

Preliminary Phycochemical and Physicochemical Evaluation of Red Seaweed *Coelarthrum Muelleri*

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Abstract: This study aimed to screen different solvents (ethanol, ethyl acetate, and n-hexane) of *Coelarthrum muelleri* for secondary metabolites and subject them to physicochemical analysis, including ash, swelling index, pH, and color tests with different reagents. Seaweeds have been recognized in the pharmaceutical industry for their broad spectrum of structural diversity and wide range of pharmacological activities for health and disease management. *Coelarthrum muelleri* from red algae was taken as a sample for this study. It was collected from the coastal range of Buleji and Kakapir beaches in Karachi, Pakistan. Three extracts of *Coelarthrum muelleri* (ethanol, ethyl acetate, and n-hexane), depending on their polarity, were subjected to phycochemicals of ten chemical compounds (glycosides, quinones, phenols, coumarins, steroids, alkaloids, saponins, flavonoids, terpenoids, and tannins) and physicochemical test for ash value, pH value, and swelling index. The dried powder of *Coelarthrum muelleri* was tested for color identification with eight other reagents. The preliminary phycochemical screening of *Coelarthrum muelleri* showed the presence of terpenoids, steroids, tannins, saponins, phenols, coumarins, glycosides, and quinones and the absence of alkaloids. Among three extracts, ethanol and ethyl acetate extracts show all compounds present except alkaloids. Next, n-hexane showed all compounds positive except for alkaloids, saponins, and quinones, which were absent. The physicochemical indicators are 38 ml of the swelling index, 2.3 pH of 1% *Coelarthrum muelleri*, and 3.16 pH of 10%. The total ash value is $24.86 \pm 0.15\%$, $8.4 \pm 0.1\%$ for acid insoluble ash, and $7.1 \pm 0.1\%$ for water-soluble ash. This study on the present seaweed will be helpful in the identification of secondary metabolites as well as be valuable for authenticating the purity and quality.

Keywords: adulteration, secondary metabolites, phycochemicals, seaweed, *Coelarthrum muelleri*.

红海藻腔肠菌的初步理化和理化评价

摘要: 本研究旨在筛选腔肠菌的不同溶剂 (乙醇、乙酸乙酯和正己烷) 中的次生代谢物, 并对它们进行物理化学分析, 包括灰分、膨胀指数、酸碱度和不同试剂的颜色测试。海藻因其广泛的结构多样性和广泛的健康和疾病管理药理活性而在制药工业中得到认可。来自红藻的腔肠菌作为本研究的样本。它是从巴基斯坦卡拉奇的Buleji和卡卡皮尔海滩的沿海地区收集的。根据其极性, 对腔肠菌的三种提取物 (乙醇、乙酸乙酯和正己烷) 进行了10种化合物 (苷类、醌类、酚类、香豆素、类固醇、生物碱、皂苷、类黄酮、萜类和单宁) 和灰分值、酸碱度值和溶胀指数的

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物理化学测试。用其他八种试剂对腔肠菌的干粉进行颜色识别测试。腔肠菌的初步物理化学筛选显示存在萜类化合物、类固醇、单宁、皂苷、酚类、香豆素、苷类和醌类，并且不存在生物碱。在三种提取物中，乙醇和乙酸乙酯提取物显示除生物碱外的所有化合物。接下来，正己烷显示除生物碱、皂苷和醌以外的所有化合物均为阳性，这些化合物不存在。理化指标为溶胀指数38毫升，1%腔肠菌酸碱度2.3，10%

3.16酸碱度。总灰分值为24.86±0.15%，酸不溶性灰分8.4±0.1%，水溶性灰分7.1±0.1%。本研究对海藻的次生代谢物鉴定有一定的帮助，对鉴定其纯度和质量具有重要价值。

关键词：掺假、次生代谢物、藻类化学物质、海藻、腔肠菌。

1. Introduction

Marine habitats are the chief source of several biological compounds that are pharmacologically active, and it is only due to this action that their needs are increasing day by day. The algae have different features from higher plants because they do not possess stems, leaves, or true roots [1]. Nowadays, they are used as the source of raw materials for many industrial products like agar and carrageenan, and they are widely consumed in the diet in many Asian countries. Edible seaweeds are also called sea vegetables [2]. Agar and seaweed carrageenan are used in the food industries as thickening, condensing, and gelatin agents and are extensively used as growth media for microorganisms [3]. Seaweeds are macroalgae. They normally exist in coastal areas and can be of many forms, dimensions, colors, and compositions. Mainly, seaweeds are of three types: brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae) [4].

