

Preliminary Data Regarding Pharmaceutical Forms Type Gels Based on Marine Algae Extracts with Antioxidant Activity

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Abstract

Particularly interest on herbs and plant extracts is due to their content of active principles with remarkable pharmacological properties. Different vegetal species contain natural antioxidants (polyphenols) used for their dermatological anti-inflammatory and healing activity, justified by their action on free radicals. The aim of this paper was to obtain selected phytopharmaceuticals formulations type gels with an increased antioxidant potential based on a mixture of fluid extracts from seaweeds species in the phylum Chlorophyta, *Cladophora vagabunda* (L.) C. Hoek, in the family Cladophoraceae, *Ulva lactuca* (L.) syn. *Ulva rigida* (L.), in the family Ulvaceae (sea lettuce) and species in the phylum Rhodophyta, *Ceramium rubrum* C. Agardh, in the family Ceramiaceae, frequently presents along the Romanian Black Sea coast. Fluid extracts concentration 10% were obtained using cold maceration extraction method, in solvents ethylic alcohol 40% and 70%. The obtained vegetal extracts were mixed in different ratio and analysed for physico-chemical properties, for the content of total phenols, chlorophylls, carotenoids, flavonoids and antioxidative capacity by photochemiluminescence method (ACL, Analytik Jena AG procedure). The selected mixed fluid extracts with greatest content of polyphenols and antioxidant activity were used for obtaining pharmaceutical forms type gels

which were analyzed for the physical-chemical properties appearance, pH, spreadability and total antioxidative capacity. Preliminary results emphasized that proposed pharmaceutical forms type gels had an appreciable antioxidant activity correlated with a good stability, an increased polyphenols content and would represent a possible new dermatological anti-inflammatory and healing preparation.

Keywords: pharmaceutical formulation, gels, marine algae, Black Sea littoral, total antioxidant capacity

Introduction

Seaweeds - are aquatic photosynthetic organisms used largely due to the variety of bioactive compounds in their composition that have begun to attract the attention of the food, pharmaceutical and cosmetic industries. Functional components extracted from algae biomass are widely used as food and health supplements with a variety of applications in food science and technology. The applications of algae in dermo-cosmetic products are increasing due to the fact that they have a high potential in various treatments for dermal diseases.

There is a growing concern for valorization marine phytobenthic bioresources in cosmetic therapy and treatments, by using new biotechnologies to obtain innovative pharmaceutical and dermo-cosmetic preparations, for biocompatible external use, based on soft fluid extracts or dried purified green algae containing natural bioactive compounds, with antibacterial, antifungal, antiproliferative, antioxidant activity, with maximum efficiency and minimal side effects, non-allergenic, regenerative and with skin protection effect against UV radiation (www.seaweed.ie/; www.irishseaweeds.com/sea-lettuce-ulva-lactuca; www.hawaii.edu/reefalgae/invasive_algae/chloro/cladophora_vagabunda.htm; www.algaebase.org/search/species/; Lindberg M., 2010; Mshigeni K.E., 1991; Drum R.; Leandro A., 2020; Noel Vinay, 2013; Kennedy J., 2020; Pooja S., 2014; Ajit Kandale, 2011)

Marine green algae species along Black Sea coast, in the phylum Chlorophyta, *Cladophora vagabunda* (L.) C. Hoek, in the family Cladophoraceae, *Ulva lactuca* (L.) syn. *Ulva rigida* (L.), in the family Ulvaceae (sea lettuce), respectively red algae species in the phylum Rhodophyta, *Ceramium rubrum* C. Agardh, in the family Ceramiaceae, are used in traditional medicine most due to their content in polyphenolic acids with increased antioxidative capacity mainly useful in anti-inflammatory diseases (Negreanu-Pirjol, 2012; Sirbu R., 2019; Negreanu-Pirjol B., 2011; Negreanu-Pirjol B.S., 2015; Negreanu-Pirjol B.S., 2018; Sirbu R., 2014; Negreanu-Pirjol T., 2019).

