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Investigations into *Sargassum* Brown Algae and the Influence of Environmental Factors on Their Yield and Composition

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Abstract

With the aim of adding value to the brown alga *Sargassum*, we studied the biochemical composition of this species in relation to ambient temperature, light intensity and rainfall rate. We observed that the overall yield of secondary metabolites from the algae is partially linked to a complementarity between temperature and rainfall. The results indicate that fluctuations also affect the concentrations of natural pigments present in the algae. Consequently, the cellular metabolism of this species could be directed towards the production of the desired metabolite(s), by selecting the right environmental conditions and timing for harvesting the algal biomass.

Keywords

Sargassum, Secondary Metabolites, Pigments, Algae, Environmental Conditions

1. Introduction

In freshwater and marine ecosystems, phytoplankton biomass is generally dominated by a succession of a few dominant species that make up the most productive part. Their growth can be attributed to physical, chemical and/or biological factors [1]. However, it has proved necessary to no longer consider the phytoplankton population as a homogeneous whole: different components can be identified depending on the physiological attributes of each species and their reactions to environmental changes [2] [3]. Martinique is a small Caribbean Island in the Lesser Antilles, with a tropical climate that alternates between wet and dry seasons. But since 2011 [4], the island has had to contend with a major climatic phenomenon: the massive invasion of *Sargassum* seaweed. This pelagic species circulates in the equatorial Atlantic and the Caribbean Sea before washing up on the Caribbean coast [5] [6]. Martinique's natural environment is already conducive to the degradation of materials [7], particularly synthetic materials. Since the arrival of the *Sargassum* beds on its magnificent beaches, the people of Martinique have seen their daily lives greatly disrupted. The consequences have been disastrous, with major impacts on health (exposure to H_2S-sulphuric acid and NH_3-ammonia), the economy (fishing, electronics, restaurants, etc.), tourism (lower tourist numbers) and the environment (damage to ecosystems) [8]. This is not an isolated phenomenon: since 2009, West Africa has also been subject to mass strandings [6] [9]. The origin of these strandings is directly linked to the eutrophication of the oceans, accentuated by the phenomenon of global warming; changes in the characteristics of this environment are leading us to fear an increase in the proliferation of this brown algae in the years to come. As a result, *Sargassum* beds will become an integral part of our Caribbean landscapes, making it even more important to draw up sustainable management plans and find long-term solutions.

The use of inhibitors of natural origin to protect materials has been around for many years and is the main technique for intervention from the environment [10]. Many plants appear to be a potential source of solutions, given the interesting properties of their chemical composition [11]. This highlights the presence of several organic families (alkaloids, flavonoids, tannins, etc.) known as 'ecological', which are available in large quantities and are renewable. Accessible and available in large quantities depending on the season, brown algae may prove to be an important and promising source of organic molecules with inhibiting properties. Consequently, the use of natural inhibitors derived from *Sargassum* seaweed would open a new way of using this biomass as a corrosion inhibitor, while at the same time addressing a research topic that is the subject of numerous publications every year [12]. The aim of this study is to develop new natural inhibitors from the species *Sargassum fluitans III*, *Sargassum natans I* and *Sargassum natans VIII*, and to solve a health problem. As other articles have already proven their effectiveness as an inhibitor, it was important for the authors to monitor the yields of these algae in order to propose the optimum conditions for exploiting this raw material [13]-[15]. At the same time, numerous studies dedicated to the ecophysiological analysis of algae have demonstrated the existence of complex relationships between light, temperature and nutrients [16] [17].

2. Materials and Methods

2.1. Material and Extraction of Algae

This work is a continuation of that already published by Lambert *et al.* and the extraction technique is therefore similar [13]. In the southern part of Martinique, at Le François and Le Diamant, the brown algae *Sargassum fluitans III* was harvested. After being washed right away, they were sorted, dried, and crushed after any remaining water was removed [18]. Then, using a green solvent made of ethanol and water, the grinding product (20 g) is extracted by reflux over the course of three hours. Three different ratios of this solvent—70:30%, 50:50%, and 30:70%—were tested in order to determine the optimal extraction solvent. A rotary evaporator is then used to evaporate the extracted solution after it has been filtered using a vacuum pump. The extract is then used for the study.

