

Full Paper

Bioactive, Microbiological, and Sensory Properties of *Sargassum cristaeifolium* and *Sargassum crassifolium* Herbal Tea

S. H. D. K Silva, D. C. Mudannayake* and B. V. A. S. M. Bambaranda*

Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

Email Correspondence: deshani@uwu.ac.lk (D. C. Mudannayake), manori@uwu.ac.lk (B. V. A. S. M. Bambaranda)

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Abstract

Sargassum is a genus of brown seaweed rich in bioactive compounds such as fucoidan which has various health-promoting properties including anti-cancer activity, anti-viral activity, and anti-inflammatory activity. This study examines the potential to develop herbal tea using *Sargassum cristaeifolium* and *Sargassum crassifolium* Seaweeds were collected from the southern coastal area of Sri Lanka and identified using a morphological key. Each seaweed type was thoroughly cleaned, blanched, and dehydrated either by oven-drying (40 °C for 48 h) or freeze-drying technique and separately ground to a coarse powder, which resulted in four treatments as oven-dried *S. cristaeifolium* and *S. crassifolium*, and freeze-dried *S. cristaeifolium* and *S. crassifolium*. Dried *Sargassum* powders were analyzed separately for moisture content, total phenolics (Folin-ciocalteu method), total flavonoids (Aluminum chloride method), and radical scavenging activity (DPPH method), total carotenoids, and fucoidan content (spectrophotometric method). Freeze-dried *Sargassum* powder samples gave significantly higher total phenolic content ($P < 0.05$) compared to oven-dried *Sargassum* powder. Total phenolics content of *S. crassifolium* and *S. cristaeifolium* were 0.73 ± 0.12 and 1.27 ± 0.32 mg gallic acid equivalents/g dry weight, respectively. Total flavonoid contents were not significantly different among the *S. cristaeifolium* and *S. crassifolium* seaweed powder considering oven drying and freeze-drying method. Freeze-dried *Sargassum* powders gave significantly higher radical scavenging activity ($P < 0.05$) than oven-dried *Sargassum* powders. Radical Scavenging activities of freeze-dried *S. crassifolium* and *S. cristaeifolium* powders were $34.6\% \pm 1.87$ and $46.83 \pm 8.30\%$, respectively. Oven-dried *Sargassum* powders gave significantly higher total carotenoid content ($P < 0.05$) than freeze-dried powders. Total carotenoid content of *S. crassifolium* was 1.88 ± 0.002 $\mu\text{g/g}$ dry weight while it was 1.86 ± 0.089 $\mu\text{g/g}$ dry weight for *S. cristaeifolium*. Freeze-dried *Sargassum* powders gave higher fucoidan content than oven-dried powders however there were no significant differences in the fucoidan content considering the four treatments and their tea liquors. Fucoidan content of the freeze-dried *S. crassifolium* seaweed powder was 30.74 ± 6.36 mg/200 mL, while that of *S. cristaeifolium* was 30.0 ± 4.09 mg/200 mL. A 5 g weighed tea bag was brewed in 200 mL distilled water for 20 min for the preparation of herbal tea. The highest overall acceptability was taken from the oven-dried *S. cristaeifolium* tea from the sensory evaluation. Results revealed that *Sargassum* powders can be successfully used as herbal tea with functional properties.

Keywords: Antioxidant activity, freeze-drying, fucoidan, herbal tea, *Sargassum*

Introduction

Seaweeds or macro-algae refers to the species of macroscopic, multicellular marine algae. Seaweeds can be categorized into three groups; brown algae, red algae, and green algae. Brown algae have been widely known as a natural source of bioactive compounds when compared with red and green algae. In general, brown seaweed contains fucoxanthin as its natural pigment, which gives a distinct greenish-brown color, from which it gets its name as discussed by Lim *et al*, 2017[1]. *Sargassum cristaeifolium* and *Sargassum*

crassifolium are two brown seaweeds rich in bioactive compounds like fucoidan and fucoxanthine, commonly found in Sri Lankan coastal areas. The bioactive compounds in the brown seaweed are known to provide potential health benefits including anti-cancer activity, anticoagulant activity, anti-viral activity, and anti-inflammatory activity as discussed by Maldeniya *et al.*, 2020 [2], Wijesinghe and Jeon 2012a [3], Wijesinghe and Jeon, 2012b [4].

Polysaccharides are polymeric molecules together with more than one monosaccharide unit connected collectively through glycosidic bonds. Fucans and fucoidans, in addition to laminarin and alginates, are regular polysaccharides located in brown seaweeds. Their high antioxidant activity makes fucoidans a powerful chemopreventive agent in opposition to oxidative stress-mediated diseases, which includes cancer, through inhibiting their initiation as discussed by Heeba *et al.*, 2015 [5] Wijesinghe and Jeon, 2012c [6].

