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Sustainable Development of Antibacterial Knitted Fabric Using *Ulva Lactuca Extract* and Cow-Urine Pretreatment

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Abstract - An increase in marine algae as natural sources of bioactive chemicals has been one of the immediate results of the growing need for sustainable functional textiles. This article assesses the antibacterial properties of Ulva Lactuca extract and its use as an environmentally friendly finishing agent on cotton-bamboo knitted fabrics. Ethanolic Soxhlet was used to extract fresh seaweed from the Kurumpanai shore in Tamil Nadu, India, following verification. Flavonoids, carbohydrates, reducing sugars, and volatile oils were found in the phytochemical screening, showing a metabolite profile with possible antibacterial importance. Characteristic absorption peaks at 280 nm and 220 nm have been recorded using UV-visible spectroscopy, which is indicative of conjugated structures and aromatic compounds that can provide UV-absorbing characteristics. The liquid extract has shown inhibition zones of 16 mm against Staphylococcus aureus and 14 mm against Escherichia coli. Similarly, when this liquid extract was applied for the determination of antibacterial activity by the Kirby Bauer method, the inhibition zones measured 16 mm against Staphylococcus aureus and 14 mm against Escherichia coli. On treatment, the cotton-bamboo fabric showed enhanced activity on being applied as a finish and thus gave rise to inhibition zones measuring 19 mm and 17 mm, respectively. This improved performance of the final fabric indicates that the bioactive components of the extract are effectively coated and stable on the textile substrate. In general, results support the potential of Ulva Lactuca as a natural antimicrobial finishing agent for environmentally friendly textile applications, especially in sportswear, undergarments, and medical textiles.

Keywords: ***Ulva Lactuca*, Antibacterial finishing, Cotton–bamboo knitted fabric, UV Visible spectroscopy, Sustainable textiles.**

INTRODUCTION

Due to increased concern about environmental pollution and imposition of harsh regulations on harmful chemicals, the textile industries around the world have begun shifting towards sustainable and ecology-friendly production methods. Such resources are observed to replace conventional synthetic finishes for their common association with toxicity, poor biodegradability, and environmental harm associated with their use (Mukherjee & Raghu, 2020). Among these resources, marine macroalgae are widely recognized as renewable sources of different bioactive compounds that show very interesting antimicrobial, antioxidant, and UV-protective activities (Holdt & Kraan 2011; Chew et al., 2008).

Green seaweeds of the genus Ulva Lactuca (Chlorophyta) represent a rich source of various biochemical constituents, including ulvans, sulphated polysaccharides, phenolic compounds, flavonoids, terpenoids, pigments, and essential minerals. These bioactive metabolites are responsible for the diverse functional properties that position Ulva species as promising candidates for pharmaceutical, nutraceutical, and biomedical applications Manivannan et al., 2008; Lahaye & Robic, 2007. In this regard, Ulva lactuca extracts were largely reported for their antimicrobial efficacy against different Gram positive and Gram-negative bacteria Kumar et al., 2008; Seenivasan et al., 2012, reflecting a high potential for their use within functional materials. In spite of such properties, there are limited numbers of studies focusing on their direct usage for textile finishing processes in order to develop sustainable antibacterial fabrics.

For reasons such as softness, moisture wicking ability, breathability, and intrinsic antibacterial properties, cotton-bamboo blended knitted fabrics are widely used in sportswear and activewear, and lately in medical textiles. Augmenting these natural benefits by incorporating bioactive marine extracts is an environmentally responsible value addition strategy in textile processing. Similarly, all-natural pre-treatment agents such as cow urine provide greener alternatives to conventional alkaline scouring agents. Cow urine contains urea, ammonia, and naturally occurring enzymes that help in the removal of waxes and impurities and enhance the absorbency of the fabric. The treatment of bamboo fabrics with bioactive marine extracts and cow urine aligns with the concept of green chemistry and sustainable textile processing. Most research into metabolite profiling from plants involves two analytical techniques: UV-visible spectroscopy and phytochemical screening. According to Harborne (1998) and Trease and Evans (2002), these techniques enable the researcher to describe the chemical makeup of seaweed extracts and thereby relate metabolite profiles to functional bioactivities. These techniques make it easy to search for phenolic compounds, proteins, carbohydrates, flavonoids, and any other UV-absorbing or antimicrobial substance.

