



PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF ETHANOLIC EXTRACTS OF *Sargassum vulgare* AND *Cystoseira spp.* COLLECTED FROM AL-HAMAMA COAST, NORTHEASTERN LIBYA

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ABSTRACT

Background: Marine brown algae contain diverse metabolites that may exhibit antioxidant and antibacterial activities. Their chemical composition and biological properties can vary with taxonomic identity, environmental conditions, sample handling, and extraction procedures. **Aim:** To characterize and compare ethanolic extracts of *Sargassum vulgare* and *Cystoseira spp.* collected from the Al-Hamama coast, northeastern Libya, with respect to phytochemical composition, antioxidant potential, and antibacterial activity. **Methods:** Algal material was reportedly collected on 15 May 2026 from a coastal depth of 0–1.5 m. Three separately processed replicates were reported for each algal group. For each replicate, 10 g of dried material was extracted with 100 g of ethanol. The planned analyses included total phenolic content (TPC) by the Folin–Ciocalteu method, total flavonoid content (TFC) by an aluminium chloride colorimetric assay, DPPH radical-scavenging activity, antibacterial activity by disc diffusion, and HPLC analysis of five target compounds. Details of several analytical conditions and quantitative results remain to be verified from the original records. **Results:** Approximate wet and dry weights were reported as 140 g and 120 g, respectively, corresponding to calculated moisture and dry-matter contents of 14.29% and 85.71%, provided that the weights represent the same material before and after drying. Preliminary HPLC transcriptions listed gallic acid, catechin, chlorogenic acid, ferulic acid, and cinnamic acid, with summed reported amounts of 0.088 mg for *Sargassum* and 0.090 mg for *Cystoseira*. These values are provisional because the amounts, percentages, and peak-area records are not fully reconciled. Verified quantitative results for TPC, TFC, DPPH activity, and antibacterial inhibition were not available for this draft. **Conclusion:** The manuscript establishes a framework for comparing two brown-algal groups from the Al-Hamama coast. Definitive conclusions about differences in chemical composition or biological activity

require verification of the original laboratory data, full methodological reporting, taxonomic confirmation, and appropriate statistical analysis.

Keyword: Brown algae; *Sargassum vulgare*; *Cystoseira* spp.; phenolic compounds; total phenolic content; flavonoids; HPLC; DPPH; antibacterial activity; Libya.

Introduction

Marine brown algae are ecologically important coastal organisms and potential sources of structurally diverse natural products. Their metabolites have attracted interest in natural-products research, food science, biotechnology, and pharmaceutical investigation because some algal extracts demonstrate antioxidant or antimicrobial activity in laboratory assays. Such activity is not uniform across taxa and may be affected by season, habitat, thallus maturity, sample processing, solvent composition, and extraction conditions.

Sargassum is a widely distributed genus of marine brown algae. The taxonomic treatment of *Cystoseira*-related algae requires particular care because the broader *Cystoseira* sensu lato complex has undergone taxonomic revision, with several closely related genera recognised in recent classifications. Species-level identification should therefore be based on diagnostic morphological features and appropriate taxonomic references; molecular confirmation may be useful when morphology alone is inconclusive.

Phenolic constituents are frequently investigated in algal extracts because some can participate in redox reactions and may contribute to measured antioxidant activity. The Folin–Ciocalteu assay is commonly used to estimate total reducing capacity expressed as phenolic equivalents, while aluminium chloride colorimetry is used to estimate selected flavonoid-like constituents. Both are operational colorimetric methods and their responses may be influenced by chemical structure and non-target substances. The DPPH assay measures the reduction of a stable radical under defined in vitro conditions; its results depend on extract concentration, reaction time, solvent, and measurement conditions.

Antibacterial screening can provide preliminary evidence of in vitro inhibition, but disc diffusion is influenced by compound solubility and diffusion through agar. Inhibition-zone diameters should not be interpreted as clinical susceptibility categories for crude algal extracts unless a validated interpretive framework applies. HPLC can complement bulk assays by separating selected target compounds, provided that identification and quantification are supported by authentic standards, calibration data, and documented chromatographic conditions.

