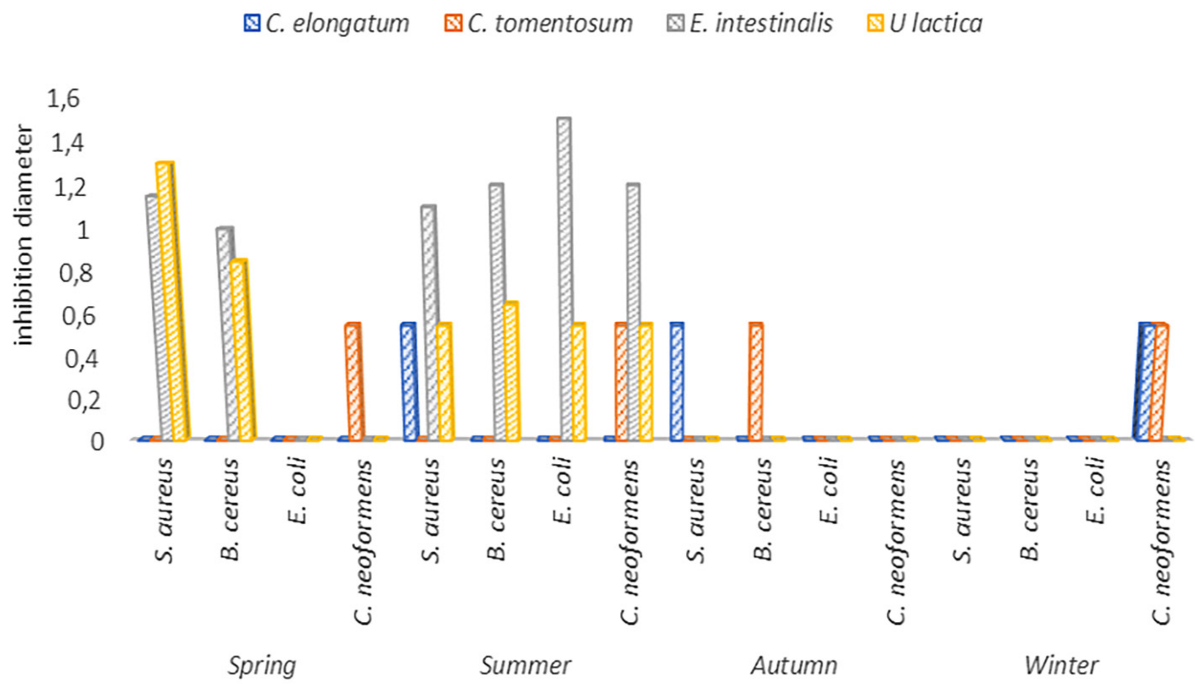

Figure 1. Variation in Chlorophyceae antibacterial activity in relation to microbial germs as a function of harvesting period

