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**Research Article****Husn-e- Yousuf (*Laminarea saccharina*); the most trending and promising natural skin care of current decade chemical characterization and antioxidant evaluation****Kiran Rafiq¹, Fatima Qamar², Safila Naveed³, Saman Usmani¹, Shafaque Mehboob¹, ShaguftaNesar⁴, Javeria Iftikhar¹, Zafar Saied Saify⁵**¹Institute of Pharmaceutical Chemistry, Jinnah Sindh Medical University, Karachi, Pakistan.²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnah University for Women, Pakistan.³Department of Pharmaceutical Chemistry, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan.⁴Jinnah College of Pharmacy, Sohail University, Karachi, Pakistan.⁵International Center of Chemical & Biological Sciences, University of Karachi, Pakistan.**ABSTRACT**

Today in the world of competition and personal grooming the skin tone matters a lot and such products are highly acceptable that accounts for the shining of skin. In this regards, the rate of skin fairness cosmetics consumption is very high in our society. However, the high composition of chemical moieties in such products is responsible for the damage of skin layers. In such circumstances the natural and herbal products can be a better choice for preventing the skin from hazardous chemicals and for gaining natural glow. Consequently, one herb is found significantly promising during study, commonly known as Husn-e-Yousuf (*Laminarea saccharina*). In present study, through chemical analysis it was determined that the herb has highly beneficial chemical composition that are friendly for skin texture and tone. Beside that strong antioxidant and radical scavenging activity proved Husn-e-Yousuf a promising skin care and an excellent choice for skin care.

Keywords: Husn-e-Yousuf; *Laminarea saccharina*; sugar kelp; acne; exfoliation.**Correspondence**

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Article History

Received: July 21, 2025

Accepted: August 22, 2025

Published: August,31, 2025

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INTRODUCTION

The objective of the present study was to chemically characterize *Laminaria saccharina* through GC-MS analysis and evaluate its antioxidant potential (using DPPH and ABTS assays) to explore its applicability as a natural and safe alternative for cosmaceutical formulations.

Among some conventional theories of beauty, the one reveals the included the belief that "beauty is in the eye of contemplate". Consequently, to make self-appearance better and attractive, the multibillion-dollar skin care product industry is under fire for promoting ideas and for the trade of such attractive products as like whitening creams, that are topical products designed to lighten the skin tone and reduce the hyperpigmentation. All beside the beautifying facts, some whitening creams may contain potentially harmful ingredients like hydroquinone, mercury, or steroids (Chopra, Tsagkaris, Matthews, Gautam, & Kamal, 2023; Hernandez, Cervantes, Luna-Vital, & Mojica, 2021; Kanlayavattanakul & Lourith, 2018). The detrimental consequences of adverse effects and reported dangers steadily steered the world towards natural resource adaptation that trend quickly acquired acceptance since a growing understanding of the potential and harmful after effects and limitations of synthetic medications, a desire for more sustainable and environmentally friendly alternatives. Among such natural herbs, Husn-e-Yusuf (*Saccharina latissima*),

known as *Laminaria saccharina* has been trending actively in the cosmetic industry in recent years (Chandra Pal, Marchiony Hunt, Diamond, A. Elmetts, & Afaq, 2016; Hu et al., 2020). It is also marketed by the names of Sugar Kelp, kombu, or Kirmali in local markets. It is obtained from the brown algae, Phaeophyceae, having high nutritional value and potential health advantages found in the rocky northern shores of the Pacific and Atlantic Ocean. By the look of this widely spread brown algae, it is also called The Devil's Apron (Burgunter-Delamare et al., 2023; Domonkos, Kis, Gombos, & Ughy, 2013; Wiese, Thiel, Nagel, Staufenberg, & Imhoff, 2009). Due to having deep moisturizing, skin nourishing and brightening effects it is being used as an active ingredient in many topical products. Some of the key chemical constituents found includes Laminarin, a polysaccharide, an important constituent of algae cell wall. The molecule potential immunomodulatory, antioxidant and prebiotic effects (Aroz Toma et al., 2023; Bonfim-Mendonca, Capoci, Tobaldini-Valerio, Negri, & Svidzinski, 2017; Neto et al., 2018; Ozanne et al., 2020). It has essential amino acids that are required for human health and tissue growth. Beside the plant has vitamin C, vitamin E, various B vitamins, and folate that make it a favorable for moisturizing and sun protection (Sapatinha et al., 2022). As the vitamin C is a potent antioxidant that helps protect the skin against free radical damage caused by environmental factors such as UV radiation and pollution, maintain the skin's firmness, elasticity, fade dark spots and brighten the skin. Vitamin E protects the skin from oxidative stress and damage. It works in moisturization by reducing water loss and strengthening the natural barrier. It has anti-inflammatory properties and soothe and skin irritation (Barbosa, Valentão, & Andrade, 2020; Elbandy, 2022; Zheng, Zhao, & Guo, 2022).