Carotenoids are the most common natural red-orange to yellow terpenoid pigments that are widely used and have interesting properties. Seaweed carotenoids mainly include β -carotene, zeaxanthin, violaxanthin, lutein, and fucoxanthin. Fucoxanthin is an orange pigment mainly found in brown algae (Phaeophyceae). The compound has a variety of biological properties, such as antioxidant, anti-obesity, anti-diabetic, anti-tumor, and antibacterial properties as discussed by Prasanna *et al.*, 2007 [7].

In this research study, herbal tea was developed by utilizing two brown seaweeds (*Sargassum crassifolium* and *Sargassum cristaefolium*). Herbal tea is a commonly consumed beverage brewed from the leaves, flowers, seeds, fruits, stems, and roots of plant species rather than *Camellia sinensis*. Herbal tea is widely used for health care and diseases prevention for centuries (Zhao *et al.*, 2013) [8]. In many previous studies, seaweed tea was prepared using the oven drying method as discussed by Lim *et al.*, 2017[1], and in this study, both oven drying and freeze-drying methods were compared to examine how drying methods affect the bioactive compounds in seaweed tea.

Freeze drying is a widespread dehydration technique to obtain high-quality dried products. However, the freeze-drying technique is complex and has high fixed and operating costs, thus the uses are usually restricted to delicate heat-sensitive materials of high value compared to the oven drying technique as discussed by Hua *et al.*, 2010[9]. Oven drying of seaweed leads to the greatest nutrient losses, probably due mainly to the effect of high temperature during drying as discussed by Ang, 1997 [10], but oven drying is more cost-effective than freeze-drying. This study examines the potential to develop functional herbal tea using *Sargassum cristaefolium* and *Sargassum crassifolium* and evaluation of its chemical and bioactive properties.

Experimental Section/Materials and Methods

Sample Collection and Identification

Sargassum cristaefolium and *Sargassum crassifolium* seaweed were collected from Dickwella (N 05° 57.599' and E 080° 41.104') and Godawaya (N 06° 35.313' and E 081° 10.098'), the Southern coastal areas of Sri Lanka. Fresh seaweeds were first washed with seawater to remove sand and other impurities and packed in Low-density polyethylene bags with seawater and transported to the laboratory. Seaweed samples were identified according to the morphological features as per the guidelines of the ABC Taxa as discussed by (Coppejans and Clerck, 2009) [11].

Pre-treatments and Preparation of Dried Seaweed Herbal Tea Powder

Sargassum cristaefolium and *Sargassum crassifolium* were washed thoroughly with fresh water and stored at -18 °C. Then seaweed samples were thawed and blanched by immersing in 88 °C warm water for 30 seconds using a water bath according to the method described by Del Rosario *et al.*, 2019[12]. Two methods were used for the drying of the seaweeds. Each seaweed type was divided into two portions, separately and each portion was dried in a hot air oven (Model: DHG-9146A) at a temperature of 40 °C for a period of 48 hours or lyophilized in a freeze dryer (Model: FD5512, ShinBioBase, Korea), until moisture content reached less than 5% (w/w). Then the dried seaweeds were separately ground into coarse powder using a grinder as described in Lim *et al.*, 2017[1] to obtain four types of seaweed powders (four treatments) as below.

Table 1. Treatments used in the study

Treatment	Description
Treatment 1 -ODS cras	Oven-dried <i>Sargassum crassifolium</i>
Treatment 2 -FDS cras	Freeze-dried <i>Sargassum crassifolium</i>
Treatment 3 -ODS cris	Oven-dried <i>Sargassum cristaefolium</i>
Treatment 4 -FDS cris	Freeze-dried <i>Sargassum cristaefolium</i>

Preparation of Herbal Tea Bags

Four types of Tea bags (5 g) were prepared using four seaweed powder types utilizing coffee filter papers as the packaging material. Prepared tea bags were stored at 4°C until further. (Lim *et al.*, 2017)[1].

Analysis of Chemical Composition

Preparation of Methanol Extraction from Dried Seaweed Powder

Methanol extractions of seaweeds were prepared according to the method described by Hamrouni-Sellami *et al.* 2013[13]. A 250 mg of dried seaweed was taken into a 15 mL polypropylene centrifuge tube and 5 mL of methanol (analytical grade) was added. The mixture was vortexed using a vortexer (Model: F202A0270) for 20 S (three times) before sonicating for 30 min at 55 °C. After sonication, tubes were shaken for 4 h on an orbital shaker (Model: OS-2000) at 300 rpm. The extract was centrifuged (Model: ST-40R) at 2500 rpm for 10 min at 24 °C, and the resulting supernatant was filtered into a separate centrifuge tube. Then 5 mL of methanol was added to the remaining solid residue, and the vortexing and centrifugation procedures were repeated. The supernatant was combined with that of the first extraction before evaporating. Then it was evaporated at the rotary evaporator (Model: R-100). The extracts were stored at -20 °C until analysis. Before analysis, extracts were re-dissolved in 5 mL of methanol.

