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Chemical Characterization of Seaweeds From Biri, Northern Samar, Philippines

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Authors' contributions

This work was carried out in collaboration between the authors. All authors read and approved the final manuscript.

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ABSTRACT

This research was carried out in an attempt to ascertain the phytochemical screening on selected seaweed species from different barangays in Biri, Northern Samar, Philippines using a descriptive research approach. It also aimed to identify the seaweed species in the study area and determine the three most and least-frequently appearing species respectively, which were subjected to phytochemical screening and the determination of extracts and its physicochemical constituents as conducted from November 2022 to April, 2023. Seaweed samples were collected, identified, and authenticated by an expert. Specimens were preserved in jars containing 10% formaldehyde and seawater in equal volumes. Fresh samples of the three most frequently appearing and the three

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least frequently appearing species were used for the screening procedures. Results revealed a total of 44 seaweed species present in selected barangays of Biri, Northern Samar. The most-frequently appearing species were *Ulva reticulata, Hormophysa cuneiformis*, and *Turbinaria conoides*, while the least-frequently appearing species were *Hydropuntia edulis, Laurencia papillosa*, and *Tricleocarpa fragilis.* Chemical characterization of these species confirmed the presence of alkaloids, saponin, phenolic compounds, and anthraquinones, although not all of them contained these metabolites. Lipids and proteins were absent in all species tested. Meanwhile, the ash content of the samples is relatively high, implying that high amounts of minerals may be found in seaweeds. Thus, the researchers recommend the replication of this research in other areas of the province to document seaweed species and their potential applications in the functional food and nutraceutical industries. This will help develop natural products with economic or commercial significance to the residents. Likewise, more specific tests for lipids and proteins in seaweeds may be used in future investigations.

Keywords: Chemical characterization; seaweeds; chemical constituents; biri; northern Samar.

1. INTRODUCTION

Seaweeds, or marine macroalgae, are non-flowering, primitive photo-synthetic macrophytes found in tidal areas of the seas and oceans that make up 71% of the world's surface [1]. The main groups of seaweeds are brown (*Phaeophyta*), red (*Rhodophyta*), and green (*Chlorophyta*) [2]. Moreover, seaweeds can be found worldwide but are restricted by light, nutrition, and a suitable substrate, [3]. Furthermore, a number of seaweed species are edible, and many are also of commercial importance to humans [4].

Meanwhile, the increasing global population and human activities have negatively impacted the natural environment that leads to shortage of bioresources for the growing population [5]. This has sparked an interest in finding novel foods that will meet the needs of a growing population and offer some health benefits [Granato, *et al.*, 2020]; [6]. Moreover, seaweeds represent a huge resource for mankind, and as the demands grow for special drugs, nutraceuticals, cosmetic products, and functional foods, there is a strong momentum for the exploration of marine biological resources in general, and seaweeds in particular, for novel compounds with health benefits [7]. Thus, recent increased interest in seaweed is motivated by attention generated in their bioactive components that have potential applications in the functional food and nutraceutical industries [8].

The phytochemicals from marine algae are extensively used in various industries such as food, confectionery, textile, dairy, pharmaceutical, and paper, mostly as gelling, stabilizing, and thickening agents [9]. Seaweeds are the richest source of proteins, lipids, carbohydrates, minerals, vitamins (A, B, C, and Niacin), and antioxidants. They also serve as low-calorie food and are valued as food supplements for people [10]. Generally, seaweeds are known to contain medicinally rich metabolites that include steroids, phenols, tannins, saponins, flavonoids, terpenoids, and glycosides, which have been extensively studied and used in the pharmaceutical industry [8].

Therefore, this present study focuses on the phytochemical screening of selected species of seaweeds in Biri, Northern Samar, specifically, to determine the active chemical constituents of three (3) most-frequently appearing and three (3) least-frequently appearing seaweeds in terms of: alkaloids, anthraquinones, cardiac glycosides, phenolic compounds, and saponin. Furthermore, it will determine the physicochemical activities of the three (3) most-frequently appearing and least-frequently appearing seaweeds in terms of: lipids, proteins, and ash content. No such investigation has yet been done in Biri, Northern Samar, hence, this study.

2. MATERIALS AND METHODS

2.1 Area of Study

Northern Samar is situated in the Eastern Visayas region. Its capital is the municipality of Catarman. It is bordered, clockwise from the North, by the Philippine Sea, Eastern Samar, Samar, Samar Sea, and San Bernardino Strait. The province is composed of 24 municipalities [11].