This study was designed to examine ethanolic extracts of *Sargassum vulgare* and *Cystoseira* spp. collected from the Al-Hamama coast in northeastern Libya. The planned analytical framework includes sample characterization, TPC and TFC estimation, DPPH radical-scavenging assessment, antibacterial screening, and HPLC analysis of selected compounds. The present draft reports only the information available in the

supplied records and identifies measurements that require verification before the findings can be interpreted conclusively.

2. Aim and Objectives

The aim was to characterize and compare ethanolic extracts of *Sargassum vulgare* and *Cystoseira* spp. collected from the Al-Hamama coast, northeastern Libya.

- 1.To document the collection and available morphological characteristics of the algal material.
- 2.To determine TPC using a validated Folin–Ciocalteu procedure and report results as mg gallic acid equivalents per gram of dry material (mg GAE/g DW).
- 3.To estimate TFC using an aluminium chloride assay and report results as mg quercetin equivalents per gram of dry material (mg QE/g DW), if quercetin was the standard actually used.
- 4.To assess DPPH radical-scavenging activity across documented extract concentrations.
- 5.To screen antibacterial activity against the bacterial strains actually tested using a documented, reproducible method.
- 6.To analyse selected phenolic compounds by HPLC using appropriate identification and quantification procedures.
- 7.To compare the two algal groups using statistical methods suited to the verified experimental design.

3. Materials and Methods

3.1. Study Area and Sample Collection

Algal material was reportedly collected from the Al-Hamama coast, northeastern Libya, on 15 May 2026, at a reported depth of 0–1.5 m. The collection environment was described as rocky coastal habitat. Three separately processed replicates were reported for each algal group. Approximate coordinates previously associated with the general sampling area were 32.91897° N, 21.62391° E; these should be confirmed against the original field records and should not be represented as exact coordinates for each replicate unless documented.

Environmental measurements such as water temperature, salinity, pH, and weather conditions should be included only if they were recorded at collection. Any permits or authorisations required for collection should be reported where applicable.

3.2. Sample Preparation and Dry-Matter Estimation

The material was reportedly cleaned to remove adhering sand and visible contaminants and then dried. The records supplied for this draft indicate approximate wet and dry weights of 140 g and 120 g per replicate, respectively. If these values refer to the same sample before and after drying, moisture content and dry-matter content can be calculated as follows:

$$\text{Moisture content (\%)} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100.$$

$$\text{Dry-matter content (\%)} = (\text{dry weight} / \text{wet weight}) \times 100.$$

Using the reported values, moisture content is 14.29% and dry-matter content is 85.71%. These calculations must be retained only after checking the original weighing records and confirming that the weights are comparable.

3.3. Preparation of Ethanolic Extracts

For each reported replicate, 10 g of dried algal material was combined with 100 g of ethanol, giving a nominal dry material-to-solvent mass ratio of 1:10 (w/w). The replicates were reportedly processed separately. The ethanol concentration, extraction duration, temperature, mixing conditions, extraction apparatus, filtration method, and extract-recovery procedure were not fully specified in the supplied text and must be recovered from the laboratory records rather than inferred.

The recovered extract should be weighed after the documented concentration and drying procedure. Extraction yield should be calculated as: $\text{extraction yield (\%)} = [\text{mass of recovered dry extract (g)} / \text{initial dry algal mass (g)}] \times 100$. No extraction yield can be reported until the final extract masses are available. Storage conditions and the solvent used to reconstitute extracts for assays should also be stated if documented.

3.4. Taxonomic Identification

The identification of *Sargassum vulgare* and the second algal group should be supported by recorded diagnostic characteristics and a cited taxonomic key or reference. Relevant characters may include thallus organisation, branching, phylloid morphology, air vesicles, reproductive structures, and anatomical features where these were examined. The second group should remain designated as *Cystoseira* spp. unless the available evidence supports a more precise identification. The taxonomist, identification reference, voucher specimen number, and any molecular methods should be reported only when applicable and documented.

3.5. Total Phenolic Content

TPC was planned for assessment using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard. The actual reagent volumes, standard concentrations, incubation time, wavelength,

sample dilution, and calibration range must be reported from the experimental protocol. The calibration equation and coefficient of determination (R^2) should be supplied from the measured standard data.

