CHEMICAL ECOLOGY OF SEAWEEDS AND THEIR RELATIONSHIP WITH PHYTAL HERBIVORES OF CIGARRAS BEACH, SÃO SEBASTIÃO, SÃO PAULO.

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ABSTRACT

Morphological characteristics, such as stem shape, size and hardness, and certain biochemical characteristics, as chemical defenses, are related to the success of sea-weeds in combating herbivorous predators. Several secondary sea-weed compounds have extensive fish and sea-urchin deterrent resources. However, in the case of mesograzers such as Amphipoda, Polychaeta and Gastropoda, success is not guaranteed. The latter play an important role in the extensive field of ecological studies through concurrence in plant and herbivore interactions. Parts of certain seaweeds produce well-known substances, such as sulfated and/or halogenated metabolites. Halogenated substances, including chlorine, bromine and iodine, are generally present in red seaweeds during metabolic processes. Some species, as ***Laurencia*** and ***Polysiphonia***, produce brominate phenols and terpenes, respectively. Nowadays several active seaweed substances are known, these including A and B Dictyol in ***Dictyota dichotoma***, carbohydrates and alginates in ***Sargassum swartzii***, delta tocotrienol in ***Sargassum tortile,*** polysaccharides in ***Acanthophora spicifera***, steroides (dehydrocholesterol) in ***Hypnea musciformis*** and sesquiterpenes in ***Laurencia***. For this study, seaweed specimens were manually collected and moistened, later being sprayed and refrigerator-stored. Species for study were selected from the Rhodophyta (***Centroceras clavulatum*** (C.Agardh) Montagne and ***Chondrophycus papillosus*** (C.Agarth) Garvary & Happer) and Phaeophyta (***Padina gymnospora*** (Kützing) Vickers and ***Sargassum cymosum*** C. Agarth). 15 g. were extracted from sprayed plant material and submitted to 250 ml of chloroform in a Soxhlet device during 30 minutes at 60ºC. The extract was collected and concentrated in a reduced pressure rotor-evaporator at ambient temperature (25ºC). The concentrated extract was purified in a Silica column, using the following solvent systems: hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), methanol, all at a 20 ml ratio. The individually collected extract was nitrogen concentrated and filtered. Constituent characterization was defined by spectroscopic chromatography in the gaseous phase (HP 5890 II Plus series), together with mass spectrometry (HP 5989B) and electronic impact (70 eV). Compound identification was done by comparing with literature and Wiley275-PC (Hewlett Packard) library mass-gotten specters. In the results, concurrence of the best extraction was observed with Methanol, whereby joined substances were Aromatical hydro-carbons, Hydro-carbons, hydrogenated phenols, esters and compounds.

The co-relationship of all individuals with 2-hexanone (-1,0; p=0.000) was highly significant, and significant with methyl 9-octadecenoate Methyl (-0,95; p=0.0051). We can deduce that with the increased concentration of both in *Centroceras clavularum* and *Chondrophycus papillosus*, there is a decrease in the amount of invading organisms. Pielou Dominance indicated significant correlationship with the compound Heptadecane(-0,95; p=0.051), as also occurred between the latter and Shannon (Hill) Diversity (-0,95; p=0.0051). Several highly significant co-relationships (p=0.000) were observed among various chemical compounds. The results therefrom are preliminary. Laboratory experiments using extracts from these macroalgae are necessary, before arriving at further conclusions According to co-relationship data, chemical compounds are strongly indicated as being intervening factors in the

plant community. The aim of this work was to chemically characterize 2 species each from the divisions Rhodophyta and Phaeophyta, based on their chemical profiles, and undertake a comparison of chemical compounds with community attributes (species richness, diversity, dominance and total number of individuals) of the four plants, through SPSS Non Parametric,bi-variate analysis.

Halogenated componds, macroalgae, secondary metabolites.

INTRODUCTION

Sea-weeds are photo-synthesizing organisms that withdraw nutrients from sea-water itself, in order to survive in the marine environment They are exposed to diverse abiotic factors, such as light, hydrodynamics, temperature and hydric-stress, besides other biotic factors as herbivores.