Karachi, on the northern edge of the Arabian Sea, has a 100-kilometer coastline with several beaches, islands, and mangrove swamps. Manora, Hawkes Bay, Sandspit, Buleji, Paradise Point, and the surrounding coastal belt clear water with various marine species may be found near Cape Monze. Seaweeds grow abundantly on these beaches, either as drift, clinging to rocks, or growing in pools [5]. *Coelarthrum muelleri* (Sonder) was discovered by Børgesen in 1931 [17]. *Coelarthrum* has eight species worldwide. Rhodophyta has more than 7000 documented classes with ongoing taxonomic revisions, which embrace the predominant algae phyla [3]. *Coelarthrum muelleri* red seaweed or algae is erect, bright red, and 15 cm in height, attached by solid, creeping, cylindrical stolons, 2 to 3 mm in diameter, thallus body with di- and trichotomously branched [6].

Table 1 Taxonomical classification

Order	Rhodymeniales
Family	Rhodymeniaceae

Continuation of Table 1

Genus	<i>Coelarthrum</i>
Species	<i>Coelarthrum muelleri</i>

Seaweeds are a rich source of structurally diverse primary and secondary metabolites such as lectins, peptides, carotenoids, polysaccharides, fatty acids, flavonoids, and phytosterols, which have a high potential for use in the food, cosmetic, and pharmaceutical industries, distinguishing them from terrestrial plants [7, 8].

Seaweeds also have many minerals and trace elements because they absorb inorganic marine materials. These minerals build up in seaweeds at a rapid rate. It may account for up to 36% of dry matter at higher levels in certain types of seaweed. The secondary metabolite of seaweed is recognized to provide many health advantages, including a decrease in coronary heart disease and anticarcinogenic and anti-inflammatory properties. Despite their diversity, research into the discovery of novel bioactive chemicals from seaweeds is an open field of study [8].

2. Material and Method

2.1. Study Area

The collection site was the coastal range of Buleji, Kakapir beaches in Karachi, Pakistan (Fig. 1).



Fig. 1 *Coelarthrum muelleri* collection site

2.2. Collection and Preparation of *Coelarthrum Muelleri*

Red algae specie *Coelarthrum muelleri* (Sonder) Borgesen (Fig. 2) was collected by hand from Kakapir and Buleji beaches, coastal areas of Karachi, Pakistan, and identified and authenticated from a herbarium sheet at the Centre of Excellence in Marine Biology (CEMB), University of Karachi, with voucher number CYM-03.



Fig. 2 Red algae (*Coelarthrum muelleri*)

The seaweed was washed with seawater at the collection site for any adhered sediments and impurities. Then, it was packed and brought to the laboratory in polyethylene bags for further study.

Seaweed was then washed with tap water to remove all salt impurities on the surface of the algae. For drying, the seaweed was spread on blotting paper for 48 hours. Then, the seaweed was kept in an oven for 4 hours and ground to reduce its particle size to 2 mm. Three solvents were used according to their polarity. For polar and non-polar ethanol, semi-polar ethyl acetate and n-hexane were used, respectively. The algal powder was soaked in an air-tight conical flask at a ratio of 1:5 for 15 days with occasional shaking on an electronic shaker. After that, it was filtered with Whatman No. 1 filter paper. The filtrate was then moved to a Rotary evaporator (RV 8V IKA) for further removal of solvents.

2.3. Chemicals Required

Distilled water, 0.1N NaOH, 0.2N NaOH, concentrated HCl, 0.2% HCl, H₂SO₄, 0.1% FeCl₃, concentrated nitric acid, and 1% acetic acid, chloroform, Mayer's reagent, 2N sodium hydroxide, 10% sodium hydroxide and 10% ammonium solution.