The island of Biri [Latitude: 12°41'N (12.6816); Longitude: 124°22' (124.3619)], a 5th class municipality, is located in its northernmost tip, facing the Pacific Ocean to the east, the famous San Bernardino Strait to the north, and to either Lavezares or San Jose, both Northern Samar's coastal towns. As of 2020, the current population of the municipality of Biri is 11,274 (Phil Atlas, 2020)., the source of livelihood were farming, fishing, aquaculture, and tourism. The municipality comprises of eight (8) barangays, four (4) of which are located in separate islands.

2.2 Sampling Technique

Purposive sampling was applied in the collection of seaweed species of seaweeds utilized for the phytochemical analyses. Specimen for the phytochemical screening were collected purposely, the basis for the collection were the three most frequently occurring seaweeds and the least three occurring seaweeds in the municipality of Biri, Northern Samar.

2.3 Data Collection

The researchers were able to secure a Prior Informed Consent (PIC) from the Municipal Agriculture Office (MAO) and from the municipal mayor and the barangay captain of each sampling site to conduct the study in their area. The researchers went to each barangay for two (2) weekends to collect seaweeds, and each was tallied to determine the three most and three least frequently appearing seaweeds.

2.3.1 Specimen collection

Specimens were collected by hand-picking and using a knife. Underwater camera for photo documentation and a field notebook were used to record the data for each specimen to serve as guide in identification. Specimens were washed with seawater to remove adhering sediments and impurities, stored in glass jars, and were brought to the College of Science for identification.

2.3.2 Preservation of the Specimens

Each specimen collected was stored in glass jars and soaked in formaldehyde solution at a ratio of 3.7% formaldehyde: 96.3% seawater.

2.3.3 Identification of the specimens

Seaweed samples were initially identified *in situ,* then, to confirm the authenticity of the seaweed samples, it was brought to the College of Science, University of Eastern Philippines

2.4 Seaweed Extraction

The six (6) selected seaweed samples were rinsed using seawater to remove dirt, epiphytes, sediments, and other debris to make sure that it will not affect the result of the phytochemical

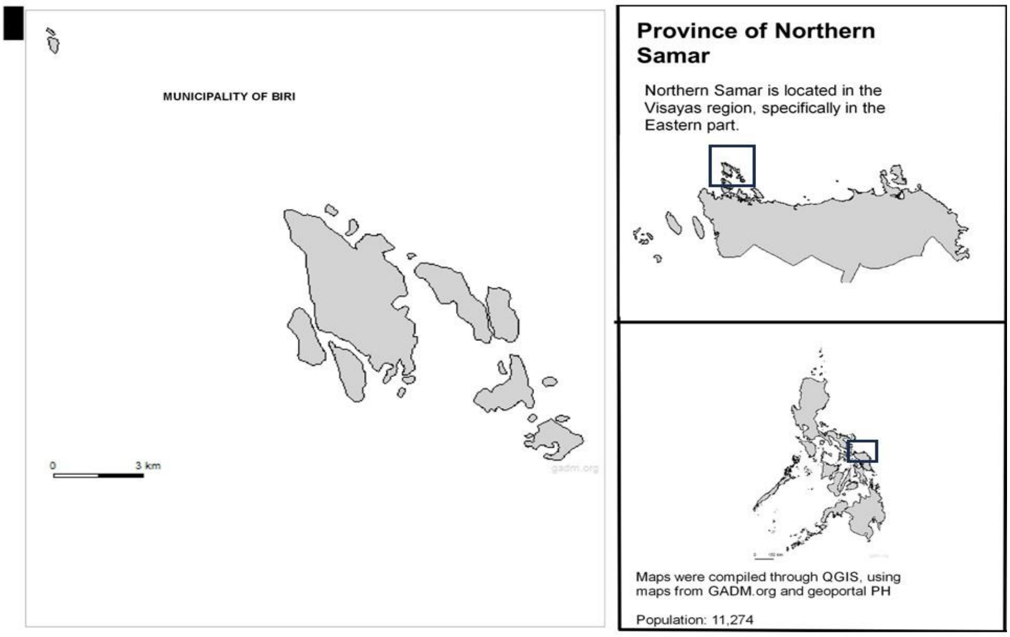

Fig. 1. Map of study location

screening and other chemical test. Manual juicer/ extractor were used for the pure extract, the extracts were then placed and contained in a clean sterile jar for phytochemical screening and characterization.

2.5 Phytochemical Screening

The chemical constituents of the three most- and three least-frequently appearing seaweeds were determined using the following procedures:

2.5.1 Tests for alkaloids

In this test, the Dragendorff's reagent and Mayer's reagents were used in determining the presence of alkaloids. A positive result was indicated by the presence of an orange precipitate in Dragendorff's reagent, and a white precipitate with the Mayer's reagent (Guevarra, 2005) as cited by Cruz [12]. Confirmatory tests for alkaloids were also performed using the protocols of Guevarra (2005).