Determination of Fucoidan Content in Herbal Tea Powder and Herbal Tea Extracts

A colorimetric method was used to analyze the fucoidan content in herbal tea powder and herbal tea extract as described by Lim *et al.*, 2017[1]. The standard curve for fucoidan was created using 0.1 to 0.5 mg/mL life extension optimized fucoidan (Maritech 926-60®). Aliquots of 1 mL of the standard were transferred to each test tube immersed in an ice-water bath. Then 4.5 mL of concentrated sulfuric acid (1:6, H₂O: H₂SO₄) was added both into the samples and standards, then were shaken in a shaker (Model: BS-06), and allowed to stand for 1 minute before transferring. The test tubes were kept in a boiling water bath (Model: YCW-010E) exactly for 10 minutes. Samples and standards were allowed to cool, and 0.1mL of 3% cysteine solution was added. Tubes were shaken in a shaker (model: os:2000) and placed at room temperature for 30 minutes.

The absorbance at 396 nm and 427 nm was measured with a microplate spectrophotometer (type: 1510). The difference ($\Delta\lambda_{396}-\Delta\lambda_{427}$) between the two absorbance values were related to fucoidan.

Determination of the Total Carotenoid Content in Herbal Tea Powder

Total carotenoid contents of the seaweed powders were determined as described by Mohy El-Din & El-Ahwany, 2016[14]. Dried seaweed powder (500 mg) was transferred into a mortar and pestle with 10 mL of acetone and was ground well. The homogenate was then centrifuged (model: st-40r) at 3000 rpm for 15 min and the absorbances were measured at 645 nm and 663 nm by using a spectrophotometer (model: SPECTROPHOTOMETER UV-2000).

Determination of the Phenolic Content in the Herbal Tea Powder

Phenolic contents of the herbal tea powders were determined as described in Hamrouni-Sellami *et al.* 2013[13]. Each seaweed methanol extract (100 μ L) was added to test tubes followed by adding of 1 mL of 10% Folin–Ciocalteu reagent in distilled water and 0.8 mL of 7.5% sodium carbonate. Then were thoroughly mixed by vortexing (Model: F202A0270) and allowed to stand for 1 h at room temperature. Calibration was carried out using gallic acid standards with concentrations ranging from 0.25 to 1.75 mg mL⁻¹. Standards and blanks were prepared using the same method as for the samples. Absorbances were measured at 765 nm by using spectrophotometer (model: SPECTROPHOTOMETER UV-2000).

Determination of Total Flavonoid Content of the Herbal Tea Powder

Flavonoid contents of herbal tea powders were determined by referring the method of Hamrouni-Sellami *et al.* 2013[13]. Each seaweed methanol extract (100 μ L) was added into test tube, and of 500 μ L of distilled water and 37.5 μ L of 5% sodium nitrite were added. After 6 minutes, 75 μ L of 10% aluminum chloride were added to each test tube, followed by addition of 250 μ L of 1 M sodium hydroxide. The samples and standards were made up to 1.5 mL with distilled water. Rutin standards (concentration range, 0.05–2 mg mL⁻¹) were used to prepare the calibration curve. Standards were prepared using the same method as for the samples. Absorbances at 510 nm were measured by using a Spectrophotometer (Model: SPECTROPHOTOMETER UV-2000).

Determination of the Radical Scavenging Activity of the Herbal Tea Powder

Radical scavenging activity of herbal tea powders were determined as described by Mutton 2012[15]. Methanol extract (100 mL) was added to a test tube, and then 1 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) in methanol was added. Samples were vortexed (Model: F202A0270) before standing at ambient temperature in the dark for 30 min. Pure methanol (100 μ L) was used as a negative control. After standing, the absorbance of each sample was measured on a spectrophotometer (Model: SPECTROPHOTOMETER UV-2000) at a wavelength of 515 nm.

Proximate Analysis - Determination of Moisture Content

Moisture content was determined by using the AOAC 2016 Method. Petri dishes were selected, cleaned and labeled. The dishes were then weighed using a calibrated digital analytical balance (model: DHG-9146A) and their initial weights were recorded. A 2 g of each sample was weighed into the labeled pre-weighed petri dishes. They were immediately transferred into a hot air oven (Model: DHG-9146A) pre-set at 105 °C to dry. The weight of samples in crucibles was measured until constant weights are obtained as final values.