2.4. Physicochemical Standardization

2–4 g of dried powder was sieved using a 40 mesh size and ash values, which include total ash, insoluble acid ash, water-soluble ash, pH determination, color

reaction with different reagents, and swelling index.

2.4.1. Determination of Ash Values

By three methods, ash values were calculated, which included total ash, insoluble acid ash, and water-soluble ash.

2.4.2. Total Ash

The crude drug was air-dried as 2–4 g were weighted in a silica dish, then incinerated at 4500°C and waited until it was free from carbon. For a half-hour, the residue was allowed to cool in a desiccator and weighed. The total ash value percentage was calculated as follows [9]:

$$\text{Total ash \%} = \frac{\text{Weight of ash obtained}}{\text{Weight of air - dried sample}} \times 100$$

2.4.3. Acid-Insoluble Ash

In 25 ml of 2M HCl, the ash was boiled for 5 minutes and filtered with the help of filter paper. The unsolved material was then washed with boiling water, burnt, cooled in a desiccator, and finally weighed. The value of acid-insoluble ash was evaluated [9].

2.4.4. Water-Soluble Ash

Ash was boiled in 25 ml of water for 5 minutes. With the help of filter paper, insoluble ash was separated and washed with boiling water. Then it was burnt for 20 minutes, cooled in a desiccator, and weighed. The percentage of the water-soluble ash was calculated [9].

2.4.5. Determination of 1% pH

In 100 ml of distilled water, 1 g of powder accurately weighed was dissolved. The sample was then filtered, and pH was determined using the table-top digital pH meter [9].

2.4.6. Determination of 10% pH

In 100 ml of distilled water, 10 g of powder was accurately weighed and dissolved. The sample was filtered, and pH was determined using a table-top digital pH meter [9].

2.4.7. Determination of the Swelling Index

1 g of fine powdered dried *Coelarthrum muelleri* was weighed and transferred into 25 ml of distilled water in a measuring cylinder and was shaken thoroughly every 10 minutes. Shaking lasts one hour, followed by three hours at room temperature. The mean value was calculated [9].

2.4.8. Powder Drug Reaction with Different Reagents

Dried powder of *Coelarthrum muelleri* was treated individually with different reagents, including 0.1N NaOH, 0.2N NaOH, concentrated HCl, 0.2% HCl, H₂SO₄, 0.1% FeCl₃, concentrated nitric acid, and 1%

acetic acid. The color detection was noted visually [9].

2.5. Preliminary Phycochemical Analysis

Phycochemical analysis was done by the standard method given by [10]. This test was carried out to identify the chemical groups naturally present in selected seaweed, such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides. These extracts' general reactions will help us identify the presence or absence of compounds.

2.5.1. Test for Alkaloids

The required quantity of hydrochloric acid in concentrated form was added to a few drops of the algal sample with Mayer's reagent (a few drops). The existence of alkaloids was indicated by the white or green precipitates.

2.5.2. Test for Terpenoids

The required quantity of chloroform with concentrated sulfuric acid was added to the required quantity of sample (0.5 ml). The presence of a reddish-brown color within the mixture indicates the existence of a terpenoid.

2.5.3. Test for Steroids

The required amount of chloroform was added with 1 ml of sulphuric acid, and then 0.5 ml of sample was added. A brown ring that appears within the mixture indicates the presence of steroids.

2.5.4. Test for Tannins

Ferric chloride was added to the algal sample. Greenish black or dark blue coloration appears to indicate the presence of tannins within the mixture.

2.5.5. Test for Saponins

The sample was added to 2 ml of DW (distilled water) in a graduated cylinder and vigorously mixed for 15 minutes. Foam appears within the mixture and indicates the existence of saponins.

2.5.6. Test for Flavonoids

The required amount of 2N sodium hydroxide was added to the algal sample (2 ml). The existence of flavonoids is indicated by the yellow color formation within the mixture.

2.5.7. Test for Phenols

2 ml of DW (distilled water) with ferric chloride (a few drops) was added to the sample. A green/blue color within the mixture showed the presence of phenol.

2.5.8. Test for Coumarins

Required quantity of 10% sodium hydroxide added to 1 ml of the sample. A yellow color appears within the mixture, indicating that coumarins are present.