This study dealt with the determination of phytochemical constituents and UV–visible spectral characteristics of the ethanolic extract of Ulva lactuca, evaluation of antibacterial efficacy of the extract against Staphylococcus aureus and Escherichia coli, and assessment of its suitability as a natural finishing agent for cotton–bamboo knitted fabrics. Such a study will encourage the development of green antimicrobial textiles that find applications in sportswear, undergarments, and medical purposes.

Objectives of the Work

1. To extract and characterize the bioactive constituents of Ulva Lactuca using ethanolic Soxhlet extraction and standard phytochemical screening techniques.
2. To study the UV–Visible absorption profile of Ulva Lactuca extract and its chromophoric and UV-absorbing compounds of relevance to textile finishing applications.
3. To determine the antibacterial potency of the ethanolic seaweed extract against Staphylococcus aureus and Escherichia coli by means of the Kirby–Bauer disk diffusion method.
4. To apply Ulva Lactuca extract as a natural finishing agent on cotton–bamboo knitted fabric and assess improvements in antibacterial performance post-finishing.
5. To investigate natural pretreatment using cow urine that can improve absorbency and facilitate the better penetration of bioactive compounds in fabrics.
6. To assess the overall suitability of Ulva Lactuca-finished cotton–bamboo fabrics for sustainable eco-friendly applications in textiles, such as sportswear, underwear, and medical textiles.

MATERIALS AND METHODS

Collection of seaweed samples

Fresh green seaweed (Ulva Lactuca) was collected from the coastal waters of Kurumpanai, Tamil Nadu, India. After washing in tap water to remove salts, sand, and other adhering impurities, the samples were spread out evenly and air-dried at room temperature until constant weight was attained. The dried material was then ground into fine powder using a mechanical grinder and was stored in airtight containers in a dry condition.

Identification of species of seaweed

The collected samples of seaweed were identified and authenticated by the experts at the Botanical Survey of India, Southern Regional Centre, Coimbatore.

Preparation of Seaweed Extract by Soxhlet Apparatus

Freshly collected samples of Ulva Lactuca were shade-dried and ground into a coarse powder. A total of 14 g of the powdered material was subjected to Soxhlet extraction with 250 mL of ethanol for eight hours. The procedure followed was according to Manivannan et al. (2008), wherein the extract, at the end, was concentrated under reduced pressure at 25–28°C in a rotary evaporator. The concentrated residue was transferred to airtight containers and kept at −20°C until further analysis.

Fabric Selection

A blended cotton–bamboo knitted fabric was chosen for sports and activewear applications because of its enhanced moisture management, breathability, and softness compared with pure cotton. The blend results in an enhanced functional performance of the fabric owing to the blending of comfort provided by cotton with the antibacterial and UV-protective properties of bamboo. Single-jersey knit structure, 30s Ne yarn count, and a fabric GSM of 160–200 were used to achieve maximum wear comfort, stretchability, and durability while acting as an apt substrate to apply bioactive extract from seaweed (Gopalakrishnan & Satheesh Kumar, 2021).

Pre-treatment of Fabric Using Natural Agents (Cow Urine)

In the case of pretreatments, the cotton-modal knitted fabric was treated using cow urine as a natural bio-alkaline agent to support eco-friendly textile processing. Fresh cow urine was filtered and diluted to the required concentration before use. The fabric was immersed in 1:5 v/v cow-urine solution and heated at 60-70 °C for 45-60 minutes to facilitate the removal of waxes, pectins, and other hydrophobic impurities, thus improving fabric wettability. Aged cow urine, which is known to have higher amounts of ammonia, demonstrating mild bleaching action, was used for natural bleaching. The fabric was then treated at 50-60 °C for 30 minutes and washed thoroughly with distilled water to remove residual odor and impurities. The pretreated samples were air-dried and then conditioned before finishing applications. This method followed procedures similar to those reported by Shukla and Tiwari (2011) and Patel et al. (2017).