Multiple Vitamin B molecules provide numerous benefits for the skin as improve the skin's moisture barrier, minimize inflammation, and regulates sebum production. Also even out skin tone, and enhance the skin's natural radiance. Vitamin B5 specifically work as humectant as it helps to hydrate irritated skin, and promotes a healthy skin barrier.

Sugar Kelp is also bunted with numerous minerals and due to the fact, the herb plays a significant role in maintaining healthy skin and are commonly utilized in skincare products. Among these, Zinc is an essential mineral that helps regulate oil production, copper is involved in the production of collagen and elastin, magnesium plays a role in various skin functions, Selenium is an antioxidant mineral that provides prevention from free radicals and oxidative damage, Silica contributes to the structural integrity of the skin, iron is essential for oxygen transport, and calcium is The herb contains fucoidan and fucoxanthin, are bioactive compounds (Buschmann et al., 2017; Dörschmann et al., 2019; Ruocco, Costantini, Guariniello, & Costantini, 2016; Silva et al., 2021). Fucoidan is a complex sulfated polysaccharide molecule, have potential health benefits, present in the composition of brown seaweeds. It has also gained attention in skincare due to its potential effects on the skin. Fucoxanthin is a natural carotenoid pigment found in brown seaweeds and has potential benefits for having anti-inflammatory effects on skin. It prominently exhibits antioxidant properties and has been studied for its potential benefits in promoting skin health and protecting against photoaging caused by UV radiation (Ayoub et al., 2015; Kadam, Tiwari, & O'Donnell, 2015). The herb has high concentration of skin-effective alginates, is large polysaccharide polymers having hydrophilic nature, as do not penetrate the lipid layer and form a thin protective layer on skin with a unique calming effect. The Husn-e-Yusuf herb also contains Laminarin, a polysaccharide molecule that serves as a lipid-reducing agent with decongestant characteristics and is most effective for acne-prone skin types. Additionally, the presence of phenolic molecules that makes it strengthful for the trapping of harmful heavy metal ions and it protects sensitive skin from toxins and other pollutants in the environment (Miao, Ishai-Michaeli, Peretz, & Vlodavsky, 1995).

Linoleate is one of the most essential components of Husn-e-Yusuf, which is used to conceal flaws such as pimples and blackheads. It is a vitamin F necessary fatty acid that is utilized in many cosmetics for its anti-inflammatory effects. The compound is beneficial to the skin because it gives it a supple bounce and antioxidant protection. It helps to maintain and restore the skin barrier by increasing moisture levels and promoting skin suppleness and smoothness. It is the primary defender of the skin barrier because it strengthens cell membranes. It also helps to protect your skin by reducing inflammation and aids in shielding your skin from damaging elements like pollutants and UV rays (Pangestuti, Shin, & Kim, 2021).

Husn-e-Yusuf herb, derived from *L. saccharina*, is used in cosmaceutical formulations like topical facial creams. Its emulsification process creates a stable emulsion that brightens the skin, reduces wrinkles, and has skin revitalizing properties. The herb is also available in dried powder, which can be mixed with rose water or milk for a thick paste. (Jesumani, Du, Pei, Aslam, & Huang, 2020).

L. saccharina is also used to formulate a facial mask used for exfoliation and promoting the dull skin complexion. The facial mask is particularly good for removing impurities and enhancing the texture of skin. The polysaccharide alginates present in Husn-e Yusuf facial mask produces a deep nourishing effect that soothes the facial skin while the phenolic

part is responsible for fighting against the harmful toxins that make the skin look aged and wrinkled. This way, Husn e Yusuf has an anti-aging factor that makes the ingredient popular among the public. It acts as a cleaner which also has unique antibacterial and anti-inflammatory properties. The facial mask promotes skin shedding, unclogging pores, and removing excess oil, resulting in smoother, brighter skin. Its effectiveness can be enhanced with antioxidant agents for tightening and reducing wrinkles. (Ale, Maruyama, Tamauchi, Mikkelsen, & Meyer, 2011).