Evaluation of the Antibacterial Activity of the Herbal Tea Liquor

Antibacterial activity was evaluated by the method described in Adamczak, 2019[16] with slight modifications. The antibacterial activity of the different concentrations of herbal tea liquors were determined by using agar disk diffusion method. Here, pure culture of *Escherichia coli* (ATCC 25922) was used as the bacterial sample. Different concentrations of herbal tea liquors were prepared according to the herbal tea preparation method described below and the concentrations were 25 mg mL⁻¹ as C₁, 50 mg mL⁻¹ as C₂, 75 mg mL⁻¹ as C₃, and 100 mg mL⁻¹ as C₄. Also, it was checked for the antibacterial effect of tea liquor (*Camellia sinensis*). Sterile water was used as the negative control and Augmentin (antibiotic) was used as the positive control. Muller Hinton agar plates were prepared and 100 µL of *E. coli* suspension was inoculated onto the agar spread plates. Herbal tea liquor with different concentrations were transferred onto sterile filter papers discs (6 mm diameter). Additionally, sterile filter papers were soaked 31.25 mg mL⁻¹ of Augmentin (positive control) and sterile water (negative control). Filter paper discs were placed on the agar plate and incubated at 35 °C for 18 h and the inhibition zone was measured. Same procedure was repeated for the tea liquor (*Camellia sinensis*) as well.

Sensory Evaluation of the Seaweed Herbal Tea Extracts of *S. cristaefolium* and *S. crassifolium*

Seaweed Herbal Tea Preparation

Tea bags were brewed in 200 mL distilled water for 20 min. Distilled water was added time to time to ensure the boiling water be at the 200 mL mark. After the specified time, the tea bags were removed from the brews, and the brews were added up to 200 mL. Then 0.3% (v/v) lemon essence was added to control the odour as described by Lim *et al.*, 2017[1].

Sensory Evaluation of the Seaweed Herbal Tea Liquor of *Sargassum cristaefolium* and *Sargassum crassifolium*

Sensory evaluation of seaweed herbal tea samples was conducted to establish preference rating of tea for appearance, color, aroma, taste, aftertaste, astringency, and overall acceptability. Sensory analysis was carried out using 30 untrained panelists. The sensory evaluation was carried out in the panel room with controlled temperature and relative humidity. The panel room was completely free of food/chemical odors, unnecessary sound, and the mixing of daylight. Judges were provided with a prescribed questionnaire to record their sensory observations. The information contained on the sensory performance was indicated as 9 = like extremely; 8 = like very much; 7 = like; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike; 1 = dislike extremely (Dzah *et al.*, 2015) [17].

Determination of Shelf Life of the Seaweed Herbal Tea

Determination of Water Activity

The water activity was determined using a moisture analyzer. Measurements were carried out in triplicate as described in Zokti *et al.*, 2016[18].

Microbial Analysis

Microbial analysis was done according to the method described in Yoon *et al.*, 2017[19] with few modifications. The selected product (oven-dried *S. cristaefolium* herbal tea powder) was stored at 4 °C for four weeks under the packaging material. Each sample (1 mL) was diluted 10-fold with 0.1% peptone solution. Total aerobic bacteria were counted using TPC agar plates that were incubated in an incubator (Model: OBI-90) for 48 h at 35°C. Yeast and mold were counted on PDA plates that were incubated for 48 h at 25 °C.

Statistical Analysis

The experimental design was Complete Randomized Design (CRD) and all experiments were performed in triplicate. One-way ANOVA and Tukey test were used to analyze the significant differences of chemical composition tests. The significant difference was considered at the level of $p < 0.05$. The sensory evaluation data was analyzed by the Friedman test. Data was analyzed by using Minitab 17 statistical software and Microsoft Excel.

Results and Discussion

Fucoidan Content of the Dried Seaweed Powders

Fucoidan is a term used for the class of sulfated fucose-rich polysaccharides found in the fibrillar cell walls and intercellular spaces of brown algae. In recent years, fucoidan have been the subject of many scientific studies aiming at assessing their potential biological activities including antitumor and immunomodulatory, antiviral, antithrombotic and anticoagulant, anti-inflammatory, and antioxidant effects as discussed by Ale *et al.*, 2011 [20]. In this study, fucoidan content of four treatments were analyzed and the results showed that there were no significant differences among the four treatments ($P > 0.05$) (Table 2). The fucoidan content is presented in mg/200 mL, as the samples were extracted in a serving size of 200 mL. The highest fucoidan content found in the freeze-dried *S. crassifolium* powder (30.74 ± 4.09 mg/200 mL), which was comparable to that of *Sargassum binderi*: 27.22 ± 0.07 mg/200 mL revealed by Lim *et al.*, 2017[1].

Fucoidan Content in Herbal Tea Liquor

There was no significant difference in fucoidan contents among the herbal tea liquors of four treatments ($P > 0.05$). Out of four treatments freeze dried *S. crassifolium* herbal tea liquor contained highest amount of fucoidan (32.5 ± 0.892 mg/200 mL) (Table 2).