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Phytochemical screening test

Preliminary phytochemical screening of the ethanolic extract from Ulva lactuca was done using standard qualitative procedures described by Harborne (1998) and Trease & Evans (2002). The various chemical tests were conducted to detect major classes of metabolites, like alkaloids by Mayer's and Dragendorff's tests, flavonoids by alkaline reagent, H_2SO_4, lead acetate, and Shinoda tests, sterols and terpenoids by Libermann-Burchard test, anthraquinones by Borntrager's test, proteins by Ninhydrin, Biuret, and Xanthoproteic tests, phenolic compounds by ferric chloride, gelatin, and ellagic acid tests, quinones by HCl and alcoholic KOH tests, carbohydrates by Molisch's and Fehling's tests, tannins by Braymer's, gelatin, and NaOH tests, saponins by froth test, cardiac glycosides by Baljet, bromine water, and Keller-Killiani tests, coumarins by fluorescence and NaOH-chloroform tests, lignin by gallic acid test, and volatile oils by fluorescence test. The presence or absence of each phytochemical mentioned above was determined based on characteristic color changes, precipitate formation, or fluorescence.

UV–Visible Spectroscopy

The double-beam UV-visible spectrophotometer was used to analyze the seaweed extract. The dried ethanolic extract was dissolved in ethanol to get a clear solution, filtered on Whatman No. 1 paper, and then scanned from 220 to 800 nm. The instrument settings included a path length of 0.651 mm with no dilution (1×), while ethanol was used as the blank for baseline correction. Absorbance readings against selected wavelengths (220-800 nm) were recorded to detect chromophoric compounds and characteristic peaks linked to phenolics, flavonoids, proteins, and other UV-absorbing metabolites. The procedures followed standard practices reported by Harborne (1998) and Trease & Evans (2002).

Antibacterial Activity – Kirby–Bauer Method

The crude extract's antibacterial activity was investigated by using the Kirby–Bauer agar well diffusion technique, according to standardized criteria (Bauer et al., 1966; CLSI, 2012). Cultures of Staphylococcus aureus and Escherichia coli were subcultured, and 3–5 well-isolated colonies of them were aseptically transferred into 4–5 mL of nutrient broth, incubated at 35°C for 2–6 hours until active growth occurred. The turbidity of the broth was adjusted with the 0.5 McFarland standard ($\sim 1–2 \times 10^8$ CFU/mL). Within 15 minutes, a sterile cotton swab dipped in the inoculum was used to lawn the surface of sterile nutrient agar plates in three directions. Six-millimeter wells were punched, and each well received 50 µL of the extract. Plates were incubated at 37°C for 24 hours, after which inhibition zones were measured in millimeters with a standard Hi-Media scale. Ampicillin was used as the positive control. All the handling and assay procedures followed the methods of Harborne (1998, p. 302) and Trease & Evans (2002, p. 585).

Results and Discussion

Phytochemical Analysis

The extract showed the presence of flavonoids, carbohydrates, reducing sugars, and volatile oils through the screening process. The findings indicate a combination of bioactive compounds that might have antioxidant and functional properties. Flavonoids indicate potential antioxidants, while the carbohydrates and reducing sugars reflect basic metabolic constituents. Volatile oils suggest aromatic components that could add to antimicrobial activity. On the other hand, alkaloids, phenolic compounds, proteins, and saponins were not present, indicating the absence of nitrogenous compounds, phenolic antioxidants, and surfactant-like metabolites. Overall, the extract is mainly rich in flavonoids and carbohydrates, containing volatile oils that could together define its biological effects.

Table 1. Qualitative Phytochemical Analysis of *Ulva Lactuca*

S.NO	Phytochemical Group	Observation	Result
1.	**Flavonoids**	Yellow → colourless (Alkaline test)	Present (+)
2.	**Carbohydrates**	Violet ring (Molisch's test)	Present (+)
3.	**Reducing Sugars**	Brick-red precipitate (Fehling's test)	Present (+)
4.	**Volatile Oils**	Pinkish fluorescence	Present (+)
5.	**Alkaloids**	No cream/reddish-brown ppt	Absent (–)
6.	**Phenolic Compounds**	No bluish-green colour ($FeCl_3$ test)	Absent (–)
7.	**Proteins**	No purple/yellow colour	Absent (–)
8.	**Saponins**	No foam formation	Absent (–)