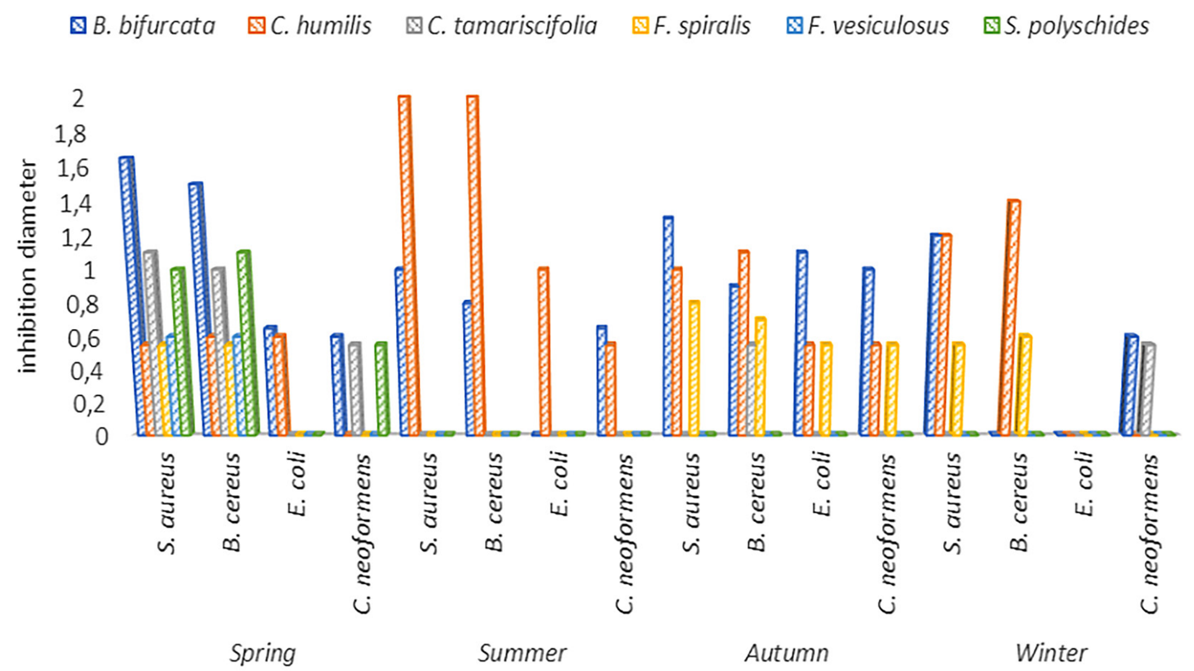

Figure 2. Variation in the antibacterial activity of phaeophyceae in relation to microbial germs as a function of harvesting period

Stirk et al. (2007) observed that seaweed extracts generally have no activity in summer and antibacterial activity in late winter and early spring. Other studies have reported that maximum antimicrobial activity values are recorded during spring and minimum values during winter for three species harvested from the Atlantic coast of Morocco (*E. compressa* et *C. dasyphylla*, and *P. complanata*) (Elkouri et al., 2004). Etahiri et al., (2003) found that the antibacterial activity of seaweed extracts harvested from the Atlantic coast of Morocco varied according to the harvesting

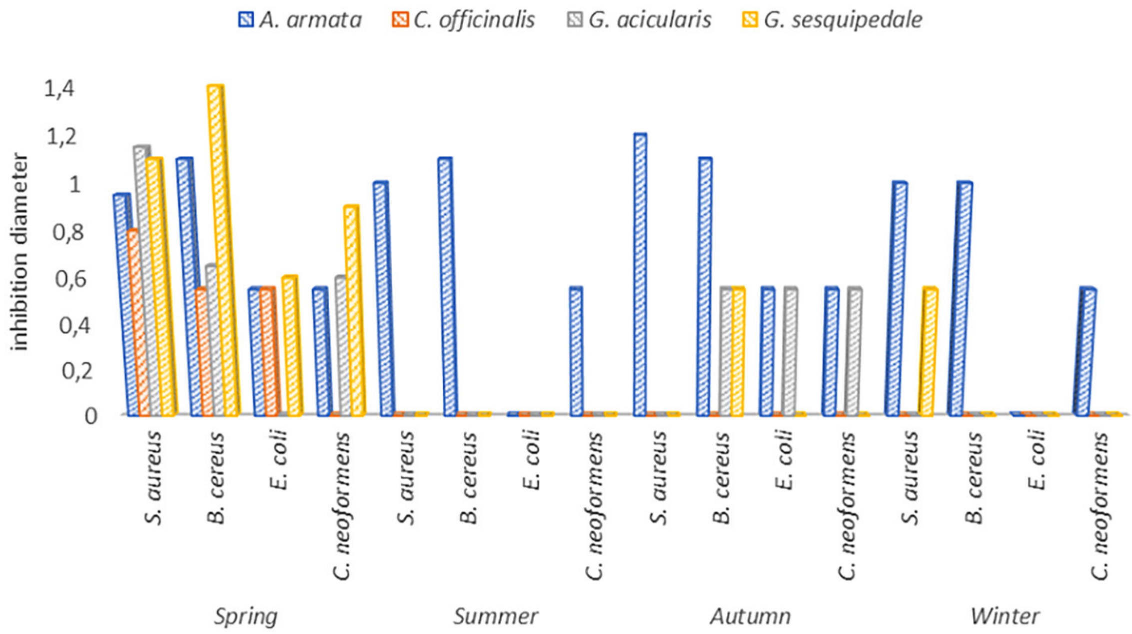

Figure 3. Variation in the antibacterial activity of Rhodophyceae in relation to microbial germs as a function of harvesting period

period, even within the same species, indicating an influence of seasonal changes. This study identified spring as the optimal harvesting period, which is consistent with the obtained results for red and brown algae.