The aim of this paper is the evaluation of the total phenols compounds, chlorophylls, carotenoids, flavonoids content in hydroalcoholic extracts of *Cladophora vagabunda*

(L.) C. Hoek, *Ulva lactuca* (L.) syn. *Ulva rigida* (L.), and *Ceramium rubrum* C. Agardh species along Romanian Black Sea coast, knowing that these three vegetal products present an increased antioxidant activity with a real phytotherapeutic potential. The selected fluid extracts with increased content of polyphenols were used for hydrogels obtaining (Negreanu-Pirjol T., 2015; Sirbu R., 2010; Negreanu-Pirjol T., 2014; Negreanu-Pirjol T., 2014). The pharmaceutical formulations type gels had a good stability in time and the most increased total antioxidant capacity, better than the fluid vegetal extracts used as raw materials.

Material and Methods

Fresh vegetal product selected for fluid extracts obtaining represented by *Cladophora vagabunda* (L.) C. Hoek (Chlorophyta, Fam. Cladophoraceae), *Ulva lactuca* (L.) syn. *Ulva rigida* (L.) (Chlorophyta, Fam. Ulvaceae) and *Ceramium rubrum* C. Agardh (Rodophyta, Fam. Ceramiaceae) species, were manual collected from the South Romanian Coast of Black Sea, sector between Mangalia – 2 Mai - Vama Veche, the medio-littoral area, in the period June - August 2019-2020 and sorted on species (Fig. 1). The three species are commonly found on the Romanian Black Sea coast, growing on rocky substrates (AOAC, 2016).

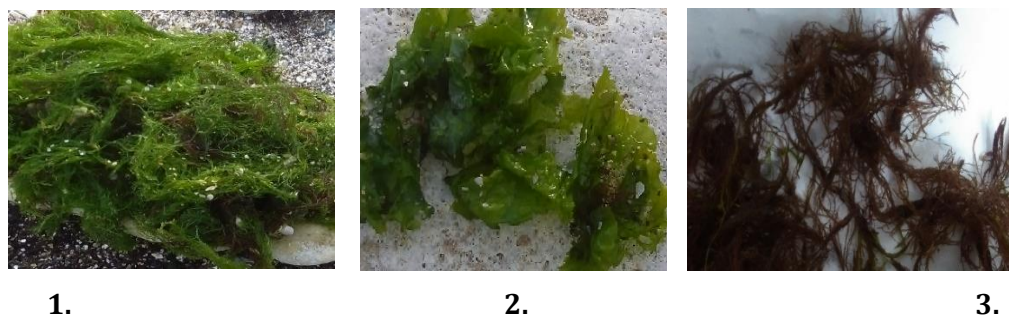


Fig. 1. Fresh vegetal material - marine algae species along Black Sea Cost

(1. *Cladophora vagabunda* (L.) C. Hoek (Chlorophyta, Fam. Cladophoraceae); 2. *Ulva lactuca* (L.) syn. *Ulva rigida* (L.) (Chlorophyta, Fam. Ulvaceae); 3. *Ceramium rubrum* C. Agardh (Rodophyta, Fam. Ceramiaceae) (photo: Dragonea)

The immediately pretreatment process after harvesting involves washing the biomass thoroughly several times with distilled water, to remove adhering sand particles and impurities and dried at room temperature, for 24 hours, in the dark. Fresh marine algae biomass was weighed to determine the loss of water through drying process. The dried algae material from three species, *Cladophora vagabunda*, *Ulva lactuca* and *Ceramium rubrum* was macerated to a fine powder (homogeneous, as well as a higher surface-to-volume ratio) and investigated for chlorophyll pigment, carotenoids, total flavonoids and total phenolic content and total antioxidant capacity, with triplicate samples of each determination.

For *fluid extract obtaining* - maceration method at cold of vegetal product was used: ethylic alcohol 40% and 70% 1:10 ratio, stir and allow soaking in the dark for 10 days in a constant temperature and stirred periodically. After 10 days, the extract is filtered and brought to a concentration of 10% with a 100 mL volumetric flask. After the 10 days obtained extract was filtered and stored at 4 °C (Negreanu-Pirjol B.S., 2015, Negreanu-Pirjol T., 2019). For the *fluid extracts*, pH, relative density were determined.