Morphological characteristics, as format, stem-size, and certain biochemical defense endowments, are related to plant-success against herbivore predation. Both the physical character of stem hardness and chemical defence itself are important in countering herbivorous action. There are cases in which stem-hardness appears to be associated to chemical self-preservation (2).

Through being more palatable, certain seaweeds are more vulnerable (26;27). Besides certain morphological and structural defence aptitudes (23;12), they also posses an arsenal of chemical deterrents (16).

Studies on secondary metabolites in macroalgae and their ecological functions are already being initiated in Brazil. The structures of around 1000 secondary compounds have already been defined (9). These compounds are difficult to work with, as they degrade quickly when used in laboratory experiments. Neverheles, their aid in reducing herbivoreous predation has been proven (16).

Certain macroalgae have the ability to concentrate calcium carbonate, to the point of this reaching 90% of their dry-weight, thus making ingestion by herbivores difficult, whereas others achieve this through having less structural material, such as cellulose, lyginin and hemicellulose.

Several substances from seaweeds are well-known, the case of sulphatate and halogenated metabolites. The most important primary sulphatate metabolites in red seaweeds are the polysaccharides. These polymers are from the galactaneae family, as are agar and carragenan (2).

Sulphatate heteroplysaccharides can be cited among brown seaweeds. Their chains are composed of fucose, xylose and glucuronic acid, and, in some species, even galactose and manose. Sulphatate polysaccharides are economically important, as they possess gelatinizing and thickening properties, besides being non-toxic. Halogenated substances are generally more often found in red seaweeds, with chlorine, bromine and iodine being included in the metabolic processes thereof. In certain genera, as for example *Laurencia* and *Polysiphonia*, some species produce, respectively, terpens and bromophenols. The bromophenols exert several biological activities, such as growth stimulation in seeds, besides being algicidal, anti-inflamatory and antibiotic (32; 2).

Many of the secondary metabolites produced by macroalgae are terpenes, acetogenins, aromatic coumpounds and polyphenols (Floratanins), which also contain important halogens in their composition. During the fertile period (the aploid or diploid phases), and due to the simultaneous composition of compounds, seaweeds can be more or less susceptible to herbivores (24).

The secondary compounds in some seaweeds have a dissuading effect on many fishes and sea-urchins. Nevertheless, in the case of certain meso-herbivores, such as the Amphipoda, Polychaeta and Gastropoda, this is not so. These plants furnish a very ample field for studies in ecology, especially for the understanding of seaweed-herbivore interaction.

Hay *et al*. (1988) studied the dissuasive effects on reef fishes and amphipods of the A and B dictyopterenes found in *Dictyopteris delicatula*. The consequences were divergent. Whereas preference by fishes dropped to intermediary, as grazing was reduced by 40%, this remained high among a mixed group of Amphipods, with no impact on predation.

Hicks (1977) observed preferential substrata of Harpacticoida Copepods. Selection of the substratum through a chemical stimulus is significant ($p \leq 0,05$) when observed in the copepod *Porcellidium dilatatum* (Porcellidiidae), with the seaweed *Zonaria turneriana* as the substratum. Further studies were undertaken by distorting algae attraction through chemical treatment. This attraction is presumed to maintain the interaction between the macro-phytic substratum and microbial film.

Hatcher (1981) studied the co-relationship of the effect of water temperature on the speed of coral-grazing in reef-dwelling fishes. If low temperatures suppress the speed of feeding more than that of reproduction, it is highly probable that edible seaweeds would elude marine vertebrates during the cold season.

Some specialist herbivores occur in marine communities, such as certain chitons, amphipods, isopods, crabs and mollusks (17, 18; 14).

Herbivores provoke a tremendous impact in the marine environment, as for example, when in the re-productive phase, reef-dwelling seaweeds of tropical-region corals generally lose from 1 to 3,5% of total biomass daily, or from 60 to 100% of total production (11; 22, 29, 13).

Herbivorous fishes may also be abundant in temperate regions (20), the other ecologically important local macroalga grazers being equinoids and gastropods (25). They have an important effect on sea-weed communities, the intensity of herbivorous predation thereabouts generally being less than in the tropics (10, 31).