Results should be expressed as mg GAE/g DW, with the calculation accounting for the calibration-derived concentration, final extract volume, dilution factors, and initial dry algal mass. Verified absorbance readings and calibration data were not supplied for this draft; therefore, no TPC values are reported.

3.6. Total Flavonoid Content

TFC was planned for estimation using an aluminium chloride colorimetric method. Quercetin should be named as the reference standard only if it was actually used. Reagent composition, standard concentrations, incubation conditions, wavelength, dilution factors, calibration equation, and R^2 must be reported from the laboratory records.

Results should be expressed as mg QE/g DW if quercetin was the standard, with calculations accounting for final extract volume, dilution factors, and initial dry material mass. This assay provides an operational estimate and should not be interpreted as a definitive quantification of every flavonoid in the extract. Verified quantitative measurements were not available for this draft.

3.7. DPPH Radical-Scavenging Assay

DPPH radical-scavenging activity was planned for assessment using documented extract concentrations and an appropriate reagent control. Sample blanks should be used where needed to account for extract colour or background absorbance. The DPPH concentration, extract concentration series, solvent, reaction time, temperature, wavelength, reference antioxidant, and replicate structure must be reported according to the procedure actually used.

Percentage inhibition may be calculated using blank-corrected absorbance values where appropriate: $\text{inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$. An IC_{50} should be estimated only if the measured concentration–response data support a reliable fit. Since verified absorbance and concentration–response data were not supplied, no inhibition percentages or IC_{50} values are reported.

3.8. Antibacterial Activity

Antibacterial activity was reported as having been investigated by disc diffusion; this method description should be retained only if it matches the procedure actually performed. The bacterial species listed in the draft are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Strain identifiers and sources should be included when available.

The final Methods section must state the culture medium, inoculum preparation and density, extract concentration, amount applied per disc, disc diameter, solvent control, positive antibiotic control, incubation conditions, and number of independent experiments. Inhibition zones should be reported in

millimetres, with an explicit statement about whether disc diameter was included. Results should include replicate-level measurements and suitable summary statistics.

Crude-extract inhibition zones should not be assigned clinical susceptibility categories unless a validated interpretive standard applies to the extract, organism, and method. Any reference to CLSI guidance should be limited to the relevant methodological principles and should accurately identify the edition consulted. Verified inhibition-zone data and control results were not supplied for this draft.

3.9. High-Performance Liquid Chromatography

The supplied records list gallic acid, catechin, chlorogenic acid, ferulic acid, and cinnamic acid as HPLC target compounds. The final method must identify the instrument and detector, column, mobile phases, elution programme, flow rate, injection volume, column temperature, detection wavelength, run time, sample preparation, and dilution factors, based on the original instrument records.

Identification should be supported by the criteria actually used, such as retention-time comparison with authentic standards and any additional confirmation. Quantification requires the relevant calibration curves, calibration range, regression equations, and appropriate validation information. The exact designation of catechin versus catechins must be confirmed against the standard and instrument report.

The preliminary transcribed sums were 0.088 mg for *Sargassum* and 0.090 mg for *Cystoseira*. These amounts cannot be interpreted as directly comparable concentrations until the basis of the quantities, calibration calculations, extract volumes, dilution factors, and sample-mass normalisation have been verified. Values should be converted to an appropriate unit such as mg/g DW only when the underlying calculations permit it.

3.10. Statistical Analysis

Statistical analysis should be conducted only after the underlying measurements and replicate structure have been verified. The independent experimental unit must be identified; repeated measurements of the same sample should not be treated as independent biological replicates. Data may be summarised as mean $\pm$ standard deviation when appropriate. A Welch independent-samples t-test, conventional t-test, or non-parametric alternative should be selected according to the design and assumptions, not automatically.

If multiple bacterial strains, concentrations, or endpoints are compared, the analysis should account for the number and structure of comparisons. Effect estimates and confidence intervals should be reported where feasible. A significance threshold such as $p < 0.05$ may be prespecified, but no inferential result should be claimed before analysis of verified data. In the current draft, no comparative statistical inference is reported.