2.2. Infrared Spectroscopy (IR) Analysis

The infrared spectra provided us with a chemical signature for each extract, enabling us to differentiate between the species studied. When subjected to IR irradiation, a molecule absorbs the radiation induced by the change in vibrational and rotational energies. This IR radiation occupies the portion of the spectrum between 4000 cm^{-1} and 400 cm^{-1} [19]. Spectra are acquired using a Bruker IR spectrometer and compared using OPUS Spectroscopy Software. The absorbance, denoted as A, is defined as the decimal logarithm of the reciprocal of transmittance, and is described by the following equation:

$$A = \log(10) \times \frac{1}{T} = \log(10) \times \frac{I_0}{I_T} \tag{1}$$

3. Results and Discussion

3.1. Half-Yearly Monitoring of Extraction Yields

Monitoring of the yield of secondary metabolites in the ground state of the species studied began in January 2023 and lasted six months. This limited duration can be explained by the low presence, or even total absence, of rafts of this species between September and December 2023. This seasonal fluctuation could be attributed to the drop in ambient temperatures at the end of the year and changes in sea currents.

Reflux extractions were carried out on all species of the *Sargassum* genus in the ground state, using three different solvent ratios. The results obtained for each species of *Sargassum* are presented in **Figure 1**. We observed significant variations in the rate of production of secondary metabolites during the first few months, with a very low rate (below 5%) in February, May and June 2023. The maximum production rate was reached in January for *Sargassum fluitans III*, with a yield of 26.84%. It is interesting to note that the biomass available at the site in January was much lower than in March and April, suggesting an increased efficiency of secondary metabolite production in this species during periods of low nutrient availability.

Nevertheless, despite the wide variations in yield, it was found that the composition of secondary metabolites remained unchanged. Only the levels of each of the families isolated varied according to the month of harvest [13] [14].

3.2. Influence of Environmental Temperature

In addition to these extractions, monthly temperature and rainfall data were

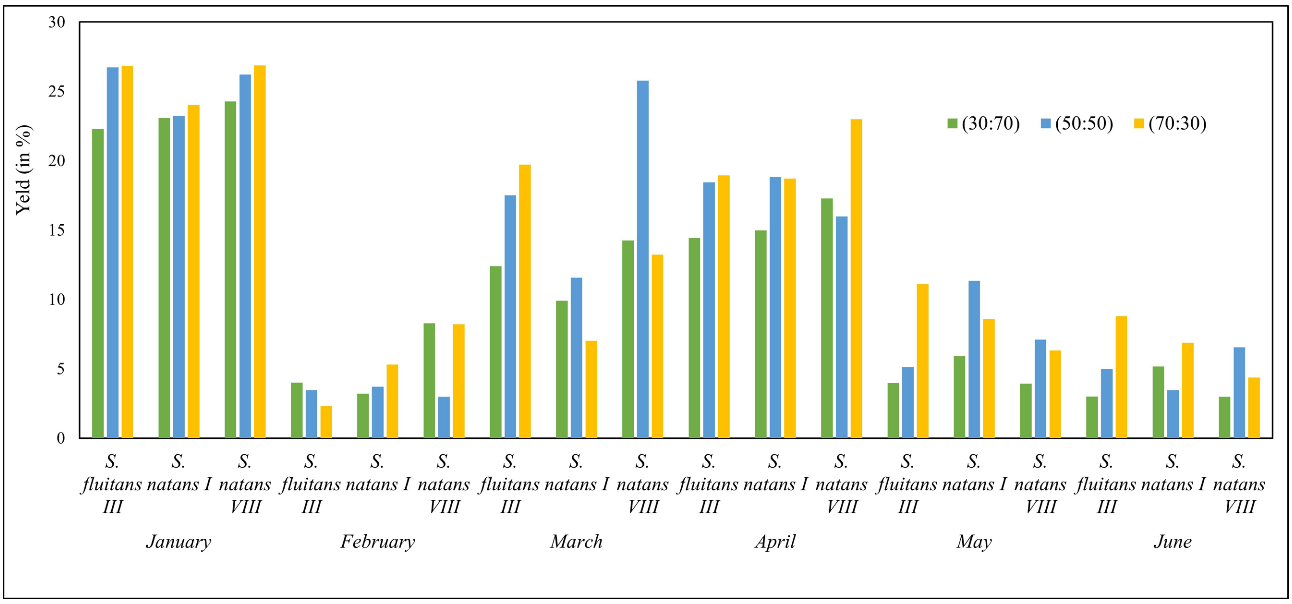

Figure 1. Six-Month yields of organic compounds extracted from *Sargassum* species.

recovered from public data on the Météo-France Martinique website (**Figure 2**). As can be seen, the maximum temperature increases over the period, reaching its maximum in June 2023, at 27˚C. Rainfall, on the other hand, is highly variable. The months of February and March saw the least rainfall, while April and May were the heaviest in terms of rainfall, with 64.6 mm in April. A comparison of the yields obtained shows that January and April 2023 gave the best yields over the chosen period. In addition, the quantities per species were greater in April. Firstly, it is assumed that rainfall plays a particular role in the reproduction and transport of seaweed rafts to our coasts. Secondly, local temperature may also play a role in algal growth and composition. Thus, a good yield of secondary metabolites would be obtained from the best temperature/rainfall complementarity.