2.5.2 Tests for anthraquinone

10mL of 10% ammonia solution, few milliliters of filtrate was added and shaken vigorously for 30 seconds. A pink, violet, or red color indicated a positive result of anthraquinone [13].

2.5.3 Test for cardiac glycosides

Keller Killiani Test was performed wherein a solution of 0.5ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract.The appearance of deep blue color at the junction of two liquids indicated the presence of cardiac glycosides [14].

2.5.4 Test for phenolic compounds

Ferric chloride Test was utilized, where ferric chloride solution was added drop wise in a test tube containing 3 mL of the given sample. The color blue, green, violet, or red indicates the presence of phenolic compounds [15].

2.5.5 Test for saponin

An equivalent of 10 g of seaweed extract was loaded to a capillary tube by immersing the tube to a height of 10 mm in the seaweed extract. Likewise, another capillary tube was loaded with distilled water. After sometime, the heights of the liquids in the two tubes were compared. If the level of the seaweed extract in the capillary tube is half or less than in the other tube containing water, then the presence of saponin may be inferred. To confirm its presence in the extract, the frothing test was administered, and a layer of foam of approximately 1cm indicated the presence of saponin (Guevarra, 2005) [13].

2.6 Physicochemical Activities

The different physico-chemical activities of the three (3) most and least frequently appearing seaweeds were determined using the following procedures:

Test for Lipids

Utilizing the emulsion test, a sample was added to 2cm^3 of ethanol, and was shaken well, allowed to settle in a test tube rack for 2 minutes, then, any clear liquid was emptied into a test tube containing 2cm^3 of distilled water. A layer of milky-white emulsion at the top of the solution indicated the presence of lipids (Guevarra, 2005).

2.6.1 Test for Proteins

The Biuret test was utilized. One to two milliliters of the seaweed solution, egg albumin, and de-ionized water was added in clean and dry test tubes. Then, 1-2 mL of Biuret reagent was added to all the test tubes, shaken well, and allowed to stand for 5 minutes. If the solution turns from blue to deep purple, proteins are present [16].

2.6.2 Ash content

Oven-dried samples were weighed, and 3 grams of sample was placed in each of 3 separate crucibles. The crucibles were then heated using a portable gas stove until the sample became completely ash. It was allowed to cool in a desiccator and weighed [17]. Ash content was calculated using the formula below:

$$\text{Ash content} = \frac{\text{weight of ash (g)}}{\text{weight of sample (g)}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Species Composition of Seaweeds in Biri, Northern Samar

There were forty-four (44) identified species of seaweeds classified into three (3) classes (Chlorophyceae [17 species], Phaeophyceae [13 species], and Rhodophyceae [14 species]).

Moreover, the seaweeds were also classified into twenty-one (21) families.

Of this number, the three (3) most-frequently appearing and widely distributed in all of the sampling sites were chosen as samples by the researchers, and they were *Ulva reticulata, Hormophysa cuneiformis,* and *Turbinaria conoides*. On the other hand, the three least-frequently appearing or those found in only one or two of the sampling sites, were likewise chosen to undergo phytochemical screening and test for the physicochemical constituents. The least frequently appearing seaweed species were *Hydropuntia edulis, Laurencia papillosa,* and *Tricleocarpa fragilis.*

Table 1. Species Composition of Seaweeds in Selected Brangays of Biri, Northern Samar, Philippines