2.5.9. Test for Quinones

Required quantity of concentrated sulphuric acid added to 1 ml of sample. The red color appears within the mixture, indicating the existence of quinones.

2.5.10. Test for Glycosides

The required quantity (3 ml) of chloroform was added to a 10% ammonium solution with 2 ml of the sample. The pink color appeared to show the presence of glycosides within the mixture.

3. Results

The total ash value of the sample indicated the amount of minerals and earthy material attached to the seaweed and was calculated to be 23.59 ± 1.28 w/w. The amount of the acid-insoluble siliceous matter present was 8.4 ± 0.1 w/w. The water-soluble ash value indicated the presence of sugar, acids, and inorganic compounds; the result was 7.1 ± 0.1 (Table 2).

Table 2 Physicochemical characterization of *Coelarthrum muelleri*

Mean pH value	pH 1% solution	pH 10% solution	
	2.3	3.16	
Swelling index	38 ml		
Ash values	Total ash %	Acid-insoluble ash %	Water-soluble ash %
	23.59 ± 1.28	8.4 ± 0.1	7.1 ± 0.1

The dried powdered sample was tested with eight different reagents (Table 3). This will be beneficial for the identification of *C. muelleri*. When the powder was reacted with 0.1N NaOH, it turned white. With 0.2N NaOH, it turned slightly yellow. With 1% acetic acid, no color change was observed. When the dried powder was reacted with 0.2% HCl and concentrated nitric acid, a slightly yellow color was observed. A dark green color was seen when the dried sample reacted with concentrated HCl, whereas H₂SO₄ turned the dried sample black. A rusty appearance was seen when the sample was reacted with 0.1% FeCl₃.

Table 3 Powdered drug reaction with different reagents

Reagents	Inferences
0.1N NaOH	White
0.2N NaOH	Slight yellow
Conc: HCl	Dark green
0.2% HCl	Slight yellow
H ₂ SO ₄	Black
0.1% FeCl ₃	Rusty appearance
Conc: Nitric acid	Slight yellow
1% acetic acid	White

Preliminary phytochemical analysis was performed on red algae (*Coelarthrum muelleri*) with three extracts (ethanol, ethyl acetate, and n-hexane) and ten chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides). Ethyl acetate showed a more significant result. Alkaloids did not show any positive results in any of the extracts (Table 4).

Table 4 Phytochemical screening of red algae (*Coelarthrum muelleri*)

Test	Ethanol	Ethyl acetate	n-hexane
Alkaloids	-	-	-
Terpenoids	*	*	**
Steroids	*	***	*
Tannins	*	*	*
Saponins	***	*	-
Glycosides	***	***	***
Phenols	***	***	***
Coumarins	***	***	*
Quinones	**	***	-
Flavonoids	***	***	***

Notes: *** abundant quantity, ** moderate, * traces, - absent

We can easily see in the above table that traces of terpenoids were found in ethanol and ethyl acetate and moderate in n-hexane extract. Steroids were present in abundant quantities in ethyl acetate, and only traces were found in ethanol and n-hexane. For tannins, only traces were found in all three extracts. Saponins were found in abundant quantity in ethanol extract, only traces in ethyl acetate, and absent in n-hexane extract. Flavonoids, phenols, and glycosides showed good results and were present in abundant quantity in all three extracts. Coumarins were present in abundant quantities in ethanol and ethyl acetate, and only traces were found in n-hexane. Quinones were present in abundant quantities only in ethyl acetate, moderate in ethanol, and were absent in n-hexane extract.

If we compare all three extracts, ethyl acetate showed good results as they had abundant amounts of steroids, flavonoids, phenols, coumarins, quinones, and glycosides.

4. Discussion

Marine creatures have the potential to be a rich source of highly bioactive secondary metabolites, which could lead to the development of novel pharmacological medicines. Compared to other algal groups, red algae are thought to be the most significant source of many physiologically active compounds. Man uses seaweed in various applications [11]. Besides health advantages, marine seaweeds are utilized in many sectors, including medicines, nutraceuticals, cattle feed, fertilizer, cosmetics, and fuel. As a result, to assure the validity of any seaweed for biological applications, it is important to

analyze the quality and standardization of algae. Such investigations should be carried out before examining any other activity the World Health Organization advises [12].