Total Carotenoid Content of The Dried Seaweed Powder

Fucoanthin is one of the most abundant carotenoids, and contributes more than 10% of the estimated total production of carotenoids especially in the marine environment. It is an orange-colored pigment, along with chlorophylls *a* and *c* and β -carotene, present in brown seaweeds and diatoms. Fucoxanthin has an unusual allenic bond and some oxygenic functional groups and is responsible for the higher antioxidant activity as discussed by Peng *et al.*, 2011[21].

There is a significant difference in total carotenoid contents of the dried seaweed powder of four treatments ($p < 0.05$). Oven dried seaweed powder showed significantly higher level of total carotenoid content ($1.88 \pm$

0.002 $\mu\text{g/g}$ in ODS- crass and $1.86 \pm 0.089 \mu\text{g/g}$ in ODS-cris) than the freeze-dried treatments ($1.52 \pm 0.148 \mu\text{g/g}$ in FDS- crass and $1.11 \pm 0.087 \mu\text{g/g}$ in FDS- cris) in this study (Figure 1).

Total Phenolic Content of the Dried Seaweed Powder

Phenolic compounds or phlorotannins are secondary defense metabolites synthesized during development as components of algal cell walls. Accumulation of these phloroglucinol-based phenolics cause a strong antioxidant activity against free radical-mediated oxidation damage and showed other potential bioactivity such as a bactericidal, antiviral, and anticancer activity and radio protective and antiallergic effects as discussed by Puspita et al., 2017[22]. Dried herbal tea powder of freeze-dried treatments showed a significantly higher ($p < 0.05$) phenolic content than the oven dried powders. Total phenolic content of freeze dried and oven dried *S. cristaefolium* was $1.27 \pm 0.32 \text{ mg gallic acid equivalents/g dry weight}$ and $0.53 \pm 0.12 \text{ mg gallic acid equivalents/g dry weight}$ respectively (Figure 2). The freeze-dried samples showed higher phenolic content than that of the oven dried samples. Past studies revealed that, total phenolic content of fresh *Sargassum sp.* was $47.60 \pm 15.05 \mu\text{g GAE/mg extract}$ (Kane et al., 2016[23]) while Puspita et al., 2017[22] showed total phenolic content of *S. muticum* enzymatic extracts were 0.9% W/W dry algal material.

Total Flavonoid Content Of The Dried Seaweed Powder

According to the results there was no significant difference in flavonoid content among the dried seaweed powder samples of four treatments ($P > 0.05$). The flavonoid content of oven dried seaweed powder of *S. crassifolium* and *S. cristaefolium* were 16.46 ± 5.07 and $12.83 \pm 2.04 \text{ mg rutin equivalents/g of dry weight}$ respectively. Total flavonoid contents of the freeze-dried seaweed powder of *S. crassifolium* and *S. cristaefolium* were 19.94 ± 2.5 and $17.1 \pm 2.58 \text{ mg rutin equivalents/g of dry weight}$, respectively (Figure 3).

Radical Scavenging Activity of the Dried Seaweed Powder

There is a significant difference in radical scavenging activity among the dried seaweed powder of four treatments. The radical scavenging activity of oven dried seaweed powder of *S. crassifolium* and *S. cristaefolium* were $23.09 \pm 2.46\%$ and $31.6 \pm 1.79\%$, respectively. The radical scavenging activity of freeze dried seaweed powder of *S. crassifolium* and *S. cristaefolium* were $34.69 \pm 1.87\%$ and $46.83 \pm 8.31\%$, respectively. According to these results freeze-dried seaweed powders showed higher radical scavenging activity (Figure 4).

Antibacterial Activity of Seaweed Herbal Tea Liquor

Results revealed that there was no significant difference in antibacterial activity among the herbal tea liquors of four treatments. According to the results freeze dried *S. crassifolium* herbal tea liquor showed the highest inhibition zone against the pure *E. coli* culture in the petri dish in all four concentrations of tea liquor than tea liquors of other three treatments (Figure 5). Tea liquor of *Camellia sinensis* did not show inhibition against the pure *E. coli* culture in the petri dishes which is apparently that it did not have antibacterial effect against the pure *E. coli* culture that used in this study. This proves the seaweed herbal tea has an antibacterial effect against the *E. coli*. In the previous study, Puspita et al., 2017[22] antibacterial activity of enzymatic extracts of *S. muticum* was determined against *E. coli* by using Phosphomycin, Ampicillin, Streptomycin antibiotics as the positive control and showed an inhibition zone of $2.2 \pm 0.1 \text{ cm}$, $1.7 \pm 0.1 \text{ cm}$ and $1.8 \pm 0.2 \text{ cm}$, respectively.