4. Results

4.1. Sample Characteristics

The supplied records describe collection on 15 May 2026 from the Al-Hamama coast, northeastern Libya, at a reported depth of 0–1.5 m. Approximate wet and dry weights were recorded as 140 g and 120 g per replicate. If these values refer to the same samples, the calculated moisture content is 14.29% and dry-matter content is 85.71%. Confirmation against the original weighing records is required.

Parameter	Recorded Information	Status
Collection date	15 May 2026	Recorded
Collection depth	0–1.5 m	Recorded
Replicates	3 per algal group	Three replicates reported per algal group
Approximate wet weight	140 g per replicate	Recorded value
Reported dry weight	120 g per replicate	Recorded value
Calculated moisture content	14.29%	Calculated from reported wet and dry weights
Calculated dry-matter content	85.71%	Calculated from reported wet and dry weights
Material used for extraction	10 g dried material per replicate	Recorded
Ethanol used	100 g per replicate	Recorded
Final extract weight/yield	Not available	Requires the actual recovered extract weight

4.2. Total Phenolic and Flavonoid Contents

Verified TPC and TFC measurements were not available in the supplied dataset. Group means, standard deviations, and statistical comparisons cannot be reported until the original absorbance readings, calibration data, dilution factors, and calculations have been checked.

4.3. DPPH Radical-Scavenging Activity

Verified DPPH absorbance readings and concentration–response data were not available. Percentage inhibition and IC₅₀ values are therefore not reported.

4.4. Antibacterial Activity

Verified inhibition-zone measurements and control results were not available for the bacterial strains listed in the Methods. No comparative antibacterial effect can be concluded from the current records.

4.5. Preliminary HPLC Record

The supplied HPLC transcription lists five target compounds in each algal extract. The reported sums were 0.088 mg for Sargassum and 0.090 mg for Cystoseira. Because the reported compound amounts, percentages, and peak-area data are not fully reconciled, these figures are presented as provisional transcriptions and not as validated comparative concentrations.

Compound	Sargassum reported amount (mg)	Cystoseira reported amount (mg)
Gallic acid	0.022	0.018
Catechin (identity/name to verify)	0.010	0.015
Chlorogenic acid	0.007	0.008
Ferulic acid	0.035	0.038
Cinnamic acid	0.014	0.011
Reported sum	0.088	0.090

Editorial verification required: the original chromatograms, calibration records, peak assignments, and calculation sheets must be checked before these values are used for quantitative interpretation. Relative peak-area percentages should not be treated as mass percentages unless the method specifically supports that interpretation.

5. Discussion

5.1. Sample Processing and Extraction

The recorded weights provide preliminary information about the material used in extraction, but the moisture calculation depends on the wet and dry weights referring to the same material and on a documented drying procedure. Final extract mass and extraction yield are essential for characterising recovery and for relating assay results to the original dry biomass. The nominal material-to-solvent ratio was reported as 1:10 (w/w), but reproducibility also depends on solvent concentration, extraction time, temperature, mixing, and recovery conditions.

5.2. Interpretation of Phytochemical Assays

TPC and TFC can provide useful comparative assay measures when performed with appropriate standards and calibration procedures. The Folin–Ciocalteu response can be influenced by reducing substances other than phenolics, while aluminium chloride response varies with compound structure. Consequently, assay-derived equivalents should be interpreted as method-dependent estimates rather than absolute inventories of all phenolic or flavonoid molecules. No comparison between the algal groups is possible until the underlying measurements are verified.

5.3. Antioxidant Activity

The DPPH assay assesses radical-scavenging response under specified *in vitro* conditions. Reliable interpretation requires a documented concentration series, suitable controls, correction for sample colour where needed, and a reproducible reaction protocol. A possible association between TPC and DPPH activity could be explored only after valid replicate-level data become available; even a statistical association would not establish that phenolics alone caused the observed response.

5.4. Antibacterial Activity

Disc diffusion provides a preliminary screen of *in vitro* inhibition, but zone size depends on both biological activity and the ability of constituents to diffuse through agar. Extract concentration, solvent, disc loading, inoculum, medium, incubation conditions, and positive and negative controls are therefore necessary for interpretation. Any future findings should be described as activity under the tested conditions and should not be presented as evidence of clinical efficacy.