The specific effects of metabolites in marine herbivorous communities are easier to check, as herbivorous predation is high-speed, whereby results are available in a matter of hours, before compounds are either lost or degraded. Many secondary macroalga metabolites are apolar and fat-soluble compounds (5,6, 7, 8), which can be dissolved in organic solvents, concentrated and used in experiments.

At present, several active substances from macroalgae are known, as for example Dictyol A and B in *Dictyota dichomata*, delta tocotrienol in *Sargassum tortile*, steroids (Dehidrocolesterol) in *Hypnea musciformis* and sesquiterpens in Laurencia (21).

Macroalgae are used as habitats by small marine herbivores requiring small amounts of energy and a reduced space for survival, thus their making use of sea-weeds, not only for habitation but also nutrition. (15).

The consequences, thereof, induce them to make alterations in the architecture and color of the plant, thereby adapting some in both size, and even the very color, to serve as dwellings (19). Populations of small herbivores are more often limited by natural enemies than by food supply (15). Consequently, they may specialize in a certain host plant due to it higher value as a secure shelter from both enemies and physical stress, rather than as a source of food. Thus, greater emphasis is placed on habitat than on food supply, specialization in this case possibly being primarily sufficient for these small herbivores to use the plant individually for this means (3,1). The host-plant more often furnishes a place where specialized herbivores can avoid, escape from and face enemies (17, 18;19), thus promoting greater diversity, richness and abundance of species.

The aim of this work is to chemically characterize species from the divisions Rhodophyta and Phaeophyta, two from each, as to their chemical profile, and co-relate them with the following community attributes: species richness, diversity, dominance and total number of individual (counts).

MATERIAL AND METHODS

Plant material

Macroalgae were collected on the northern rocky-coast of Praia das Cigarras in São Sebastião , São Paulo State. The seaweeds with intact stems and holdfasts were manually collected. Afterwards, they were washed with distilled water and dried in the shade, to latter be pulverized and stored in a refrigerator. Species studied are listed as follows:

Division Rhodophyta
Centroceras clavulatum (C. agardh) Montagne
Chondrophycus papillosus (C. Agardh) Garbary & Harper

Division Phaeophyta
Padina gymnospora (Kützing) Sond
Sargassum cymosum (C. Agardh).

Extraction and identification of terpenes

15g of pulverized material from each of the plants was submitted to extraction with 250ml of chloroform in a Soxhlet apparatus for 30 minutes at 60ºC. This extract was collected and concentrated in a retro-evaporator at reduced pressure and room temperature (25ºc). The concentrated extract was purified in a Silicate column with the following solvent systems: hexane, hexane:chloroform (1:1), chloroform, chloroform: methanol (1:1), all in 20ml doses. Individually collected eluates were concentrated in nitrogen and then filtered. Determination of constituents consisted of spectroscopic studies by chromography in the gas-phase (HP 5890 ser. 11 Plus) linked to mass spectrometry (HP 5989B), and by electro-impact (70eV). Compound identification was done through comparison with mass spectrums obtained from literature and the Wiley 275-pc (Hewlett Packard) library.

RESULTS

According to results, the best solvent-system was methanol, the substances found therein being Hydrocarbonates, phenols, alcohols, esters, ketones and hydrogenated compounds. Selection of chemical compounds extracted from seaweeds (*Centroceras clavulatum, Padina gymnospora, Chondrophycus papillosus* and *Sargassum cymosum*) was based on repeat-occurrence.

Tabele 1 – Values of the Community attributes: species richness, diversity, dominance and total count.