Sensory Evaluation of Herbal Tea Liquors

Sensory evaluation was carried out by using 30 untrained panelists. In the sensory evaluation, preference rating of herbal tea for appearance, color, aroma, taste, aftertaste, astringency and overall acceptability was estimated. *S. crassifolium* oven dried tea liquor showed the highest preference in terms of appearance, colour and astringency. *S. cristaefolium* freeze dried tea liquor scored highest preference for aroma. When considering the taste and after taste all samples showed the same preference rate. *S. cristaefolium* oven dried herbal tea scored highest overall acceptability (Figure 6), thus was selected as the best product out of four treatments.

Shelf Life of the Seaweed Herbal Tea

Water Activity (a_w) and Total Bacteria Counts

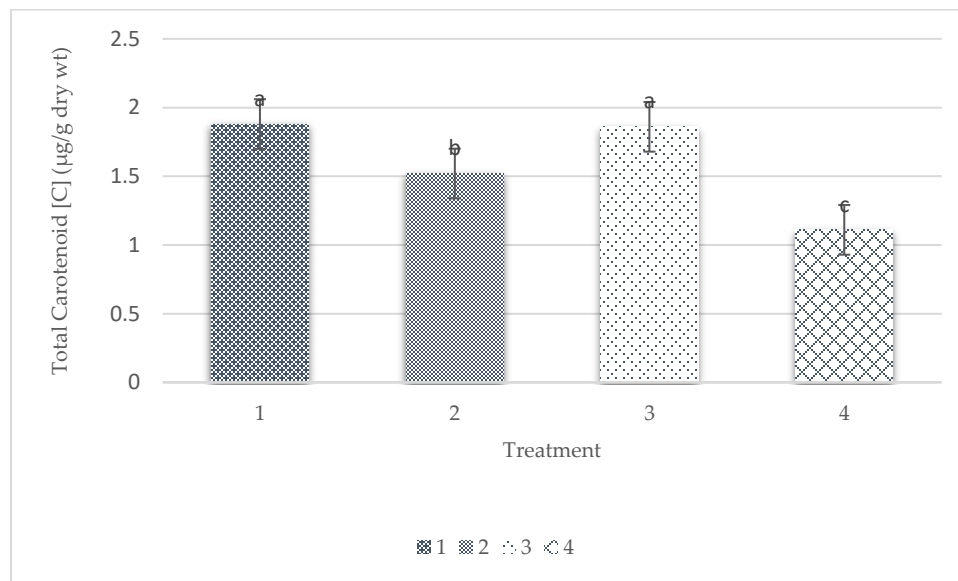
Water activity of the product was determined up to one month under the packaging material. In the day 0 no aerobic bacteria were observed. In the day 7, 14 and 21 the bacteria counts were gradually increased within the acceptable limits.

Yeast and Mold Count

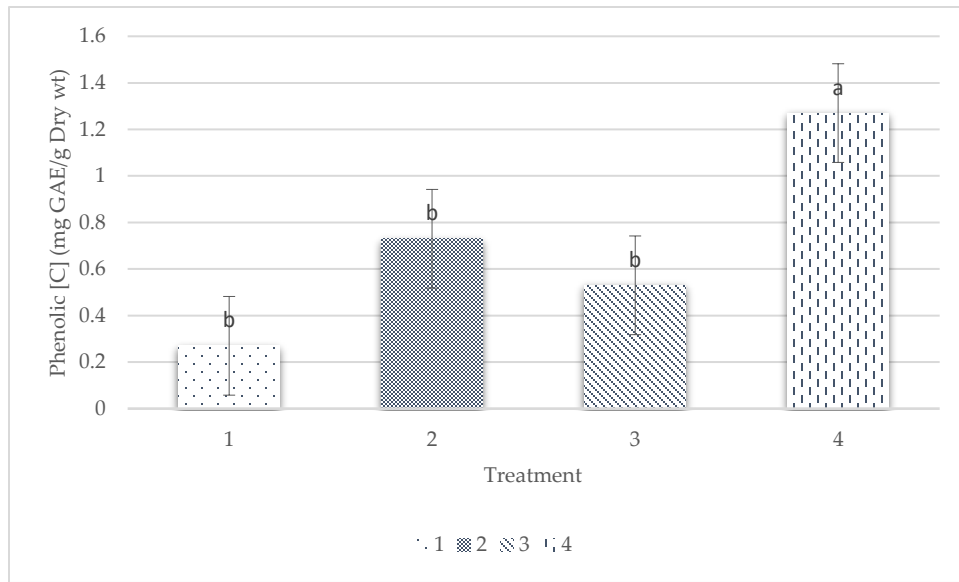
Yeast and mold counts were determined up to one month under the packaging material. In the day 0 and day 7 no yeast and mold were observed. In the day 14 and 21 the counts were ranged between 4.82 log cfu/g, and 5.12 log cfu/g.

