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Development of Bioactive Non-Implantable Products using Brown Seaweed for Hygienic Textiles

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Abstract: *The aim of the investigation was to evaluate the anti-bacterial, bioactive compounds and anti-oxidant property of fibrous brown marine algae Sargassum wightii. Antimicrobial finish has been imparted to the cotton fabric using extracts of seaweed by microencapsulation using pad-dry-cure method. Both in vitro and in vivo studies have demonstrated, how this Sargassum wightti acts as antioxidant, and antibacterial properties and it also shows good antiviral and anti-carcinogenic activities. Bioactive compounds are inhibited the growth of microorganisms present in human body. Bioactive compounds were confirmed by means of the spectrum in the GC-MS spectroscopy. Antioxidant activities were evaluated using the DPPH method. The total phenolic content was determined with the folin-ciocalteu method. The methanol fraction of treated fabric had the highest antioxidant activity (42.5±1.21%), because of the phenolic content trap the reactive oxygen species and develops the cells present in the skin. Parallel streak method was to evaluate the antibacterial activity of seaweed treated fabric. The results showed that higher inhibition zone of 40 mm. The treated fabrics are most widely used in a wide range of health care, pharmaceutical and hygienic textiles.*

Keywords: *Antibacterial textiles, Microencapsulation, Bioactive compounds, Cotton fabric.*

INTRODUCTION

The development of natural based antimicrobial finishes is directly applied to the cotton fabric to produce value added textile product [1]. Many natural based herbal products have antimicrobial properties. In recent years particularly chitosan a natural occurring biopolymer act as an antimicrobial agent in the field of textiles [2]. Many of the plants contain bioactive compounds like phenol, terepenoids, flavonoids, alkaloids, polypeptide and polyacetylenes etc. which are acting as antibacterial properties. Some of these bioactive compounds are act as bactericides and some of them act as bacteriostatic [3]. In order to maintain good hygienic environment, seaweed extracts act as an antimicrobial agent for curing most of the degenerative diseases.

Sargassum wightii is one of the species of brown seaweed and it is a nature's power of herbal plant, used in folkloric medicine for the treatment of various diseases, widely cultivated in the gulf of manner region. Seaweeds are rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides [4]. Beneficial effects from the use of seaweed extracts as natural regulators have induced increased crop yield and plant vigor to withstand adverse environmental effects [5]. Seaweeds are one of the important marine living organisms could be termed as the futuristically promising plants and it is a major source of food and medicine [6]. Bioactive natural products are commonly distributed in the plant kingdom, and extract derived from different plants and also from red, green and brown macro and micro algae can be used as biological products [7]. A marine alga represents an infinite resource of raw materials used in pharmaceutical, medicine, food industries and cosmetics [8]. Marine algae act as an important source of bioactive natural substances [9, 10].The extracts and active bio components of various marine algae have an excellent antibacterial

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activity against Gram-positive and Gram-negative bacteria [11]. The functions of these secondary metabolites are protecting against fouling organisms and pathogens and also play a key role in UV protection and act as allopathic agents [12]. Seaweeds collected from diverse regions have been evaluated for a broad variety of biological activities like anti-bacterial, anti-viral, anti-fungal, anti-algal, anti-tumour, anti-inflammatory, anticoagulant and anti-oxidant activities [13]. Anti-bacterial and anti-oxidant activities are most commonly investigating properties in seaweeds in all over the world [14].

Sargassum wighitii has strong antioxidant and antimicrobial properties, recent studies have demonstrated that the carotenoids are an important group of natural pigment with specific applications as colourants, feed supplements, and nutritious medicine, cosmetic and biotechnological purposes. [15]. In addition, sargassum wighitii extract with an abundance of flavonoids and tannins has been shown to have a highest antioxidant activity. Phenolic compounds are important components of many medicinal applications [16].

Materials and Methods

Materials

Bio-scoured and Bio-bleached 100% cotton socks fabric (60 Course/inch, 40 Wales/inch) was used for the application of antimicrobial finish. The plant of Sargassum wighitii of edible brown seaweed was used for anti- microbial finish. They were freshly collected from thonidurai coast of mandapam and were rinsed in seawater and packed in aseptic bags and brought to the laboratory for further processing.

Methods

Extraction Process

In this method, 20g of powdered algal sample contained in a what man No.1 filter paper thimble was placed into an extraction chamber. The extraction chamber was then connected to a flask containing 200ml organic solvent with increasing polarity; hexane, ethyl acetate, chloroform and methanol, subsequently (1:10,w/v). Constant heat source was supplied for this procedure (40-50°C). All the extracts were concentrated under reduced pressure using a rotary evaporator and left air dried in a fume cupboard to obtain paste extract. The dried paste extracts were then stored at 4°C for further bioassay.