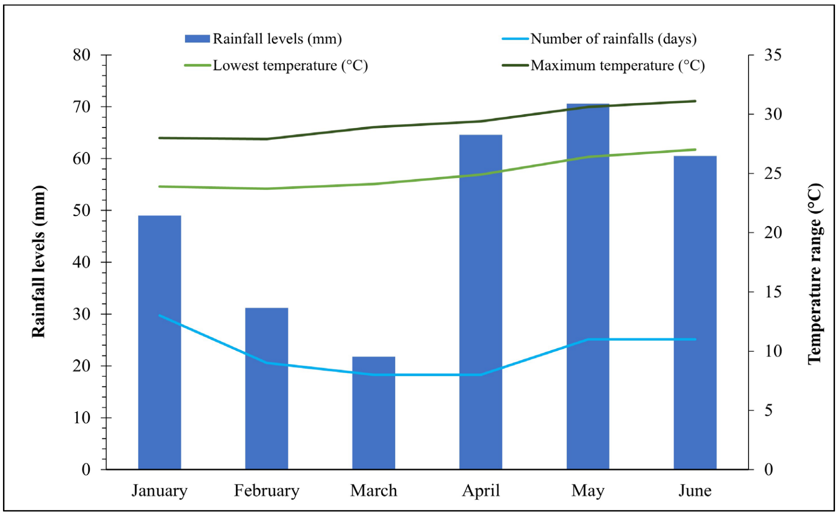

Figure 2. Data on weather variations in Martinique over the first six months of 2023.

3.3. Variation in Algae Pigments

A variation in the colour of the raw extracts was also noted during the monitoring (**Figure 3**). Visual analysis of the extracts from February 2023 revealed a green coloration of the species *Sargassum fluitans III* and *Sargassum natans I*. The extraction solvents whose filtrates showed a green or yellow coloration were composed of 70% and 30% ethanol respectively. The polarity of these solvents therefore allowed fucoxanthin and chlorophyll to be solubilised. These two pigments are known to act synergistically to give the algae its brown colour.

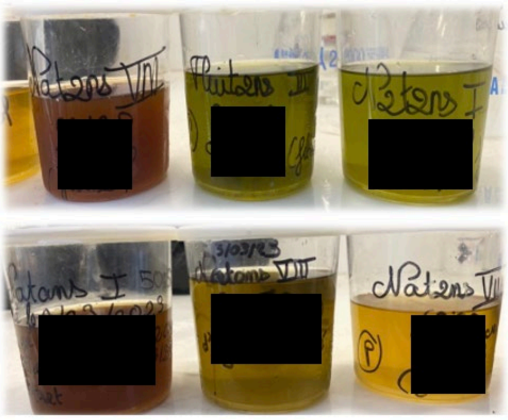

Figure 3. Colour range obtained after total extraction.

The bibliography states that carotenoids (or tetraterpenoids), which are derived from the polymerisation of fifty isoprenic units with an aliphatic or alicyclic structure, belong to the chemical family of terpenoids. They are synthesised by many plants, including algae, green plants and certain fungi [20]. Unlike terpenes, which are basic hydrocarbons, terpenoids have additional functional groups that give them numerous properties, particularly as antibacterials. Terpenoids follow metabolic pathways like those of lipids. However, algae follow the methylerythritol phosphate (MEP) biosynthetic pathway, the end product of which is DMAPP (dimethylallyl pyrophosphate), a precursor of carotenoids, steroids, chlorophylls, etc.

The colour of brown algae depends mainly on a xanthophyll pigment from the carotenoid family, fucoxanthin (**Figure 4**). Fucoxanthin can absorb wavelengths in the 500 - 560 nm range and is highly soluble in ethanol and ether. As carotenoid pigments are fat-soluble, they can be incorporated directly into certain membranes [21]. Consequently, their solubility in water can only occur when they are bound to other molecules, to water-soluble macromolecules or by the presence of acid functions leading to the formation of water-soluble salts.

Around 60 different carotenoid pigments have been identified in algae, 4 or 5 of which are found in all classes of algae [23] [24]. The xanthophyll pigments that mask chlorophyll is found in the chloroplasts or chromoplasts of algal plant cells. Thus, the fucoxanthin molecule plays the role of [25]:

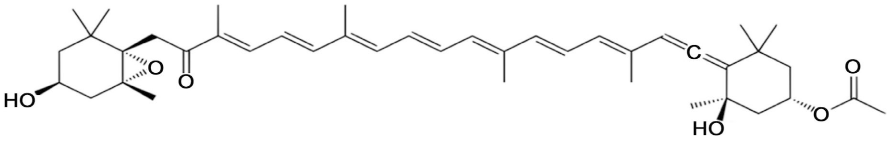

Figure 4. Representation of the Fucoxanthin molecule [22].