Semisolid gels based on Cladophora - Ulva - Ceramium (C-U-C) fluid extracts quality control standard tests were the follows: appearance, homogeneity, pH and spreadability, initial and after 30 days of obtaining (Negreanu-Pirjol T., 2015, Negreanu-Pirjol T., 2014).

pH was achieved using a Consort electronic pH-meter with temperature sensor

For *relative density*, the pycnometer method was used

For *chlorophyll and carotenoid content*, 0.1 g ground dry algal material was extracted in 10 mL 80% acetone (triplicate samples for each species), 1% final, through UV-Vis spectrophotometric analysis (using a S106 WPA spectrophotometer) at 470, 647 and 663 nm wavelenghts. The concentrations of chlorophyll *a*, chlorophyll *b* (only in chlorophytes) and total xanthophyll and carotin were calculated by using the trichromatic equations (Lichtenthaler & Buschmann, 2001, Miazek K., 2011)

For *total flavonoid* determinations, 1 g ground vegetal tissue was extracted in 5 mL methanol and filtered (triplicate samples). 0.5 mL of extract was diluted in 4 mL water and 8 mL methanol mixture, and the spectrophotometric absorbance was read against a methanol blank at 340 nm wavelength (Szabo I., 2012).

For determining the concentration of *total phenolic compounds*, a spectrophotometric version of the Folin-Ciocalteu method was used. 0.1 g fruit tissue was extracted in 10 mL methanol and filtered. 1 mL of extract was reacted with 5 mL Folin-Ciocalteu reagent (10%) and 4 mL sodium bicarbonate solution (7.5%) for 30 minutes. Spectrophotometric absorbance was read against a blank at 765 nm. A calibration curve was prepared, by using different gallic acid concentrations [10-12]. The resulting concentration was expressed as mg/kg dry weight and mg/kg DW gallic acid equivalent (GAE), (Stanković M.S., 2011; Stanković, 2011; Siddiqui N., 2017).

Semisolid gels preparation based on Cladophora - Ulva - Ceramium (C-U-C) seaweeds species fluid extracts was as follows (Tables 1, 2) (Popovici I., 2011):

Table 1. Samples Codification

Seaweeds C-U-C fluid extracts	Gels concentration 10%
Maceration in 40% alcohol (1:1:1) ratio	G1
Maceration in 70% alcohol, 1:1:1) ratio	G2
Maceration in 40% alcohol, (1:2:2) ratio	G3
Maceration in 70% alcohol, (1:2:2) ratio	G4

Table 2. Carbopol gel 1% formula based on C-U-C fluid extracts (G1 -G4)

Ingredients	Quantities (g)	
	G1 - G2	G3 - G4
Carbopol 940	1	1
Triethanolamine	0.8	0.8
Propylenglicole	5	5
C-U-C fluid extracts (1:1:1) ratio	7.5	-
C-U-C fluid extracts (1:2:2) ratio	-	7.5
Preservative solution ad	100	100

Carbopol 940 1% gel based on C-U-C fluid extracts obtaining: disperse carbopol 940 in the preservative solution, shake vigorously (with a high speed electric stirrer) to avoid the formation of agglomerates. The dispersion was neutralized gradually with triethanolamine solution so as not to include air. Fill with preservative solution to 100 g add and mixed the fluid extracts.

Total Antioxidant Capacity (TEAC) by photochemiluminescence method

Apparatus: photochemiluminometer PHOTOCHEM, Analytik Jena AG, Germany

Samples preparation:

Stock solutions for seaweeds C-U-C fluids extracts, were prepared as follows: 10 g powder mixture of seaweeds (molar ratio C-U-C 1:1:1, respectively molar ratio 1:2:2) were diluted with 100 mL ethyl alcohol concentration 40%, respectively 70%. From the stock solutions, were taken 10 µL working volume, according with Antioxidative Capacity in Lipid-soluble substances (ACL) (Hermann H., 2004).