Microencapsulation by Ionic Gelatin Process

Microcapsules containing extract were prepared employing sodium alginate. Then 30 ml of extract and 10 ml of Tween 20 were added to the polymer solution and mixed thoroughly to form smooth viscous dispersion. This was sprayed into calcium chloride solution by means of a sprayer. The droplets were retained in calcium chloride for 15 minutes. The microcapsules were obtained by decantation and repeated washing with isopropyl alcohol followed by drying at 45°C for 12 hours. The cotton socks was immersed in the microencapsule solution using three bowl padding mangle, squeezed and then dried at 90-100°C in an oven. The microcapsules were also applied on the fabric by using a binder (8% citric acid).

Quantitative Determination of Total Polyphenols

In this procedure, 100 μl aliquot of stock sample (extract concentration 1000 μg/ml of water) was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then 100 μl of 50% Folin-Ciocalteau phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720nm using spectrophotometer (Milton Roy Spectronic 1201).The total phenolic contents of the samples were expressed as mg Gallic acid equivalent per gram(mg GAE/g). Gallic acid was used as a positive control.

Gas Chromatography-Mass Spectrometry (GC-MS)

Sargassum wightii was shade dried at room temperature and 5 g of the powdered sample was soaked in 98% methanol for 16 hr. Then the extract was filtered through Whatman No 1 filter paper along with 0.5 g of sodium sulfate solution to remove the sediments present in the solution filtrate. The solution filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar components of the brown seaweed. An aliquot of 2 µl of this solution was used for GC-MS analysis [17, 18].

Assessment of the Antioxidant Activity

DPPH Radical Scavenging Activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Yen and Chen (1995). Briefly, a 2.0 ml aliquot of test sample was added to 2.0 ml of 0.16 ml DPPH methanolic solution. The mixture was shaken vigorously then left to stand at room temperature for 30 min in darkness. Changes in the absorbance of the samples were measured at 517nm using a spectrophotometer (Milton Roy Spectronic 1201).

Antibacterial Analysis

The AATCC plates were prepared by pouring 15ml of AATCC media into sterile Petri plates. The plates were allowed to solidify for 5min and the bacterial culture was inoculated as single line followed by the four lines without refilling the inoculation loop. The fabric was cut into 5 ×2.5 size and immersed in treatment bath containing herbal and antimicrobial agents with the M:L ratio of 1:1:1 for 15 minutes and air dried at room temperature. The finished fabric with the diameter of 2.5 cm was placed over the inoculated bacterial species. And the plates were kept for incubation at 37ºC for 24 hours. At the end of incubation, zone of incubation formed around the fabric was measured in millimeter and recorded.

Scanning Electronic Microscope (SEM)

The surface morphology of treated and untreated fabrics was investigated by the JEOL JSM-6490 Scanning electron microscope (SEM) at the magnifications of ×1000.

Fabric Characteristics

The mechanical and comfort properties of treated fabric were performed according to the respective ASTM standards, ISO standards, IS standards and AATCC test methods. All the tests were carried out at standard atmospheric condition (27°C, 65± 2% RH). The bursting strength of knitted fabric was measured as per standard ASTM D3786 (2009). The stiffness in terms of bending length was measured as per standard ASTM D1388 (2008). The rate of air flow passing perpendicularly through a known area of fabric is adjusted to obtain a prescribed air pressure difference between the two fabric surfaces was measured according to standard ASTM D737(1996). The ability of vertically aligned treated fabric specimens to transport liquid through them was measured according to standard AATCC TM 197(2011)

Results and Discussion

Qualitative Assessment of Bio-Active Components of dyes using GC-MS Spectroscopy

The qualitative GC-MS fingerprint profile methanol extract of Sargassum wighitii (Fig.1) was picked a wavelength from 100 to 700 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at the mm of 670, 610 and 416 with the absorption 0.347, 0.118 and 1.841 respectively. The GC-MS spectrum of the purified compound was recorded and its absorption maximum (λmax) was compared with the fucoxanthin standard, the results confirmed that the standard and purified compounds exhibited the same spectroscopic profile with similar λmax (331, 446, and 468 nm). Fucoxanthin, flavonoids, phenols, tannin and saponins shows a characteristic bioactive absorption pattern (λmax) in this region of spectrum present in treated fabric.