- Light collector, by transferring absorbed light energy to the chlorophyll;
- Photoreceptor, recovering energy from the chlorophyll if there is excess light and shade.

Variations in the environment (light intensity, environmental fluctuations, etc.) play a key role in the concentration of carotenoid pigments in the algae [26] [27]. As the concentration of chlorophyll and fucoxanthin is variable, the photoreceptor role of fucoxanthin in brown algae consists of absorbing light energy and transmitting it to chlorophyll, which is present in small quantities [28] [29].

As far as our extracts are concerned, phytochemical tests carried out on the extraction ratios concerned revealed the following in the terpene family:

- The absence of triterpenes and the low presence of steroids and unsaturated sterols for *Sargassum fluitans III* and *Sargassum natans I*.
- And the absence of triterpenes and steroids, as well as the high presence of unsaturated sterols, for *Sargassum natans VIII*.

In addition, the IR test results for these same extracts confirm these results. The chemical profiles of the green and yellow extracts are similar but differ from the IR spectra of the brown extracts usually obtained (**Figure 5**).

Comparison of the spectra (30:70) of *Sargassum fluitans III*, *Sargassum natans I* and *Sargassum natans VIII* gave similarities of 57.17%, 89.52% and 98.87% respectively. We can therefore assume that the bonds normally established between the molecules present in the algae and the extraction solvent were either too strong or too weak for the *Sargassum fluitans III* and *Sargassum natans I* species. This difference is particularly noticeable for the C=C bonds at 1.645 cm^{-1}; C-O at 1.044 cm^{-1} and C-C 880 cm^{-1}.

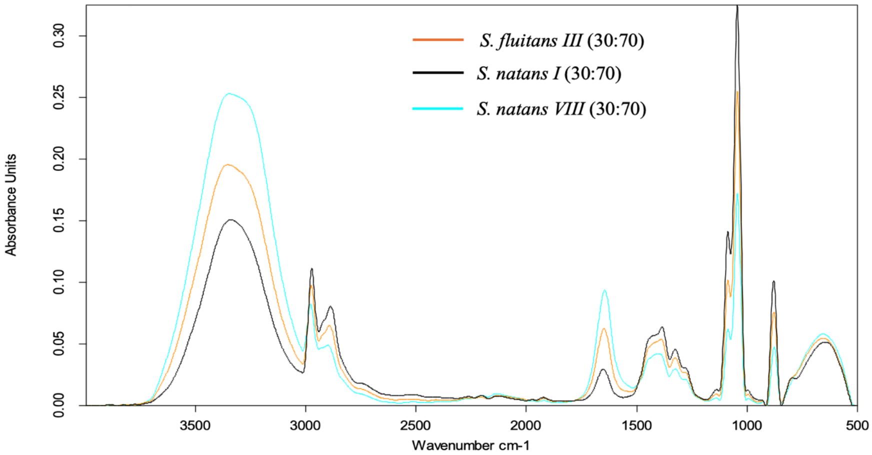

Figure 5. Infrared spectrum of Sargassum extracts from February 2023.

As the brown colour of *Sargassum* algae depends largely on the xanthophyll pigments that mask the chlorophyll, obtaining a green or yellow coloured filtrate gives rise to several hypotheses:

- The fucoxanthin molecule did not act as a light collector and/or photoreceptor for the species affected.
- The non-integration of fucoxanthin on the algal membrane, leading to insolubilization of the molecule despite the presence of ethanol. This non-integration could be due to the rate of established bonds.
- Chlorophyll deficiency due to a magnesium deficiency in the water (specific to the yellow colour).

In order to confidently identify the origin of the observed colour change, it would be necessary to confirm these results using gas chromatography–mass spectrometry (GC-MS) analysis. Additionally, the concentrations of chlorophyll and fucoxanthin in each species should be accurately measured, as these pigments are likely to play a significant role in the colour variation.

4. Conclusion

It must be emphasized that the general biochemical characterization of *Sargassum* represents only an initial phase of this experimental research, and that a more in-depth examination of the cellular components is necessary before these algae can be considered for use. Nonetheless, the results of this study have furthered our knowledge of Sargasso seaweed. The compounds present in its molecular composition, such as alkaloids and phenolic compounds, have already shown significant inhibitory activity. However, it is important to note that the composition of the algae can vary depending on the season and the surrounding climate. Thus, the integration of a wider range of environmental factors should facilitate an in-depth understanding of the potential offered by this alga. All in all, this study has highlighted the potential of *Sargassum* seaweed as an effective and environmentally-friendly supplier of natural inhibitors, paving the way for the use of this seaweed in optimal conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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