Effect of temperature and salinity on seasonal variation in bioactivity

Marine algae constitute an enormous reservoir of potentially active natural molecules, and several studies have pointed to various causes, including the role of ecological parameters induced by climatic factors on the biology and physiology of the species, particularly on the production of secondary metabolites (Hornsey & Hide, 1985; Mouradi et al., 2010; Xu et al., 2018). For the algae harvested from the Oualidia lagoon, these products are synthesized mainly during the spring for the three classes of algae and during the summer for chlorophyceae. Figure 4 shows that the maximum antibacterial activity of chlorophyceae against

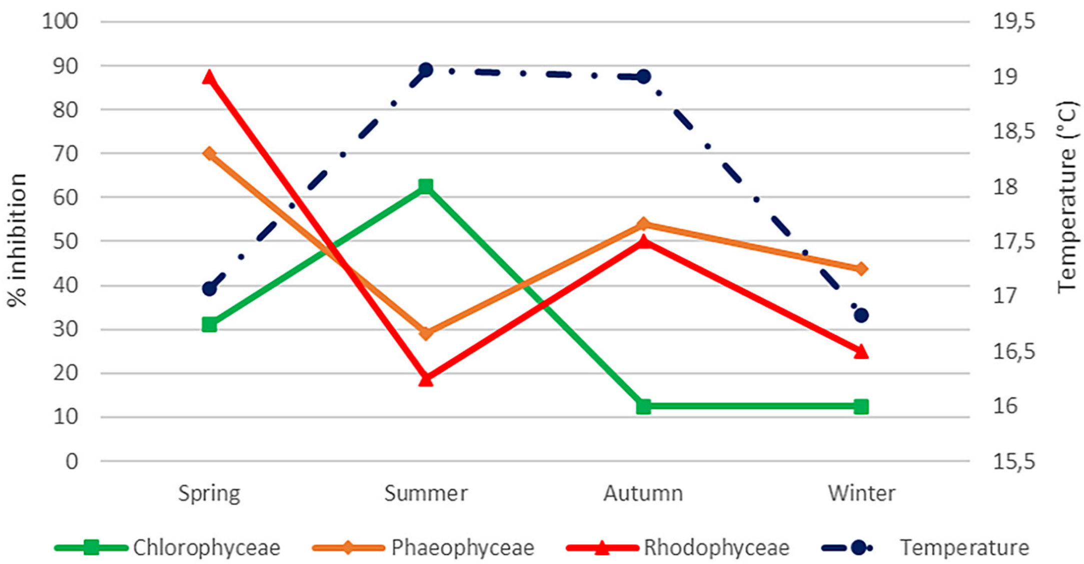

Figure 4 . Variation in the seasonal antimicrobial activity of algal extracts as a function of temperature variation

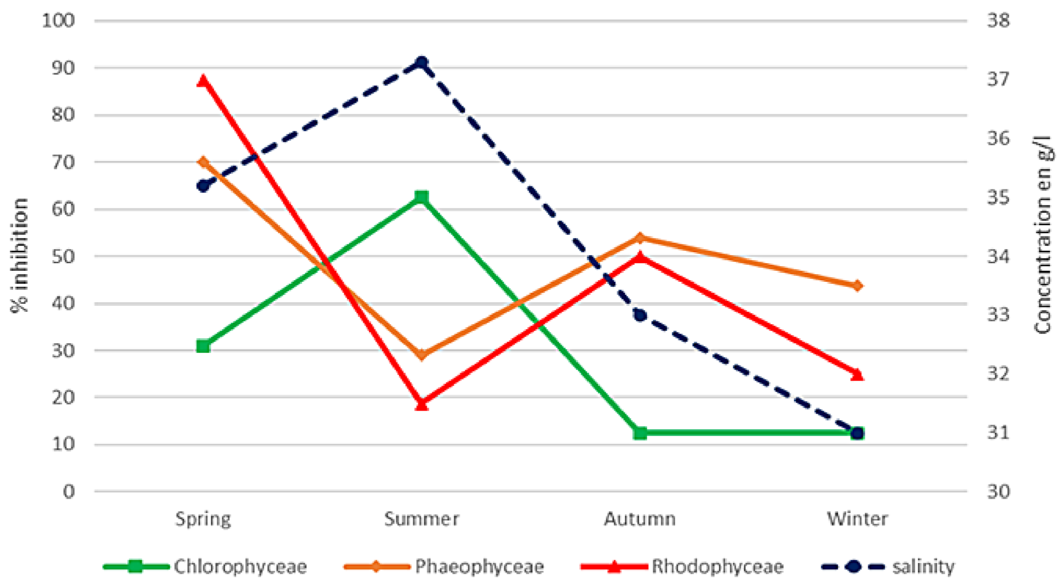

Figure 5. Variation in the seasonal antimicrobial activity of algal extracts as a function of salinity variation

microbial germs and antifungal agents was recorded during the summer season, coinciding with the maximum temperature of the environment (19°C). In the case of phaeophyceae and rhodophyceae, this biological activity peaks in spring. However, this activity decreases during autumn and winter for chlorophyceae, whereas it diminishes during summer for other red and brown algae.