Attributes	*C. clavulatum*	*P. gymnospora*	*C. papillosus*	*S. cymosum*
Richness	5.05	8.45	8.3	8.85
Shannon (Hill)Diversity	3.44465	4.17925	4.4904	3.8643
Pielou Dominance	0.20005	0.31715	0.2944	0.4135
Total count	5452	6330	5246	9617
Fractal Dimension	1.7359219	1.785165	1.809499	1.780464
(-)-Trans pinane Bicyclo[3.1.1]	70000	200000	0	120000
1-(1-3) Butadiene-2-yl)-Cyclopentan	0	0	0	100000

1,1-Dichloro-2,2,3,3,4,4,4,-heptafl	0	0	0	900000
1,2 Benzenedicarboxylic acid, bis	230000	0	0	0
1,2-Benzenedicarcoxilic acid, dibu	200000	0	0	0
1,4,8-Dodecatriene	0	250000	0	0
10,13-Octadecadienoic acid, methyl	0	0	0	250000
10-Undecenoyl chloride	100000	0	0	0
13-Octadecenal,(Z)cis-13-oct	0	0	0	100000
15-Tetracosenoic acid, methyl ester	0	200000	0	0
1-hexyl-2-nitrocyclohexane cycl	0	0	100000	0
2 - Hexanone	230000	180000	250000	0
2 - Pentadecanone, 6,10,14-trimethyl	80000	0	0	0
2-(4-hydroxybutyl)-2-nitrocyclodec	0	0	211000	0
2,2-Dideutero octadecanal	210000	0	0	0
2-exo, 3exo, Epoxy-5-6-dimethylideneb	0	0	300000	0
3 octanol	150000	0	0	0
3,4-Benzo-CIS-6,7-Dimethyl-5-Ethox	0	0	0	300000
3,4-dihydrothienyl (3,4,b)-5-carbox	0	0	0	900000
5 Octadecenal	70000	0	0	0
7 Octadecenoic acid, methyl ester	0	480000	300000	0
7 Octen-2-one, 6 methyl	80000	0	0	0
7-Hexadecenoic acid,methyl ester	0	0	0	110000
8-Octadecenoic acid, methyl ester	0	480000	0	0
9,12 Octadecadienoic acid, methyl	100000	250000	0	0
9,12, 15-Octadecatrienoic acid, met	0	250000	100000	0
9,12-Octadecadienoic acid (Z-Z)	100000	250000	100000	250000
9-Hexadecenoic acid methyl ester	100000	210000	111000	110000
9-Octadecenoic acid	170000	200000	210000	0
9-Octadecenoic acid (Z)-, methyl	120000	210000	300000	300000
Benzerazide	170000	0	0	0
Benzerazide Serine 2-(2,2,4,-tr	170000	0	0	0
Bicyclo [5.1.0]oct-3-ene	0	250000	0	0
Butanoic acid n-Butiryc	0	200000	0	0
Butanoic acid, 3 methyl	130000	0	0	0
Chloroform Methane, Trichloro	160000	250000	220000	900000
(+)-bicyclo[5.1.0] octan-2-one	70000	0	0	0
CIS - 1-Ethinyl-2 Methyl-1-Cyclohexa	70000	0	0	0
Cyclodecane	0	0	100000	0
Cyclohexanol, 5 methyl-2-(1-methyl	70000	0	0	0
Dihydromethienyl-[3,4,B]-5-CARBO	0	0	0	
Eicosenoic acid, methyl ester	0	200000	0	0
Ethane, 1,1,2,2-tetrachloro - (CAS)	0	0	0	900000
Glycine, methyl ester	80000	0	0	0
Hahnfett	70000	0	0	0
Heptadecane	180000	0	100000	0
Heptadecanoic, 16-methyl	0	10000	0	0
Hexadecanoic acid - Palmiti	200000	510000	580000	220000
Hexadecenoic acid	0	0	0	0
Hexadecenoic acid, methyl ester	0	1000000	900000	550000
Hidrazine, 1,2 dimethyl	0	200000	0	0
I-Hexadecyne (CAS)	0	0	0	100000