Class	Seaweed Species	Sampling Sites				
		1	2	3	4	5
	Anadyomene plicata C. Agardh	×	×	×	/	/
	Avrainvillea erecta (Berkeley) A. Gepp & E. Gepp	/	×	×	×	×
	Boergesenia forbesii (Harvey) J. Feldmann	/	/	×	×	/
	Bornetella sphaerica (Zanardini) Solms-Laubach	/	/	×	×	/
	Caulerpa brachypus Harvey	×	×	×	×	/
	Caulerpa racemosa (Forsskål) J. Agardh	/	/	×	/	×
Chlorophyceae	*Caulerpa serrulata* (Forsskål) J. Agardh	/	/	×	/	×
	Caulerpa taxifolia (Vahl) C. Agardh	/	/	×	×	×
	Chaetomorpha crassa (C. Agardh) Kutzing	/	/	/	/	/
	Dictyosphaeria versluysii Weber-van Bosse	×	/	×	×	/
	Halicoryne wrightii Harvey	×	×	×	×	/
	Halimeda macroloba Decaisne	/	/	/	/	/
	Halimeda opuntia (Linnaeus) Lamoureux	/	/	/	/	/
	Ulva clathrata (Roth)	/	/	×	×	×
	Ulva intestinalis Linnaeus	×	/	×	×	/
	Ulva lactuca Linnaeus	/	/	/	/	/
	Ulva reticulata Forsskål	/	/	/	/	/
	Canistrocarpus cervicornis (Kutzing) De Paula and De Clerk	/	/	×	/	/
	Dictyota dichotoma (Hudson) Lamoureux	×	/	×	×	×
	Hormophysa cuneiformis (J.F, Gmelin) P.C. Sliva	/	/	/	/	/
Phaeophyceae	*Padina gymnospora* (Kützing) Sonder	/	/	/	×	/
	Padina minor Yamada	/	/	/	/	/
	Sargassum aquifolium	×	/	×	/	/
	Sargassum cinctum	/	/	×	/	/
	Sargassum gracillimum Reinbold	×	/	×	×	/
	Sargassum hemiphyllum (Turner) C. Agardh	/	/	×	×	×
	Sargassum oligocystum Montagne	/	/	/	/	/
	Sargassum polycystum C.A. Agardh	/	/	/	×	×
	Sargassum siliquosum J. Agardh	/	/	×	×	/
	Turbinaria conoides (Turner) J. Agardh	/	/	/	/	/
	Acanthapora spicifera (Vahl) Borgesen	×	/	/	×	/
	Amphiroa foliacea J.V. Lamouroux	/	/	/	/	/

Class	Seaweed Species	Sampling Sites				
		1	2	3	4	5
Rhodophyceae	*Coelothrix irregularis* (Harvey) Borgessen	/	×	×	×	×
	Eucheuma denticulatum (N.L.Burman) Collins and Harvey	×	×	/	/	×
	Euthora cristata (C. Agardh) J. Agardh	×	×	×	/	×
	Gelidiella acerosa (Forsskål) Feldmann and Hamel	/	/	×	/	×
	Gracilaria salicornia (C. Agardh) Dawson – with four (4) haplotypes	/	/	/	/	/
	Hydropuntia edulis (S.G. Gmelin) Gurgel & Fredericq	/	/	×	×	×
	Hypnea servicornis J. Agardh	×	/	/	×	×
	Laurencia tronoi Ganzon-Fortes	/	/	×	/	×
	Palisada perforate (Bory) K.W. Nam	×	/	×	/	×
	Mastophora rosea (C. Agardh) Setchell	/	×	×	×	/
	Portieria hornemannii (Lyngbye) P.C. Silva	/	/	×	×	×
	Tricleocarpa fragilis (Linnaeus) Huisman & R.A. Townsend	×	/	/	×	×

***Legend**:*
/ - present
× - absent

Sampling sites:
1 - Barangay Progress
2 - Barangay Pio del Pilar
3 - Barangay McArthur
4 - Barangay San Pedro
5 - Barangay San Antonio

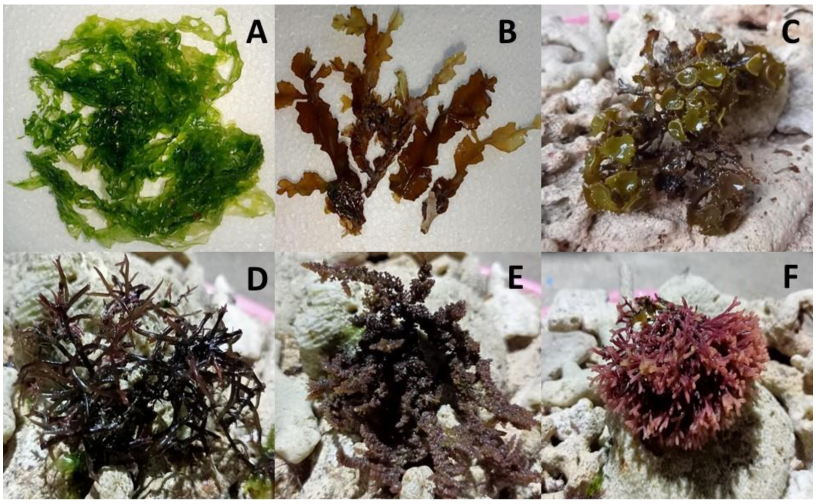

Fig. 2. The frequently occurring and the least occurring seaweeds for Phytochemical screening. (A). *Ulva reticulata;* (B). *Hormophysa cuneiformis;* (C). *Turbinaria conoides;* (D). *Hydropuntia edulis;* (E). *Palisada perforata;* (F): *Tricleocarpa fragilis*