Figure 5 illustrates the variation in the antimicrobial activity of algal extracts as a function of salinity. The winter period has the lowest salinity, reaching a maximum value of 37.3 g /l during the summer. Maximum Chlorophycea activity is recorded when salinity reaches its peak. On the other hand, Phaeophyceae and Rhodophyceae activity reaches a minimum. However, it is interesting to note that when activity reaches its minimum, Chlorophyceae activity also decreases. These results are in line with other studies that have reported that the ability of algae to increase antimicrobial synthesis is mainly linked to fluctuations in temperature and salinity (Xu et al., 2018).

CONCLUSIONS

The conducted study shows seasonal variations in antimicrobial activity in the three classes of algae due to variations in environmental factors such as temperature, concentration, and salinity. The green algae harvested during the summer and spring for red and brown algae are the most active. This season must correspond to the period when these factors interact with each other, providing the best conditions for the development and growth of the algae. In conclusion, certain species of algae in the Oualidia lagoon are good sources of active molecules.

REFERENCES

1. Aamiri, A., Rezzoum, N.-E., Touhamia, Y., Zidane, H., Benhassan, L., Pascal, P.-Yves., & Bellahcen, T. O. 2023. Biochemical and heavy metals composition of the invasive seaweed Sargassum muticum (Yendo) according to season and sites along Moroccan Atlantic Coast [Preprint]. In Review, 20. https://doi.org/10.21203/rs.3.rs-3026174/v1
2. Bahammou, N., Raja, R., S. Carvalho, I., Cherifi, K., Bouamama, H., Cherifi, O. 2021. Assessment of the antifungal and antioxidant activities of the seaweeds collected from the coast of Atlantic Ocean, Morocco. Moroccan Journal of Chemistry, 9(3), 639-648. https://doi.org/10.48317/IMIST.PRSM/MORJCHEM-V9I3.25910
3. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493-496.
4. Boujaber, N., Oumaskour, K., Hassou, N., Lakhdar, F., Assobhei, O., Etahiri, S. 2016. antimicrobial effect of two marine algae Gelidium sesquipedale and Laminaria ochroleuca collected from the coast of El Jadida-Morocco. J.Bio.Innov, 5(1), 16-23.
5. Caccamese, S., Azzolina, R. 1979. Screening for

antimicrobial activities in marine algae from eastern Sicily. Planta Medica, 37(12), 333-339. https://doi.org/10.1055/s-0028-1097346