Exfoliation by phenolic groups removes toxic impurities, making the facial skin sensitive to environmental impact. Aginates create a protective layer, while laminarin eliminates oil for acne. This results in a hydrated, smooth complexion, radiance, and youthful elegance. (Wang, Jayawardena, Yang, Lee, & Jeon, 2020).

MATERIALS AND METHODS

Collection of Sample and Extraction

Dry seeds of *Laminaria saccharina* were washed, further air dried, grinded to powder and sequentially extracted with hexane, and methanol, twice at room temperature for three days. The extracts were filtered and evaporated under reduce pressure using rotary evaporator to obtain a concentrate masses of LS-H and LS-M. LS-H was analyzed through GC-FID and GC-MS spectroscopy.

GCMS

Chromatographic Condition

Chemical profiling was done using gas chromatography-flame ionization detection (GC-FID) a Shimadzu GC-17 A equipped with a capillary column SPB-5® with dimensions (30 m × 0.25 mm ID and 0.25 µm). Carrier gas helium with a flow rate of 1 mL/min was used. The oven was kept initially at 70 °C for 5 min, programmed to 260 °C at a rate of 5 °C/min and kept isothermal for 30 min. Temperature and splitting ratio of injector was set to 280 °C and 1:30, respectively. For GC-MS, Hewlett-Packard 5890 (Bunker Lake Blvd, Ramsey, MN) gas chromatograph coupled to Jeol JMS-600 mass spectrometer and equipped with ZB-5MS® capillary column (30 m × 0.25 mm ID and 0.25 µm df) was used, keeping analytical conditions similar as described for GC-FID. EI ion source was kept at 250 °C and 70 eV.

Total Phenolic Content

By making minor adjustments to the folin-ciocalteau spectrophotometric technique, the total phenolic content of LS-H and LS-M was determined (Idris, Wintola, & Afolayan, 2019; Javanmardi, Stushnoff, Locke, & Vivanco, 2003; Qamar, Sana, Naveed, & Faizi, 2023). The standard curve was plotted using a concentration range of 4 to 40 g/ml with gallic acid as the reference. One millilitre of the sample was obtained, and 2.5 milliliters each of the folin-Ciocalteau reagent and 7.5% Na₂CO₃ were then added. The absorbance at 740 nm was measured in comparison to a reagent blank after 30 minutes of incubation at room temperature and in the dark. Gallic acid equivalents were used to represent the results.

Total Flavonoid Content

For the purpose of determining flavonoids, an aluminium chloride colorimetric approach was employed (Avoseh, Mtunzi, Ogunwande, Ascrizzi, & Guido, 2021; Sellimi et al., 2018). 0.1 ml of aluminium chloride 10% and 0.1 ml of 1 M potassium acetate were added to 1 ml of sample and held for 5 minutes. The reaction mixture's absorbance at 415 nm was measured after the mixture stood in the dark at room temperature for 5 minutes. Rutin equivalent measurements were made for the total flavonoid content.

DPPH Colorimetric Assay

With a few minor modifications, the method of Fatima et al. (2023) was used to measure the DPPH radical scavenging activity (Qamar et al., 2023). In separate test tubes, extract samples (ranging from 0.1 to 4 mg/ml) were collected, and 3.5ml of DPPH solution was added. For 30 minutes, the reaction mixtures were incubated in the dark. Each sample's 517 nm absorbance was measured using methanol as a blank. All of the observations were made in triplicate and the ascorbic acid calibration curve was generated. Ascorbic Acid Equivalent Antioxidant Capacity (AAE) g/100g of dry extract was used to express the results.

The antioxidant activity of the extracts was performed, percentage inhibition was calculated and results were comprehended as IC₅₀.

ABTS Cation De-Colorization Assay

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation de-colorization test technique, as published by Fatima et al. in 2023 with minor variations (Lichterfeld et al., 2015), was used to measure antioxidant activity. ABTS solution and potassium per sulfate was mixed at room temperature and the mixture was Potassium per sulphate and ABTS solution were combined at room temperature, and the mixture was incubated for 16 hours in the dark at room

temperature to create a dark-green solution. Several quantities of extract solutions were made, and 3.5ml of the extract solution was combined with 3.5ml of the ABTS solution before being incubated for 6 minutes. Thereafter, readings were taken in triplicates at 743nm to record the absorbance. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) g/100g of dry extract using trolox as a reference.