Table 2. Fucoidan content [C] (mg/ 200 mL)

	ODS-Crass (T ₁)	FDS-Crass (T ₂)	ODS-Cris (T ₃)	FDS-Cris (T ₄)
Seaweed powder	26.12± 4.87	30.74± 6.36	27.5± 2.31	30± 4.09
Herbal tea liquor	28.98± 7.3	32.5 ± 0.892	28.15± 3.9	32.22± 6.92



Means with different superscript letters differ significantly (p<0.05)
Figure 1. Total carotenoid content of the oven dried and freeze-dried seaweed powders (Treatments; 1 -ODS cras, 2- FDS cras, 3- ODS cris, 4 -FDS cris)



Means with different superscript letters differ significantly ($p < 0.05$)

Figure 2. Total phenolic content of the oven dried and freeze-dried seaweed powders (Treatments; 1 -ODS cras, 2- FDS cras, 3- ODS cris, 4 -FDS cris)

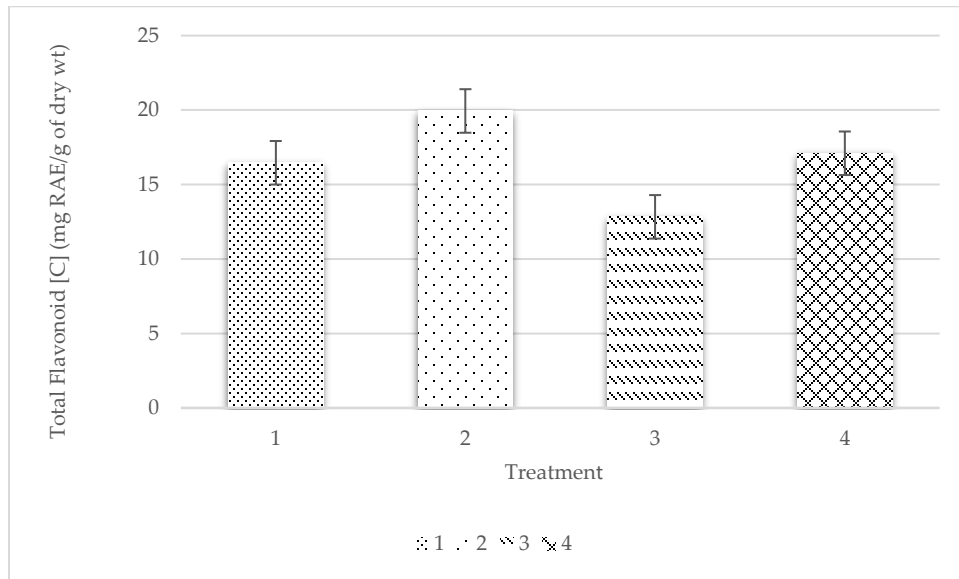
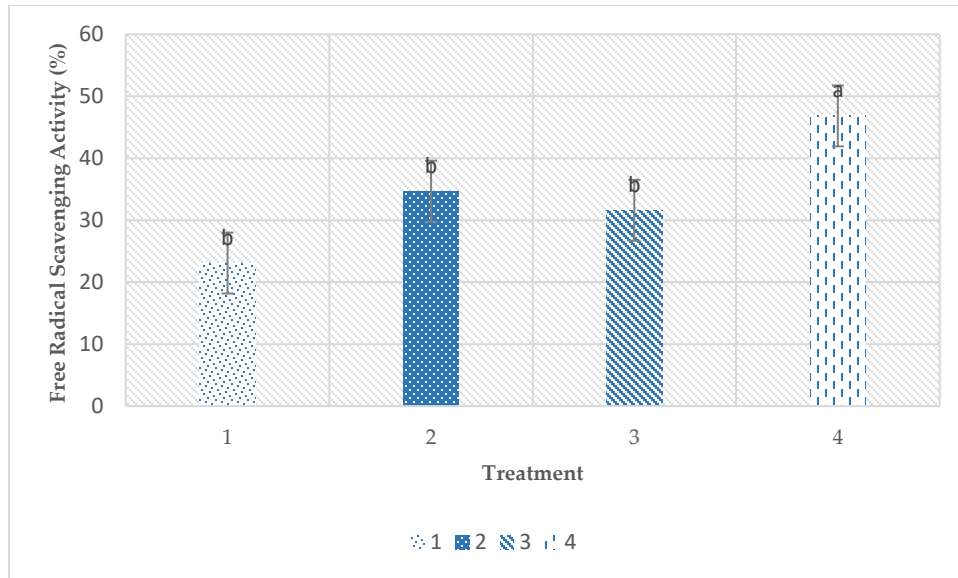


Figure 3. Total flavonoids content of the oven dried and freeze-dried seaweed powders (Treatments; 1 -ODS cras, 2- FDS cras, 3- ODS cris, 4 -FDS cris)



Means with different superscript letters differ significantly ($p < 0.05$)