Stock solution for semisolid pharmaceutical forms type gels, were prepared as follows: 5 g of C-U-C gels were diluted with 5 mL ethyl alcohol 95%. From the stock solutions, were taken 10 µL working volume, according with Antioxidative Capacity in Lipid-soluble substances (ACL) (procedure, Analytik Jena AG, 2004).

The total antioxidant capacity of the samples were quantified by comparison with the standard Trolox (constructing a calibration curve with Trolox®) (Fig. 2) and were quantified as equivalent units of standard substance Trolox equivalent antioxidant activity (TEAC) is a measurement of antioxidant strength based on Trolox, (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble derivative of vitamin E, in units called Trolox Equivalents (TE). Due to the difficulties in measuring individual antioxidant components of a complex mixture, Trolox equivalency is used as a benchmark for the antioxidant capacity of such a mixture, *nmol/sample* (Shahidi F., 1999; Popovov I., 1999; Le Tutour B., 1990; Döll M., 2003; Winnefeld K., 1995).

From stock solutions were prepared the samples, as follow (Table 3):

Table 3. Working scheme (volumes in μL), (Hermann H, 2004)

Reagent	R1	R2	R3	R4	Sample	
Blank	2.300	200	25	0	0	
Calibration curve	2.300 (μL)	- vol.	200	25	vol. (μL)	0
Measurement samples	2.300 (μL)	- vol.	200	25	0	vol. (μL)

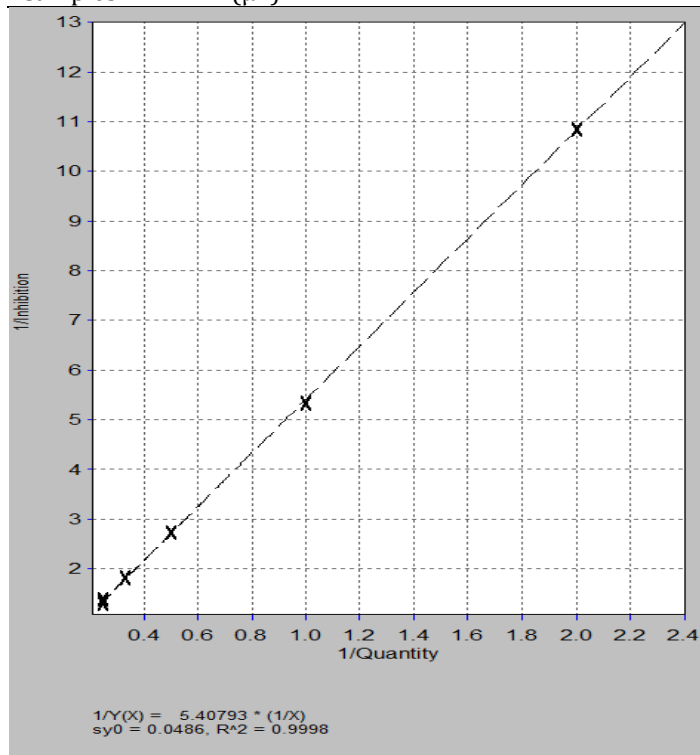


Fig. nr. 2. Trolox standard calibration curve (ACL method, Analytik Jena AG)

Results and Discussions

The values of the $p\text{H}$ and relative density parameters did not significantly modified, proving a good compatibility between the selected bases and C-U-C seaweeds fluid extracts (Table. 3).