Composition and Characterization

Laminaria saccharina extract showed various well-known compounds using GCMS technique on comparing with NIST database. The observed compounds belong to diverse classes of chemical compounds as shown in Table-1. Compound with significant abundance includes fatty acid, fatty acid esters, hydrocarbons and hydrocarbons derivatives.

The extract showed the presence of twenty-one (21) compounds out of which 16 compounds were identified while 5 of them remain unidentified. Higher percentages of fatty acid esters were identified. Compounds Methyl 8-methyl-nonanoate (1), Methyl 10-methylundecanoate (4), Methyl myristylate (6), Methyl palmitate (9), Methyl linoleate (11), Methyl 11-octadecenoate (12), Methyl stearate (13) were the prominent fatty acid esters. Fatty acids such as Palmitic acid (10), Linoleic acid (14), cis-Vaccenic acid (15) and Stearic acid (16) were also observed. The only hydrocarbon that was identified during the study was Tetradecane (2). Compounds 2- methyl-tetradecane (3), 2,2-Dimethyltetradecane (5), and 2,6,10-Trimethyltetradecane (7) were the hydrocarbon derivatives. Interestingly (16) were also observed. The only hydrocarbon that was identified during the study was Tetradecane (2). Compounds 2- methyl-tetradecane (3), 2,2-Dimethyltetradecane (5), and 2,6,10-Trimethyltetradecane (7) were the hydrocarbon derivatives.

RESULTS AND DISCUSSION

The GC-MS analysis revealed twenty-one compounds in *Laminaria saccharina*, with fatty acid esters being the most abundant class (notably methyl palmitate, methyl linoleate, and methyl stearate). The antioxidant assays demonstrated that LS-M extract had superior radical scavenging activity (IC₅₀ = 1.68 mg/ml, 86% inhibition) compared to LS-H (IC₅₀ = 2.5 mg/ml, 68% inhibition). This indicates a higher antioxidant potency in methanolic extract, making it more suitable for cosmetic formulations. The strong antioxidant values further validate the protective role of *Laminaria saccharina* against oxidative skin damage, supporting its objective as a natural cosmeceutical candidate.

The present study is designed to identify the significant constituents that account for the outstanding skin care of the herb and for the purpose the plant extract was examined through GCMS to identify the composition of compounds and the method worked to analyze the Phenolic and Flavonoid content.

In cosmetology, *Laminaria saccharina* has diverse applications. Its extracts are incorporated into anti-aging creams, sunscreens, and moisturizers owing to their ability to enhance skin hydration and elasticity. Polysaccharide-rich fractions are used in exfoliating masks, improving cell turnover and reducing hyperpigmentation. Fucoidan-based

Table1. Compounds identified by GC/MS in *Laminaria saccharina* extract.

S. No	Compound	MF	RT	RI L	% Match	Class of Compounds
1	Unidentified	-	9.861	-	-	-
2	Methyl 8-methyl-nonanoate (1)	C ₁₁ H ₂₂ O ₂	12.149	-	86	Fatty acid esters
3	Tetradecane (2)	C ₁₄ H ₃₀	13.197	236.21	92	Hydrocarbon
4	2- methyl-tetradecane (3)	C ₁₅ H ₃₂	14.482	1467	80	Hydrocarbons derivative
5	Methyl 10-methylundecanoate (4)	C ₁₃ H ₂₆ O ₂	14.777	-	86	Fatty acid esters
6	Unidentified	C ₂₇ H ₄₈ O	15.448	3115	-	-
7	2,2-Dimethyltetradecane (5)	C ₁₆ H ₃₄	15.678	1515	78	Hydrocarbons derivative
8	Methyl myristylate (6)	C ₁₅ H ₃₀ O ₂	17.082	1706	90	Fatty acid ester
9	2,6,10-Trimethyltetradecane (7)	C ₁₇ H ₃₆	17.872	1555	80	Hydrocarbon derivative
10	Pentadecylphenol (8)	C ₂₁ H ₃₆ O	18.579	2499	77	Phenol