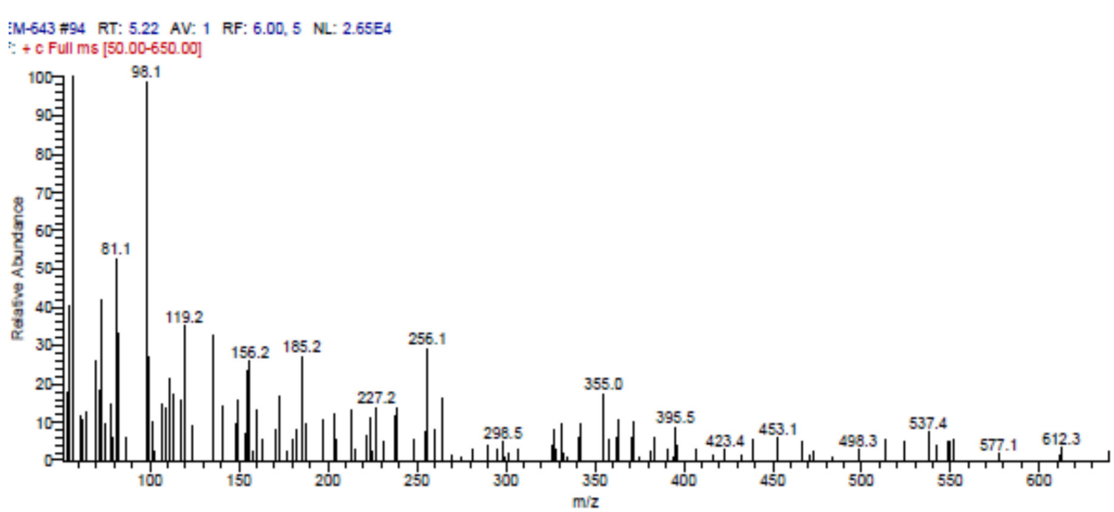

Figure.1. Analyze of bioactive compounds using GC-MS spectroscopy for Treated fabric

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Total Phenolic Content

The Phenolic content was determined using folin-ciocalteu reagent and was expressed as Gallic acid equivalents as shown in Table 1. The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenols obtained in the treated fabric.

Table.1. Total Phenolic content (mg Gallic acid equivalents (GAE/g extract) of treated fabric

Concentration of the Methanol Extract (ml)	mg/g of Gallic acid
1.5	20.45
2.0	30.70
2.5	40.20
5.0	52.10
12.5	61.78
25.0	103.28

DPPH Radial Scavenging Activity

The radial scavenging activity of treated fabric was analysed by DPPH method. The method helps to assess the percentage of scavenging activity present in the treated fabric. The test results are shown below in Table 2.

Table.2. Antioxidant activity of the different solvent extract of the Sargassum wightii

S. No	Seaweeds Name	Concentration (μg/ml)	DPPH Free Radial Scavenging Activity % Inhibition			
			Methanol	Aqueous Extract	Petroleum Ether	Ethyl Acetate
1.	Sargassum wighitii	50	42.5$\pm$ 1.21	40.11 $\pm$ 1.65	38.65$\pm$ 1.42	39.23$\pm$1.12

The test results showed that the pressure of antioxidant scavenging activity was same for seaweed Extraction and treated fabric. The antioxidant activities were more important for medical fabric, because of trapping the free radial of oxygen species and inhibit the cell damage and develop the cell growth present in the skin.

Analysis of Antibacterial Activity

The antibacterial activities are tested on the standard of AATCC 147. It was determined mainly by two bacterial species i.e. Staphylococcus aureus and E.Coli. Both samples are cut in the size of 1.5x108cfc/ml. In this two bacterial species Staphylococcus aureus has an excellent antibacterial activity when compared to E.Coli. This shows that an excellent antibacterial property is achieved in evenly coated fabric.

Table.3. Zone of Inhibition of coated fabrics

S.No	Fabric Sample	Seaweed Sample	Zone of inhibition (mm)			
			S.aureus		E.Coli	
			Before washing	After washing	Before washing	After washing
1	100% cotton	Brown seaweed	40	38	35	32

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The graph shows that the antimicrobial properties of the selected sample of Sargassum wightii. Treated fabrics had maximum zone of inhibition of around 40 mm for Staphylococcus aureus than E.Coli.

Figure.3. Zone of Inhibition of two bacteria

The above figure 3 clearly shows the better zone of inhibition against Staphylococcus aureus, than E.Coli.

Scanning Electronic Microscope (SEM) Test

The surface of microencapsulated seaweed coated cotton fabric was morphologically observed by scanning electron microscope. The surface of the seaweed coated material had long and narrow line. From the SEM analysis, it is clear that the seaweed coated cotton fabric surface had some morphological form which is different from the untreated cotton fabric that showed smooth surface.