Table 3. Seaweeds C-U-C fluid extracts quality control results

Seaweed extract	Fluid $p\text{H}$	Relative density
C	5.5 - 5,9	1.0276 - 1.0281
U	5.6 - 5.8	1.0260 - 1.0269

C 5.8 – 6,1 – 1.0388

- *Macroalgae fluid extracts chlorophylls pigments content determination*
- Chlorophylls pigments contents of green algae fluid extracts at 10% concentration of algal extracts, emphasize an increased values for both species, as follows: Chlorophyll *a* concentration was the highest in *Cladophora* (488,427 mg/kg), while maximum *chlorophyll b* content was found in *Ulva lactuca* (321,700 mg/kg), Fig. 3.
- The *chlorophyll a* content was highest in the case of *Ulva lactuca*, (918,988 mg/kg), than *Cladophora vagabunda* (488,427 mg.kg) and *Ceramium rubrum* (119,597 mg/kg), Fig. 3.
- *Macroalgae fluid extracts carotenoids pigments content determination*
- High carotenoid concentrations were found in *Cladophora vagabunda* (196 mg/kg) and *Ceraminum rubrum* (90 mg/kg), compared with a decreased content registered for *Ulva lactuca* (11,366 mg/kg), Fig. 4
- *Macroalgae fluid extracts flavonoids content determination*
- Dry *Cladophora vagabunda* tissue contain the highest amount of flavonoids (2,707 mg/kg), compared with a decreased content of *Ulva lactuca* (44,590 mg/kg) and *Ceraminum rubrum* (19,110 mg/kg), Fig. 5.
- *Macroalgae fluid extracts total phenols content determination*
- Total phenols content was less variable, with the highest value in *Cladophora* tissue (5,888 mg/kg GAE) and a decreased values for *Ceraminum rubrum* (4,201 mg/kg) and *Ulva lactuca* (3,780 mg/kg), Fig. 6.

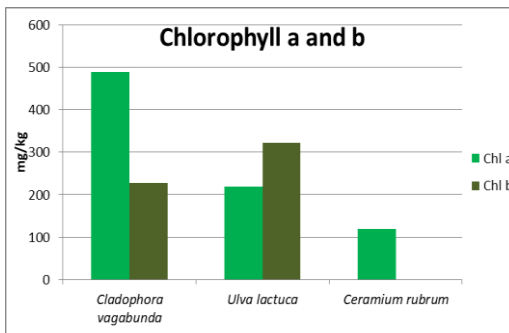


Fig. 3. Chlorophyll content in dry algal tissue (mg/kg)

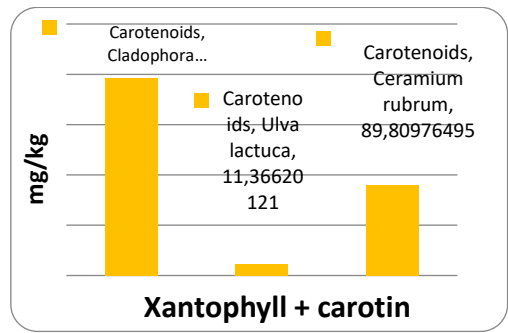


Fig. 4. Total carotenoid content in dry algal tissue (mg/kg)

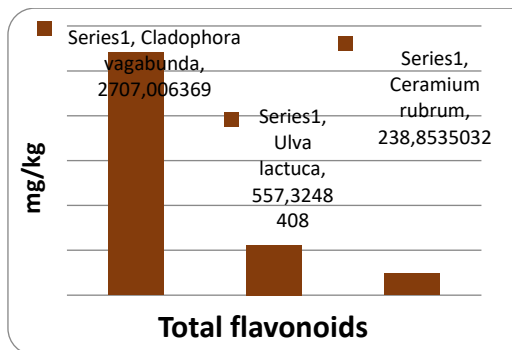


Fig. 5. Total flavonoid content in dry algal tissue (mg/kg)

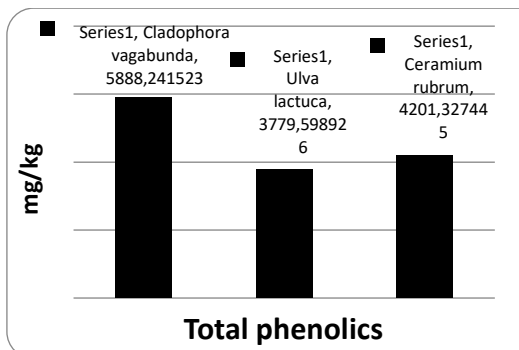


Fig. 6. Total phenolic content in dry algal tissue (mg/kg)

The values of the determined physical-chemical parameters did not significantly modified, proving a good compatibility between the selected pharmaceutical bases and seaweeds C-U-C fluid extracts (Table 4).