Figure 4. Radical scavenging activity of oven dried and freeze-dried seaweed powders (Treatments; 1 -ODS cras, 2- FDS cras, 3- ODS cris, 4 -FDS cris)

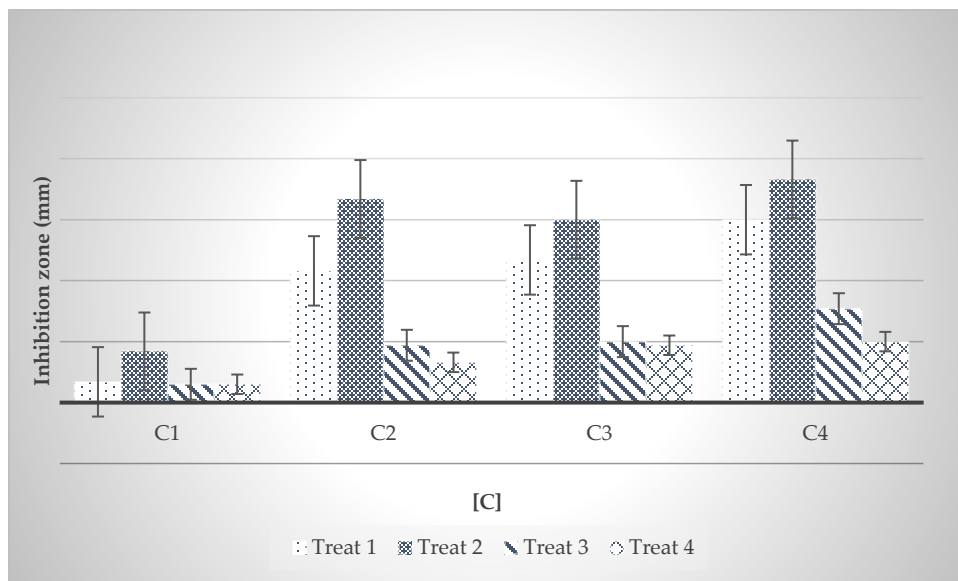


Figure 5. Antibacterial activity of different of herbal tea liquor concentrations: C1-25mg/mL, C2-50mg/mL, C3-75mg/mL, C4-100mg/mL. (Treatments; 1 -ODS cras, 2- FDS cras, 3- ODS cris, 4 -FDS cris)

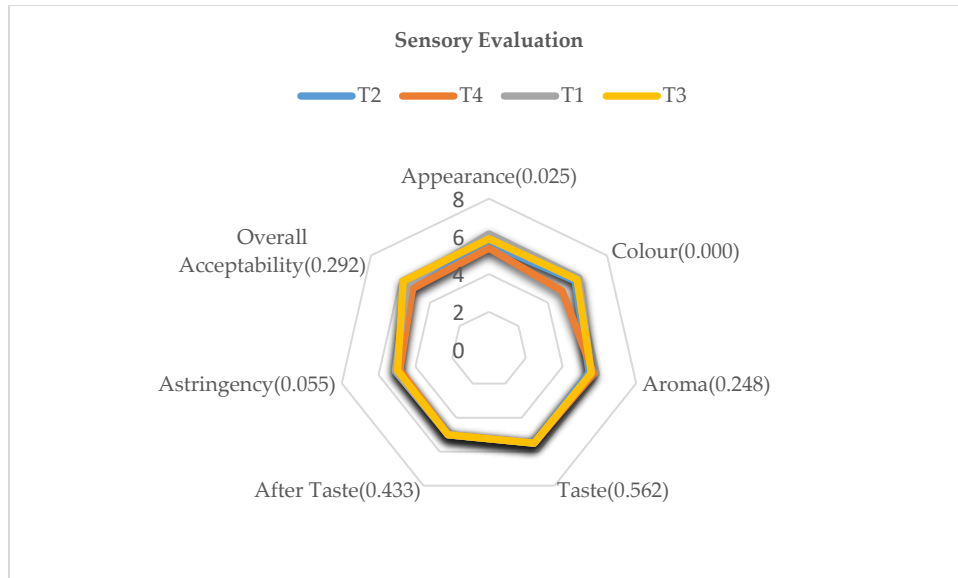


Figure 6. Sensory attributes of different herbal tea liquors

- T1- Oven dried *S.crassifolium* herbal tea
- T2- Freeze dried *S.crassifolium* herbal tea
- T3- Oven dried *S.cristaefolium* herbal tea
- T4- Freeze dried *S.cristaefolium* herbal tea

Conclusion

The study revealed that *S. cristaefolium* and *S. crassifolium* are rich in bioactive compounds including fucoidan, carotenoids, phenolics and flavonoids. Herbal tea developed from two *Sargassum* species showed high antioxidant activity and antibacterial properties. Thus, the two *Sargassum* species can be successfully used as herbal tea which give functional health benefits.

Conflicts of Interest

The authors declare no conflicts of interest.

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