(+) Presence of the compound, (-) absence of the compound

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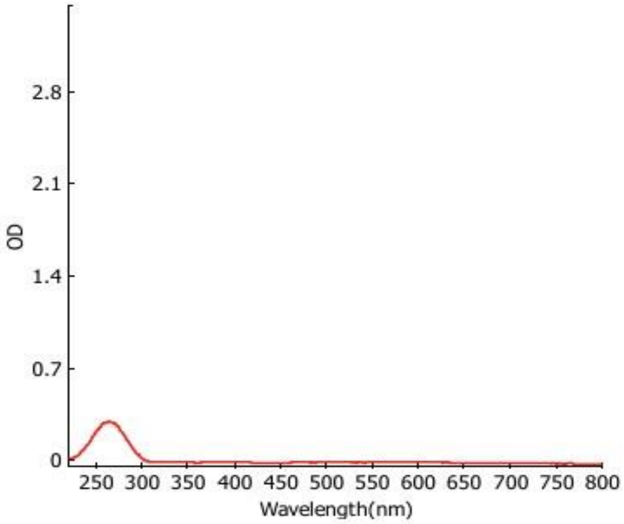
UV Absorption Characteristics

The UV–Visible scan of the ethanol extract from Ulva lactuca, between 220–800 nm, presents a strong peak at 280 nm (OD = 0.199), indicating aromatic compounds or conjugated carbonyl groups (C=O) probably from carbohydrate derivatives or small conjugated structures. A smaller signal at 220 nm (OD = 0.016) represents the $\pi \rightarrow \pi^*$ transitions related to the conjugated double bonds in carbohydrates and other unsaturated molecules, thus being in agreement with the preliminary phytochemical screening. The absorbance becomes minimal or negative in the visible range of 400–800 nm, indicating the absence of any pigment such as anthocyanin, chlorophyll, and anthraquinone. This confirms that the extract is colourless or almost colorless. Overall, the main absorbance in the UV region suggests the presence of UV-absorbing bioactive compounds in the extract, which may be dominantly carbohydrates along with some aromatic derivatives, and an absence of brightly colored secondary metabolite. The extract is thus colorless, showing suitability for textile applications.



Axes:

- X-axis: Wavelength (nm), 220–800 nm
- Y-axis: Absorbance (OD)

Antibacterial Activity

The antibacterial activity of *Ulva lactuca* was assessed in both its liquid extract form and as a finishing treatment applied to cotton–bamboo (CB) fabric against *Staphylococcus aureus* and *Escherichia coli*. The liquid extract exhibited moderate antibacterial efficacy, producing inhibition zones of 12 mm and 14 mm against *S. aureus* and *E. coli*, respectively, indicating the presence of bioactive compounds capable of effectively penetrating Gram-negative bacterial membranes. The finished CB fabric treated with the extract demonstrated identical inhibition values (19 mm for *S. aureus* and 17 mm for *E. coli*), confirming that the active constituents were successfully transferred to the textile substrate and retained their antimicrobial potency after finishing. The similarity between the liquid extract and finished fabric results suggests that the phenolic compounds, volatile oils, sulfated polysaccharides, and carbohydrate derivatives present in *Ulva lactuca* remain stable during the application process and continue to exhibit broad-spectrum antibacterial activity. Overall, the consistent performance of both sample forms highlights the potential of *Ulva lactuca* as a natural, eco-friendly antimicrobial finishing agent suitable for sustainable textile applications such as sportswear, undergarments, medical textiles, and functional everyday fabrics.

Sample	Test Organism	Zone of Inhibition (mm)
Ulva Lactuca liquid extract	Staphylococcus aureus	16
Ulva Lactuca liquid extract	Escherichia coli	14
Finished CB fabric (with Ulva)	Staphylococcus aureus	19
Finished CB fabric (with Ulva)	Escherichia coli	17

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CONCLUSION

The research work carried out showed that Ulva lactuca can be used as an efficient and eco-friendly antimicrobial finishing treatment on cotton-bamboo knit fabrics. The result obtained from phytochemical analysis pointed out that Ulva lactuca contains flavonoids, carbohydrates, reducing sugars, and volatile oils. The result obtained from the UV-Visible spectroscopy matched with the result obtained from the previous analysis, as it showed characteristic peaks at 280 nm and 220 nm. Ethanolic extract showed considerable antibacterial activity with zone inhibitions of 16 mm on Staphylococcus aureus and 14 mm on Escherichia coli. But it significantly improved on the finished cotton-bamboo fabric with zone inhibitions of 19 mm and 17 mm, respectively, and clearly confirmed that there was efficient adsorption and stability of extraction on the fabric. Moreover, natural pretreatment with cow urine effectively improved absorbency on fabrics and aided easy entry of bioactive compounds and eco-friendly processing. Results clearly pointed out that Ulva lactuca can be an unconfined natural source for developing eco-friendly antimicrobial textile materials for sportswear, intimate wear, medical use, and hygiene-based functionality.

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