Lauryl acetate	0	0	100000	0
Linoleic acid	100000	0	0	0
Methyl 9-octadecenoate Methyl	150000	0	300000	0
Methyl dihydromalvalate	160000	0	0	0
methyl tetradecanoate	40000	200000	111000	0
Mukolactone Oxacyclohexadecan	0	150000	0	0
Neophytadiene 2,6,10-trimetyl	0	200000	200000	120000
Octadecanoic acid (CAS) Stearic	0	0	111000	0
Octadecanoic acid, methyl ester	160000	10000	111000	120000
Oleic Acid	0	150000	0	0
Oxiraneundecanoic acid, 3 penthyl-	0	200000	0	0
Palmitic acid	220000	510000	580000	0
Pentanoic acid Valeric ac	130000	200000	0	0
Phenol, 2,6-bis (1,1-Dimethylethyl	60000	0	0	0
Silane, methoxytrimethyl	80.000	0	0	0
Tetradecanoic acid, methyl ester	40000	200000	111000	0
Tetradecanoic acid, Myrist	60000	190000	80000	0
Tetrahydroxycyclopenadienone - tri	210000	10000	0	0
Triosulfuric acid, S-(2-a	0	0	111000	0

Chemical compounds with community attributes (species richness, diversity, dominance and individual count) were compared through SPSS Non-Parametric bivariate analysis, whereby the following correlationships were encountered.

Individual counts indicated a highly significant co-relationship with 2-hexanone (-1,0; p=0.000) and significance with methyl l 9-octadecenoate Methyl (-0,95; p=0.0051). Thus, we can deduce that an increase in concentration of these compounds in *Centroceras clavularum* and *Chondrophycus papillosus* is accompanied by a corresponding drop in attendant organism abundance.

The correlationships of Pielou Dominance and Shannon (Hill) Diversity with the compound Heptadecane were both significant (-0,95; p=0.051). Several highly significant co-relationships (p=0.000) were observed among various other chemical compounds.

The results herewith are preliminary. Laboratory experiments using extracts from identical macro-algae are necessary to reach further conclusions

According to results, chemical compounds are highly indicated as being one of the interfering factors in plant communities. The high correlationship with individual counts conveys this. Several species of macroalgae produce chemical compounds either for defense or to attract organisms.

Apart from other compounds, Hoppe (1982) encountered Dictyol A and B and pachydichol in *Dictyota dichotoma*. Likwise, as in *Acanthophora spicifera* from the Philapene coast, he found the polysaccharide 22-dehydrocholesterol in *Hypnea musciformis* from the west coast of India, and Lambda-carragenana in *Chondrophycus papillosus* from Tasmania.

For Pereira (2002), brown seaweeds have a series of diterpenes, such as Dictyol B and its corresponding acetate, pachydictol A, dictyol E and Dictyol H, as efficient forms of chemical defence, each expressing itself differently against herbivores. Certain of these plants also produce hidrocarbonates and polyphenols (floratanines) which are also active against herbivores.

In red seaweeds, such as *Laurencia,* sesquiterpenes (desbromolaurinterol) are outstanding in reducing herbivore activities. Other compounds which so operate are Elatol, Cielolaurane and Polisadine.

The results of this work are preliminary. Further tests involving chemical compounds and organisms from the macro and meiofauna, need to be done.

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RESUMO

Características morfológicas como forma, tamanho, dureza do talo e determinadas características bioquímicas como defesas químicas estão relacionadas ao sucesso das algas em relação aos efeitos predadores dos herbívoros. Os compostos secundários de algumas algas têm efeito dissuasor sobre muitos peixes e ouriços. Entretanto para alguns mesoherbívoros, como por exemplo, Amphipoda, Polychaeta e Gastropoda, não causam o mesmo efeito. Essas algas desempenham um campo de estudo muito amplo em ecologia no entendimento das interações algas e herbívoros.