Table 4. Physical-chemical parameters for semisolid pharmaceutical preparations type gels based on

C-U-C seaweeds fluid extracts

Parameter	Gels based on UCT fluid extracts (G1 – G4)
appearance	translucent mass, yellow – greenish color, sweet taste, characteristic smell
homogeneity	homogenous aspect without air bubbles, drops or particle clusters
pH	5.6 – 6.5
spreadability (cm)	10.56 – 32.448

Total antioxidant capacity (*TEAC*) for seaweeds C-U-C fluid extracts, respectively for gels G1 – G4 based on seaweeds C-U-C fluids extracts in presented in Table 5.

Table 5. Total antioxidant capacity (*TEAC*) of seaweeds C-U-C fluids extracts and gels based on seaweeds C-U-C fluid extracts

N o.	Sample type (stock solution)	Volum e sample (µL)	Inhibition value	Quantity mean (<i>TEAC</i>) (nmol equiv. Trolox/ volume sample)
1	Seaweeds C-U-C fluid extracts (1:1:1) ratio in ethyl alcohol 40%	10	0.280	1.112
2	Seaweeds C-U-C fluid extracts (1:1:1) ratio in	10	0.410	3.526

ethyl alcohol 70%						
3	Seaweeds	C-U-C	fluid	10	0.387	2.879
extracts (1:2:2) ratio in ethyl alcohol 40%						
	C-U-C	fluid	extracts	10	0.443	3.702
(1:2:2) ratio in ethyl alcohol 70%						
4	Gel 1			10	0.123	1.127
5	Gel 2			10	0.224	1.430
6	Gel 3			10	0.359	2.341
7	Gel 4			10	0.389	2.991

The results obtained for **total antioxidant capacity (TEAC)** determinations, emphasize the follows:

at the working solution volume (10 μ L) according to the procedure, it was observed a valuable *TEAC* for all the analyzed semisolid pharmaceuticals preparations type gels (G1 - G4), between 1.127 - 2.991 nmol equiv. Trolox / volume sample, but more decreased compare with the raw material - seaweeds C-U-C fluid extracts. The most increased total antioxidant capacity value for sample Gel 4 (2.991 nmol equiv. Trolox / volume sample), was registered.

at the working solution volume (10 μ L) according to the procedure, it was observed an increased *TEAC* for seaweeds C-U-C fluid extracts samples, with values between 1.112 - 3.702 nmol equiv. Trolox / volume, more increased compare with the registered values of gels (G1 - G4) obtained from the same seaweeds C-U-C fluids extracts. The most increased total antioxidant capacity value for sample C-U-C fluid extract (1:2:2) ratio in ethyl alcohol 70% (3.702 nmol equiv. Trolox / volume sample), was registered.

Conclusions

Gels based on C-U-C seaweeds fluid extracts did not have modifications in quality parameters change during 30 days of observation,

Total antioxidant capacity was highest for semisolid pharmaceuticals preparations Gel 4 and seaweeds C-U-C fluids extracts used for obtaining G4 were extracted under maceration with alcohol 70%, mixed in 1:2:2 ratio.

Method of extraction, nature of used vegetable products, fluid extracts ratio determined good values for total antioxidant capacity, in this case, nature of the pharmaceutical base used for gels obtaining, did not interfere with total antioxidant capacity, which was increased for semisolid pharmaceuticals preparations Gel 4.

The obtained semisolid preparations G4 could be further submitted to other tests for its antioxidant properties valorification.

Pharmaceutical formulations type gels based on marine algae, had an increased total antioxidant capacity correlated with a good stability and increased polyphenols content and would represent a possible new dermatological anti-inflammatory and healing preparations with antioxidant potential against free radicals action.

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