Physicochemical studies may be an invaluable source of information and are often used to determine the purity and quality of a drug. The ash value is particularly important for detecting the kind of adulterant introduced to the medicine for adulteration and determining the test sample's impurities, authenticity, quality, and purity [13]. Generally, the ash value measures the number of inorganic salts in the drug sample and the residue left after cremation. The total ash content of the algae sample shows the presence of mineral content, salts, and some essential trace elements needed in human nutrition. The acid-insoluble ash test may be used to assess the presence of silica or calcium oxalate in the crude medication. The swelling index indicates the presence of mucilage, pectin, and hemicellulose [14].

The extraction yield is significantly dependent on the extraction technique and solvent used. Preliminary phytochemical screening of *Coelarthrum muelleri* extracts revealed the presence of flavonoids, tannins, saponins, phenolic compounds, terpenoids, steroids, glycosides, coumarins, and quinones in the three extracts of *Coelarthrum muelleri* and the absence of alkaloid. Among all the three extracts, ethyl acetate and ethanol extract showed a significant amount of secondary metabolites.

A literature survey has proved that the highest amount of flavonoids and phenols existed in *Coelarthrum muelleri* with different solvents [5]. However, in this study, flavonoids and phenols were also present in all three extracts. Marine phenols are usually more powerful than phenols from terrestrial plants because they have a wide range of chemical structures. Phenols are responsible for various biological and physiological functions, including antioxidant, anti-mutagenic, anticarcinogenic, and anti-inflammatory [15]. Flavonoids possess antioxidant properties and may be employed in foods containing fats to preserve them and prevent the production of harmful molecules induced by lipid oxidation. Immunomodulatory, anti-inflammatory, antibacterial, antiviral, anticancer, and antithrombotic actions are also biological activities of flavonoids [16].

In our result, only an abundant quantity of saponin was identified in ethanol, whereas traces were found in ethyl acetate, and hexane has not shown saponins. Compared to other studies, this one has shown that marine-derived saponins, particularly those derived from Rhodophyta species, are a rich source of agar and carrageenan, both of which are responsible for gel formation and interference in analyses. Compared to terrestrial saponins, seaweed-derived saponins are still

untouched in terms of biological activities and quantitative and qualitative chemical analyses. As a result, the prospects for discovering new therapeutic leads from marine-derived saponins are increasing [5]. Only traces of tannins were found in each extract. Other studies have shown that tannin has anticarcinogenic, antimicrobial, and antioxidant properties.

These extracts highlighted the presence of steroids, coumarins, quinones, glycosides, and terpenoids reported for the first time in *Coelarthrum muelleri*. The seaweed might possess anticoagulant activity due to the presence of coumarin. Terpenoids in seaweeds have a broad range of nematocidal, cytotoxic, and antitumor activities. Seaweed steroid has been shown to have insecticidal, antiparasitic, antibacterial, and cardiogenic effects. These extracts are being studied further to isolate, identify, and elucidate the structure of the bioactive chemicals that are responsible for their pharmacological action.

5. Conclusion

Seaweeds are growing in popularity among scientists due to their bioactive components and qualities such as antiviral, antitumor, anti-inflammatory, and anti-lipidemic. Identifying such potential seaweed is crucial in medicine. So it becomes necessary to study the phycochemical and physicochemical characteristics before their use in research and pharmaceutical formulation. It also aids in the differentiation from other related species and adulterants. From the present study, it can be concluded that biologically active phycochemicals were present in the ethyl acetate and ethanol extracts of red seaweed (*Coelarthrum muelleri*). Steroids, coumarins, quinones, glycosides, and terpenoids were reported for the first time in this specie. In other words, the findings established the existence of a therapeutically active compound in (*Coelarthrum muelleri*). Standardization of novel medicinal seaweeds is accomplished by chemical examination and comparison. Further investigations are still required to ensure and isolate the secondary metabolites that will be helpful in various fields, including pharmacy, agriculture, and nutraceuticals.

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