3.2 Phytochemical Screening Results

Results of the phytochemical screening reveal that phenolic compounds were present in all species tested, while saponin was present only in 4 if the samples. Moreover, alkaloids were detected only in *Hormophysa cuneiformis* and *Turbinaria conoides*. On the other hand, anthraquinones were present only in *Tricleocarpa fragilis,* while all 6 seaweed species tested negative for cardiac glycosides. These results imply that the coastal waters of the municipality of Biri, Northern Samar, specifically the sample barangays, has a diversity of seaweed species which could be harnessed economically through their possession of phytochemicals that could be the bases for drug discovery and development aside from the common use as food or source of alginates.

3.3 Physicochemical Activities of Seaweed Extracts

The six identified seaweed species were subjected to tests to determine their physicochemical activities or constituents, specifically lipids, proteins, and ash content. Results reveal that lipids and proteins were absent in all of the species tested. However, test for the ash content revealed a relatively high percentage of ash content. This result implies that there is a potentially high amount of minerals.

Table 2. Summary result of phytochemical screening of the three most- and three least-frequently appearing seaweeds in Biri, Northern Samar

Secondary metabolite test	Seaweed extracts					
	1	**2**	**3**	**4**	**5**	**6**
ALKALOID						
Dragendorff's Test	+	+	+	+	+	+
Mayer's Test	-	+	+	-	-	-
Confirmatory Test	-	+	+	-	-	-
ANTHRAQUINONE	-	-	-	-	-	+
CARDIAC GLYCOSIDES	-	-	-	-	-	-
PHENOLIC COMPOUNDS	+	+	+	+	+	+
SAPONIN						
Capillary Test	-	-	+	+	+	+
Frothing Test	-	-	+	+	+	+

LEGEND:
+ = Tested compound is present in the seaweed extract
- = Tested compound is absent in the seaweed extract
1 = Ulva reticulata
2 = Hormophysa cuneiformis
3 = Turbinaria conoides
4 = Hydropuntia edulis
5 = Palisada perforata
6 = Tricleocarpa fragilis

Table 3. Summary result of the determination of physicochemical activities of three most and three least frequently appearing seaweeds in Biri, Northern Samar

Physicochemical activities	Seaweed extract					
	1	**2**	**3**	**4**	**5**	**6**
LIPID	-	-	-	-	-	-
EMULSION TEST						
PROTEIN	-	-	-	-	-	-
BIURET TEST						
ASH CONTENT	22.60%	29.20%	33.07%	33.96%	38.37%	63.17%

LEGEND:
+ = Tested compound is present in the seaweed extract
- = Tested compound is absent in the seaweed extract
1 = Ulva reticulata
2 = Hormophysa cuneiformis
3 = Turbinaria conoides
4 = Hydropuntia edulis
5 = Laurencia papillosa
6= Tricleocarpa fragilis

4. CONCLUSION

Utilizing the purposive sampling technique, this descriptive research to set to assess the seaweed resources in the Municipality of Biri, Northern Samar, Philippines. From the results, the three most - and the three least-frequently appearing seaweeds were collected, identified, and subjected to phytochemical screening and determination of its physicochemical constituents.

There were 44 species of seaweeds in five sampling sites in Biri, Northern Samar. These belong to three classes, and 21 families, implying that Biri, Northern Samar harbors a diversity of species of seaweeds.
The three most-frequently appearing seaweeds are: *Ulva reticulata, Hormophysa cuneiformis,* and *Turbinaria conoides*. Meanwhile, the three least- frequently appearing seaweeds are: *Hydropuntia edulis, Laurencia papillosa,* and *Tricleocarpa fragilis.*

Although not all secondary metabolites were present in any one seaweed species, the most commonly recorded secondary metabolite were phenolic compounds, while the least common metabolite was anthraquinone, which was present in only one sample. This implies that seaweed species available in the study area contain several chemical constituents or secondary metabolites which could have economic significance or potential applications for drug discovery and development.

In terms of the physicochemical activities of the seaweed samples, lipids and proteins turned out to be negative in all samples; however, the ash content of all the samples were relatively high, which implies that the seaweed species tested have a potentially rich mineral content.

Considering the limited tests done in this research, it is recommended that the seaweed extracts may be subjected to further testing to verify the existence of other chemical constituents or secondary metabolites that could have potential medical applications and/or can be used to create natural products.

The use of other methods of preparations, tests, and analyses for phytochemical screening and physicochemical constituent determination should be done to further test the availability of secondary metabolites and physicochemical constituents on seaweeds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

As per international standards or university standards, respondents’ written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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