11	Methyl palmitate (9)	C ₁₇ H ₃₄ O ₂	19.143	1921	94	Fatty acid ester
12	Palmitic acid (10)	C ₁₆ H ₃₂ O ₂	19.502	1970	92	Fatty acid
13	Unidentified	-	20.525	-	-	-
14	Methyl linoleate (11)	C ₁₉ H ₃₄ O ₂	20.74	2096	94	Fatty acid ester
15	Methyl 11-octadecenoate (12)	C ₁₉ H ₃₆ O ₂	20.809	-	95	Fatty acid ester
16	Methyl stearate (13)	C ₁₉ H ₃₈ O ₂	21.031	2128	93	Fatty acid ester
17	Linoleic acid (14)	C ₁₈ H ₃₂ O ₂	21.117	2173	92	Fatty acid
18	<i>cis</i> -Vaccenic acid (Asclepic acid) (15)	C ₁₈ H ₃₄ O ₂	21.168	-	93	Fatty acid
19	Stearic acid (16)	C ₁₈ H ₃₆ O ₂	21.351	2170	89	Fatty acid
20	Unidentified	-	22.633	-	-	-
21	Unidentified	-	22.636	-	-	-

Table 2. DPPH colorimetric assay and ABTS cation decolorization assay.

Extracts	DPPH scavenging activity	IC ₅₀ (mg/ml)	AA % 100ug/ml	TEAC (mM/100g)
LS-H	56 %± 0.03	2.5 ± 0.05	68%± 1.3	32.36 ± 0.05
LS-M	72%± 0.25	1.68 ± 0.08	86%± 0.2	68.38 ± 0.08

formulations are being developed in cosmeceuticals for their anti-wrinkle, skin-brightening, and anti-inflammatory properties. Moreover, the herb's natural UV-protective effects make it suitable for photoprotective cosmetic products, further supporting its role as a valuable natural ingredient in modern skincare.

It was observed that the plant has different fatty acid, fatty acid esters, hydrocarbons and hydrocarbons derivatives (table-1). Among the fatty acid esters, Methyl palmitate, Methyl linoleate, Methyl 11-octadecenoate and Methyl stearate were the prominent moieties and due to the benefit of such significant molecules the herb is utilised in skincare products. Most importantly these fatty acid esters work as emollients and meaning they help soften and smooth the skin's surface. They establish a protective barrier assisting in moisture retention, avoiding dryness while maintaining skin suppleness (Avoseh et al., 2021). Another essential aspect of fatty acid esters is that they are moisturizing and can aid increase skin moisture. They form a lightweight layer on the skin, minimizing water loss and increasing moisture retention, making them ideal for dry and parched skin. Additionally, the component improves the texture of skincare products. They contribute to a rich and smooth feel when applied to the skin and stabilize the product, preventing other ingredients from oxidizing. The molecules have non-comedogenic ability, so the formulation having the herb extract do not interfere they not interfering with pores, leaving behind blemishes and blackheads (Sellimi et al., 2018).

In analysis the Husn-e-Yousuf plant was also found to have different fatty acids such as Palmitic acid, Linoleic acid, *cis*-Vaccenic acid and Stearic acid as a major part and interestingly the presence of these molecules is highly accountable for making the herb a stupendous cosmaceutical as palmitic, linoleic, vaccenic, and stearic acids have unique skincare properties. Palmitic improves skin hydration and maintains moisture levels, while linoleic is anti-inflammatory and beneficial for acne and dermatitis. Linoleic acid can regulate sebum production and prevent clogged pores in acne-prone individuals. Whereas *cis*-vaccenic acid is an anti-inflammatory and barrier function enhancer, reducing inflammation and redness. Stearic acid is a cleansing and emulsifying agent and it is used in skincare products for removing dirt and impurities, and providing skin conditioning. These fatty acids are also found in natural oils like coconut oil, shea butter, and plant-based oils, and are often incorporated into skincare formulations due to their beneficial properties. Hence the naturally occurrence of these fatty acids in *Laminaria saccharina* proves it an excellent dermal care source (Lichterfeld et al., 2015).