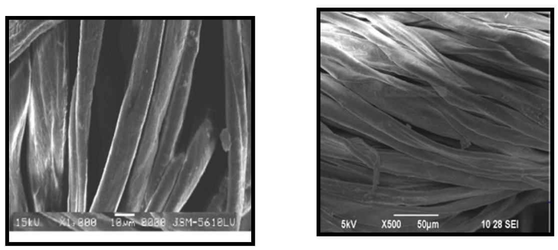

Figure.4.SEMPhotographs of Microcapsules treated fabric

The scanning electronic microscope (SEM) of fabric was tested for the untreated fabric. The result shows that the absence of coated particle on the surface of the fabric. In the case of treated cotton fabric, the result clearly indicates that the microencapsulated seaweed extract is evenly coated to the surface of the fabric.

Effect of Air Permeability for Treated and Untreated Fabric

Air permeability is an important factor in the performance of textile materials which provide an indication of the breathability of fabrics. Socks fabric basically open loop structure weaves, so the air can easily pass through the fabric to the surrounding and the amount of air passes through sq/cm of fabric would result in permeable characteristics of the fabric.

Table.4. Air permeability test for treated and untreated fabric

S.No	Untreated fabric (Cm^3/sec)	Treated fabric (Cm^3/sec)
1	1.463	0.937
2	1.482	1.101
3	1.511	1.112
4	1.499	0.999
5	1.501	1.105

The above table shows that if the cover factor of the fabric increases the air permeability get increased for the untreated fabric, but in case of treated fabric due to the microcapsules coated on the interstices between the yarns then the air permeability is slightly decreased.

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Effect of Wick Ability Characteristics for Treated and Untreated Fabric

The table.5 shows that the amount of water absorbed by the treated fabric will be slightly less than the untreated sample, because the seaweed microcapsules coated on the interstices will allow some amount of water from surface to the intermolecular structure and it will spread evenly like untreated fabric and therefore the coated particles was not affect the water wickable characteristics of the treated fabric.

Table.5. Wicking test for treated and untreated fabric

S.No	Time in sec	Untreated fabric (Cm)	Treated fabric (Cm)
1	5	2	1.8
2	10	2.1	1.9
3	15	2.5	2.1
4	20	3.0	2.8
5	25	3.2	3.0

Effect of Bending Length for Treated and Untreated Fabric

Fabric bending indicates the resistance of the fabric to bending and it is a key factor for the study of handle and drape. This test works on the principle called cantilever. This method is used to determine the bending length, flexural rigidity and bending modulus of the treated and untreated fabrics.

Table.6. Bending length test for treated and untreated fabric

S.No	Untreated fabric (Cm)	Treated fabric (Cm)
1	2	1.8
2	2.5	2.1
3	2.8	2.3
4	2.9	2.5
5	2.5	2.3

The above table shows that the bending length will not have much difference in both treated and untreated material. The bending modulus of the treated fabric increases due to coating on the fabric s and the flexural rigidity decrease for the treated fabric. Therefore it would decrease the bending length to a minimum level for the treated fabric compared with untreated fabric. The fabric stiffness of the treated material will not be affected due to seaweed coated on the fabric.

Effect of Treatment on Bursting Strength in Fabric

The bursting strength was more important for knitted fabric, because of unstable dimensional stability and it extended in width and lengthwise direction. The test results are given below in Table.6

Table.6 Bursting strength of untreated and treated fabric.

S.No	**Untreated Fabric (Kg/Cm^2)**	**Treated Fabric (Kg/Cm^2)**
1	5.5	5.9
2	5.6	6.0
3	5.9	6.5
4	5.8	6.3
5	6.0	6.2

The test result shows that microcapsules covers the fabric surface evenly and it gives better results in the treated fabric compared to untreated fabric. This is mainly due to absorption of microcapsules on the fabric surface excreted more pressure during application of liquid to the material. Further, in terms of bursting strength, the results from Table 6 also suggest that much significant difference ($P < 0.05$) in bursting strength value was recorded for the untreated and treated fabrics.

Conclusion

The sargassum wightii extracted with methanol solvent in a soxhlet apparatus had functional bioactive compounds such as flavonoids, fucoidan, tannin, saponin, and polyphenols. The confirmation of bioactive compounds in the treated fabric was confirmed by using GC-MS spectrum. The micro-capsulated treated fabric has an excellent antioxidant property that has been proved by DPPH radical scavenging activity test for trapping the free radicals present in the skin for avoiding cell damage and fast growth of cell tissue present in humans. This analysis proved that higher level of bioactive compounds present in the seaweed extract and treated fabric helps to improve the antioxidant activity**.** The treated fabric has no significant difference in water absorbency and bending properties but it has a significance difference in the air permeability, washing fastness and rubbing fastness. Future scope of this treated fabric is most widely used for wound dressing, bandage, health and hygienic textiles.

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