6. Cagalj, M., Skroza, D., del Carmen Razola-Díaz, M., Verardo, V., Bassi, D., Frleta, R., Mekinié, I., Tabanelli, G., Šimat, V. 2022. Variations in the composition, antioxidant and antimicrobial activities of Cystoseira compressa during seasonal growth. Mar. Drugs, 20(64), 14.
7. Chibi, F., Rchid, H., Arsalane, W., Nmila, R. 2019. Screening of the antioxidant activity of crude extracts in 86 algae species from El Jadida coast (Morocco). Int. J. Pharm. Pharm. Sci, 54-61.
8. Elkouri, A.E., Bultel-Poncé, V., Assobhei, O., Etahiri, S. 2004. Etude de la variation saisonnière de l'activité antimicrobienne et anti-inflammatoire chez quelques espèces d'algues marines de la côte Atlantique Marocaine. Review of Biol. and Biotechnol 29-36.
9. Elshouny, W., Gaafar, R., Ismail, G., Elzanaty, M. 2017. Seasonal variation of the antibacterial activity of some seaweeds against multi drug resistant pathogenic bacterial strains. The Egyptian Journal of Experimental Biology (Botany), 13(1): 341– 351. https://doi.org/10.5455/egyjebb.20170821061756
10. Etahiri, S., Bultel-Poncé, V., Elkouri, Abd Elkbir, Assobhei, Omar, Zaoui, Driss, Guyot, M. 2003. Antibacterial activities of marine algae from the Atlantic coast of Morocco. Marine life, 13 (1-2), 3-10.
11. García-Bueno, N., Dumay, J., Guerin, T., Turpin, V., Paillard, C., Stiger-Pouvreau, V., Pouchus, Y.-F., Marín-Atucha, A.A., Decottignies, P., Fleurence, J. 2015. Seasonal variation in the antivibrio activity of two organic extracts from two red seaweed : Palmaria palmata and the introduced Grateloupia turuturu against the abalone pathogen Vibrio harveyi. Aquatic Living Resources, 28(2-4), 81-87. https://doi.org/10.1051/alr/2016003
12. Grozdanić, N., Zdunić, G., Šavikin, K., Đuričić, I., Kosanić, M., Mačić, V., Matić, I., Stanojković, T. 2019. Seasonal variation in biopharmaceutical activity and fatty acid content of endemic Fucus virsoides algae from Adriatic Sea. Acta Poloniae Pharmaceutica - Drug Research, 76(5), 833-844. https://doi.org/10.32383/appdr/108920
13. Heavisides, E., Rouger, C., Reichel, A., Ulrich, C., Wenzel-Storjohann, A., Sebens, S., Tasdemir, D. 2018. Seasonal variations in the metabolome and bioactivity profile of fucus vesiculosus extracted by an optimised, pressurised liquid extraction protocol. Marine Drugs, 16(12), 503. https://doi.org/10.3390/md16120503
14. Hornsey, I. S., Hide, D. 1985. The production of antimicrobial compounds by British marine algae. IV. Variation of antimicrobial activity with algal generation. British Phycological Journal, 20(1), 21-25. https://doi.org/10.1080/00071618500650041
15. Ibtissam, C., Hassane, R., José, M.-L., Francisco, D.S.J., Antonio, G.V.J., Hassan, B., Mohamed, K. 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. 8(7), 1258-1262.
16. Karkhaneh yousefi, M., Seyed Hashtroudi, M., Mashinchian Moradi, A., Ghasempour, A. 2020. Seasonal variation of fucoxanthin content in four species of brown seaweeds from Qeshm Island, Persian Gulf and evaluation of their antibacterial and antioxidant activities. Iranian Journal of Fisheries Sciences, 19(5), 2394-2409. https://doi.org/10.22092/ijfs.2020.122396
17. Kumar, K.S., Kumari, S., Singh, K., Kushwaha, P. 2021. Influence of seasonal variation on chemical composition and nutritional profiles of macro- and microalgae. In: G. Rajauria & Y. V. Yuan (Éds.), Recent Advances in Micro and Macroalgal Processing, 1, 14-71. Wiley. https://doi.org/10.1002/9781119542650.ch2
18. Mouradi, A., Chikhaoui-Khay, M., Akki, S., Akallal, R., Hrrimle, I., Givernaud, T. 2010. Analyse structurale des fractions polysaccharidiques extraites de laparoi cellulaire d'Hypnea musciformis (Rhodophyceae, Gigartinales). Afrique Science: Revue Internationale des Sciences et Technologie, 2(2) , 226-244. https://doi.org/10.4314/afsci.v2i2.61166
19. Padmakumar, K., Ayyakkannu, K. 1997. Seasonal Variation of Antibacterial and Antifungal Activities of the Extracts of Marine Algae from Southern Coasts of India. Botanica Marina, 40(1-6), 507-515. https://doi.org/10.1515/botm.1997.40.1-6.507
20. Stengel, D. B., Connan, S., Popper, Z. A. 2011. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. Biotechnology Advances, 29(5), 483-501. https://doi.org/10.1016/j.biotechadv.2011.05.016
21. Stirk, W. A., Reinecke, D. L., Van Staden, J. 2007. Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. Journal of Applied Phycology, 19(3), 271-276. https://doi.org/10.1007/s10811-006-9134-7
22. Trigui, M., Gasmi, L., Zouari, I., Tounsi, S. 2013. Seasonal variation in phenolic composition, antibacterial and antioxidant activities of Ulva rigida (Chlorophyta) and assessment of antiacetylcholinesterase potential. Journal of Applied Phycology, 25(1), 319-328. https://doi.org/10.1007/s10811-012-9866-5
23. Xu, P., Tan, H., Jin, W., Li, Y., Santhoshkumar, C., Li, P., & Liu, W. 2018. Antioxidative and antimicrobial activities of intertidal seaweeds and possible effects of abiotic factors on these bioactivities. Journal of Oceanology and Limnology, 36(6), 2243-2256. https://doi.org/10.1007/s00343-019-7046-z