5.5. HPLC Record and Quantitative Interpretation

The preliminary HPLC record identifies five target analytes, with ferulic acid having the largest transcribed amount in each extract. However, the internal consistency of the amounts, percentages, and peak-area information has not been established. The apparent difference between the summed reported amounts is too preliminary to support a conclusion about comparative phenolic composition. Confirmatory interpretation requires the original chromatograms, authentic-standard records, calibration equations, dilution calculations, and normalisation to initial dry biomass.

5.6. Overall Significance

The study addresses an under-documented local algal resource and proposes an integrated framework for phytochemical, antioxidant, antibacterial, and chromatographic assessment. Its scientific contribution will depend on transparent reporting and traceable measurements. The present draft should be considered a work in progress rather than evidence that one algal group has superior antioxidant or antibacterial properties.

6. Strengths and Limitations

6.1. Strengths

- The study focuses on two brown-algal groups collected from the same general coastal region.
- The proposed framework combines bulk phytochemical assays, an antioxidant assay, antibacterial screening, and HPLC analysis.
- The draft identifies uncertainties in the supplied data rather than treating unverified values as definitive findings.
- The use of separately processed replicates could support comparison if their biological and technical independence is clarified.

6.2. Limitations

- Verified quantitative results for TPC, TFC, DPPH activity, and antibacterial inhibition were not available for this draft.
- Final extract masses and extraction yields were not supplied.
- Several extraction and analytical conditions require confirmation from original laboratory records.
- The identity of the *Cystoseira* material has not been confirmed at species level in the supplied information.
- The HPLC amounts, percentages, and peak-area values require reconciliation with the original instrument records.
- The number and independence of replicates need clarification; a small sample size may limit statistical power.
- Sampling at one location and on one date limits inference about seasonal and geographic variation.

7. Future Directions

The immediate priority is to verify the laboratory notebook, raw assay readings, calibration curves, extract weights, and original HPLC files. The taxonomic identification should be confirmed and supported by an identification reference and, where available, a voucher specimen. Once the dataset is complete, TPC, TFC, DPPH, and antibacterial results should be calculated using documented procedures and analysed according to the true replicate structure.

Future studies could include additional collection dates and sites, broader chemical profiling, validated quantification of target compounds, concentration-dependent antibacterial assays, and additional

antioxidant assays. Such work could help assess the reproducibility and generalisability of any observed activity, without assuming that in vitro findings translate directly into therapeutic benefit.

8. Conclusion

This study was designed to characterise ethanolic extracts of *Sargassum vulgare* and *Cystoseira* spp. collected from the Al-Hamama coast, northeastern Libya, using phytochemical assays, DPPH radical-scavenging assessment, antibacterial screening, and HPLC analysis. The records supplied for this draft include preliminary sample-weight information and provisional HPLC amounts for five target compounds. However, the quantitative assay dataset is incomplete and the HPLC records require verification. Definitive conclusions about differences in chemical composition, antioxidant potential, or antibacterial activity should therefore be withheld until the original measurements are validated, taxonomic identification is adequately supported, and appropriate statistical analyses are completed.

Declarations

Ethics Approval and Consent to Participate: Not applicable to laboratory analysis of algal material if no human participants or vertebrate animals were involved. Any applicable collection permits or institutional approvals should be reported.

Consent for Publication: Not applicable.

Funding: The authors should identify all funding sources. A statement that no external funding was received should be included only if accurate.

Conflicts of Interest: The authors should disclose relevant financial or non-financial competing interests. A no-conflict statement should be used only if factually correct.

Author Contributions: Contributions to conceptualisation, methodology, sample collection, laboratory investigation, data analysis, and manuscript preparation should be assigned according to each author's actual work.

Data Availability: The availability of raw laboratory measurements, calibration data, chromatograms, and supporting records should be stated accurately. Data may be deposited in an appropriate repository or made available upon reasonable request if this reflects the authors' actual arrangements.

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Use of Artificial Intelligence: No

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