Algumas substâncias produzidas por macroalgas são bem conhecidas como os metabólitos sulfatados e/ou halogenados. As substâncias halogenadas são geralmente mais encontradas em algas vermelhas, incluindo cloro, bromo e iodo nos processos metabólicos destas algas. Em alguns gêneros, como por exemplo, espécies de ***Laurencia*** e ***Polysiphonia,*** produzem terpenos e fenóis bromados, respectivamente. Atualmente são várias as substâncias ativas conhecidas das macroalgas, como por exemplo: Dictyol A e B em ***Dictyota dichotoma***, carboidratos e alginatos em ***Sargassum swartzii***, delta tocotrienol em ***Sargassum tortile***, polissacarídeos em ***Acanthophora spicifera***, esteróides (dehidrocolesterol) em ***Hypnea musciformes***, sesquiterpenos em ***Laurencia.***

Para este estudo as macroalgas foram coletadas manualmente e secas à sombra, sendo posteriormente pulverizadas e armazenadas em geladeira. As espécies usadas foram: Divisão Rhodophyta, ***Centroceras clavulatum*** (C. Agardh) Montagne e ***Chondrophycus***

papillosus (C.Agarth) Garvary & Happer; Divisão Phaeophyta***,*** ***Padina gymnospora*** (Kützing) Vickers e ***Sargassum cymosum*** C. Agarth.

De cada material vegetal pulverizado, 15 g. foram submetidos à extração com 250 mL de clorofórmio em aparelho de Soxhlet por 30 minutos a 60ºC. O extrato foi coletado e concentrado em rotaevaporador em pressão reduzida a temperatura ambiente (25ºC). O extrato concentrado foi purificado em coluna de Sílica com os seguintes sistemas de solventes: hexano, hexano:clorofórmio (1:1), clorofórmio, clorofórmio:metanol (1:1), metanol, todos na proporção de 20 mL. Os eluatos, coletados individualmente, foram concentrados em nitrogênio e filtrados. A determinação dos constituintes foi caracterizada por estudos espectroscópicos por cromatografia em fase gasosa (HP 5890 ser. II Plus) acoplada a espectrometria de massas (HP 5989B) por impacto eletrônico (70 eV). A identificação dos compostos foi realizada através de comparação com espectros de massas obtidos da literatura e da biblioteca Wiley275-pc (Hewlett Packard).

De acordo com os resultados a melhor extração foi observada com Metanol, onde as substâncias encontradas foram: Hidrocarbonetos (Alcanos, Alcenos, Ciclano), Hidrocarbonetos aromáticos, fenóis, álcoois, ésteres, cetonas e compostos hidrogenados.

De acordo com os resultados o melhor sistema de solvente foi o Metanol, onde as substâncias encontradas foram: Hidrocarbonetos (Alcanos, Alcenos, Ciclano), Hidrocarbonetos aromáticos, fenóis, álcoois, ésteres, cetonas e compostos hidrogenados. Dentre os compostos químicos extraídos das algas (***Centroceras clavulatum, Padina gymnospora, Chondrophycus papillosus e Sargassum cymosum)*** foram selecionados os que apresentaram pelo menos duas ocorrências (Tabela 58).

Comparação dos compostos químicos com os atributos da comunidade (riqueza de espécie, diversidade, dominância e número total de indivíduos) das quatro macroalgas, através do SPSS para análise Não Paramétrica, bivariada foram realizadas e encontramos as seguintes correlações.

O número total de indivíduos teve correlação altamente significativa com: 2 – Hexanone (-1,0; p=0.000) e significativa com: Methyl 9-Octadecenoate Methyl (-0,95; p=0.051). Podemos inferir que quando aumentam as concentrações dos compostos: Methyl 9-octadecenoate Methyl e 2- Hexanone em ***Centroceras clavulatum e Chondrophycus papillosus*** a abundância dos organismos presentes nas algas diminui.

A dominância de Pielou teve correlação significativa com o composto Heptadecane (-0,95; p=0,051) e a diversidade de Shannon (Hill) teve correlação significativa com o composto químico Heptadecane (-0,95; p=0,051). Foram observadas várias correlações altamente significativas (p=0.000) entre alguns compostos químicos.

O resultado deste trabalho é preliminar para maiores conclusões experimentos em laboratório, usando os extratos destas macroalgas, são necessários.

O Objetivo desse trabalho foi caracterizar quimicamente 2 espécies da divisão Rhodophyta e 2 da divisão Phaeophyta quanto ao seu perfil químico, e correlacioná-los com os atributos da comunidade: Riqueza de espécie, diversidade, dominância e número total de indivíduos.

Macroalgas, substâncias halogenadas, compostos secundários.