Fucoidans, exhibit poor shear-thinning properties and poor viscoelastic properties, are biocompatible, non-toxic, and biodegradable, as well as possessing antioxidant and antiradical capabilities. Fucoidans have been shown to increase skin firmness, elasticity, brightness, hair development, safety, cleanliness, rigidity, and gloss. They prevent and treat skin photoaging by inhibiting wrinkle-related enzymes (e.g., collagenase, gelatinase, elastase), increasing collagen synthesis, regulating matrix metalloproteinases, and preventing extracellular matrix degradation. Laminarins (also known as laminarans) were discovered in North Atlantic laminaria and have a degree of polymerization of 15-40 and a molecular weight (Mw) of 2-10 kDa. They are called -(13)-d-glucans. Laminaribioses is a diholosidic repeating unit composed of (16)-d-Glcp. Laminarins are biocompatible, have low cell toxicity, are biodegradable, and exhibit antimicrobial activity (Ozanne et al., 2020).

Hydrocarbons are organic compounds with hydrogen and carbon atoms, used in skincare for their occlusive and emollient properties. They form a protective layer, reduce water loss, and enhance moisture retention, improving skin hydration and softness. They enhance the skin's natural barrier function, providing soothing and calming effects. Hydrocarbons are compatible with various ingredients, improving formulation stability and enhancing the absorption of active ingredients. Some hydrocarbons, like isododecane, have low comedogenic potential, making them suitable for oily or acne-prone skin types.

Laminaria saccharina extract is high in natural polysaccharides, which help to hydrate the skin and provide anti-aging benefits. It is utilised to promote a plump and supple complexion in moisturisers, serums, and facial masks. Antioxidants, such as vitamins C and E, found in seaweed protect the skin from free radical damage. It also has anti-inflammatory and calming characteristics, such as fucoidans, which soothe sensitive skin and promote an even tone. *Laminaria saccharina* extract is also recognized for its detoxifying abilities, which help to purify the skin by pulling out impurities and toxins.

Antioxidants are compounds that help protect cells from damage caused by harmful molecules called free radicals. Free radicals are highly reactive molecules that can cause oxidative stress in the body, leading to various health problems and accelerating the aging process. In the analysis of *Laminaria saccharina* through DPPH Colorimetric assay ABTS and Cation De-Colorization assay, the herb was established excellent at antioxidant response through percentage inhibition at IC50 (table-2) (Ozanne et al., 2020).

As because *Laminaria saccharina* is rich in bioactive compounds with antioxidant properties, including phlorotannins, fucoxanthin, vitamins, and minerals. Fucoxanthin, a carotenoid pigment with possible health advantages, scavenges free radicals and reduces oxidative stress. Vitamins and minerals such as vitamin C, E, beta-carotene, and beta-carotene also contribute to *Laminaria saccharina*'s antioxidant ability. The antioxidant activity, however, may differ based on the cultivar, harvesting season, and processing processes. Based on such an outstanding composition, *Laminaria saccharina*, has been adopted in skin care as an antioxidant and anti-ageing, as the skin antioxidant system has an enormous surface exposed to the environment to protect and, as a result, is very vulnerable to exogenous radical attack, making the defense mechanism continually challenged. As a result, cosmetology performs an important role in the prevention and reduction of cutaneous ageing through the research of compounds with effective antioxidant activity that may be included into cosmetic products for daily care.

CONCLUSION

The present study demonstrated that *Laminaria saccharina* possesses a rich composition of fatty acids, esters, and bioactive compounds that contribute to its strong antioxidant activity as evidenced by DPPH and ABTS assays. These findings confirm its potential as a natural dermo-cosmetic agent with applications in skin hydration, anti-aging, and protection against oxidative stress. Thus, *Laminaria saccharina* can be recommended as a promising natural ingredient for the formulation of effective and safe skin care products, aligning with the increasing demand for herbal alternatives in cosmetology. The plant is naturally gifted with vitamins and such compounds that have already proven for their promising effects on skin and due to that, this seaweed has a great potential to be used as a source of natural skin care remedy with no adverse effects.

AUTHOR'S CONTRIBUTION

Dr. Kiran Rafiq designed the study after literature search and contributed in all research bench work. Dr. Fatima Qamar analyzed the spectra and data. Dr. Safila Naveed did write up. Dr. Saman Usmani and Dr Shafaque Mehboob analyzed the results and also did write up. Dr. Shagufta Nesar and Javeria Iftikhar collected sample and performed all tests. Dr. Zafar Saied Saify did data analysis.

FUNDING

No funding was received for the present study.

AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of present research is available from the corresponding author, upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

ACKNOWLEDGEMENT

We are grateful our students for the sample collection from local and international markets.

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