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Extraction of algal pigments and their suitability as natural dyes

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Abstract
With increase in water toxicity due to textile effluents from synthetic dyes, there is a need for alternate sources that do not have a toxic effect. Algae possess colour pigments which can be used by the textile industry for dyeing fabric and also as a food colourant, since these pigments are found to have various anti-microbial and antioxidant properties. In due to antimicrobial properties of the pigments, these can be used for producing medi-textiles that would be extremely beneficial for use in hospitals. In the present work, pigments were extracted from red, green and brown algae and tested for their ability to resist growth of common pathogenic microbes and to study how well they adhere to fabrics and retain colour. The adherence and colouring properties of these were tested and compared by using different natural mordants.

Keywords: *Phycobilins, Natural dyes, Antimicrobial, Textile, Algal pigments*

Introduction

Natural dyes are in existence since ancient times for coloring and printing fibers (Qinguo *et al*., 2008). Dyes and Colorants pigment from natural sources are environmentally friendly and in recent times, gaining importance mainly due to health and environmental issues. Natural dyes are pigments derived from mineral, animal or plant sources (Ansari and Thankur, 2000) and can be obtained from any part of the plant, such as leaves, fruits, twigs, seeds, flowers, bark root, etc. (Gogoi N and Kalita, 1999). The two biggest disadvantages of plant pigments production are represented by the need of a wide land for their cultivation and the CO2 emissions (IPCC, 2007).
In recent years, significant interest has been developed in the commercial utilization of algae, based on their valuable bioactive compounds with applications in various industries like, food, cosmetic, agri- and horticultural sectors and in human health. The bioactive compounds with special interest, include pigments, lipids and fatty acids, proteins and polysaccharides (Stengel *et al*., 2011). Algae contain a wide range of photosynthetic pigments. Three major classes of photosynthetic pigments are chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins. Macroalgae contain phycoerythrins and carotenoids, which represent valuable pigments for the textile finishing industry. Phycoerythrin is a water-soluble, light harvesting protein, which is specialized in the energy transfer chain (Liu *et al*., 2005). In recent times, the usage of natural dyes in textile coloration as UV-protectant and as antimicrobial agent has been explored by many researchers (Ali *et al*., 2011; Ali *et al*., 2014; Ali *et al*., 2015; El-khatib and Ali, 2011; El-Mollaa *et al*., 2011).The use of natural dyes instead of synthetic dyes would be an alternate solution but most natural dyes are expensive as the extraction process is often expensive. This calls for the search of better natural dyes that are economical and have a relatively easier extraction process. Algal pigments would serve this purpose and they have the additional benefit of having some antimicrobial activity. Hence the research was proposed to utilize algae as a source of natural dye and to analyze their antimicrobial activity.

Materials and Methods

Three different algal samples viz., *Spirulina platensis*, *Turbinaria conoides* and *Halymenia dilatata* were employed in this study for extraction of pigments and analyzing their antibaterial activity.

1. Processing of algal samples
1.1. *Spirulina platensis*

The *Spirulina* was cultured in Zarrouk's medium. The fully-grown *Spirulina* was then filtered using a muslin cloth, collected and sun dried and processed in a mechanical blender and made in to fine powder.

1.2. *Turbinaria conoides*
The fresh samples of *Turbinaria* were collected from Ramanathapuram coast and the excess moisture was removed and dried for 4 hours. *Turbinaria* sample was cut into small pieces and incubated overnight in hot air oven at 40˚C. Dried sample of *Turbinaria* were made into powder.

1.3. *Halymenia dilatata*
The *Halymenia* samples were also collected from Ramanathapuram coast and same sample processing procedure was followed as with *Turbinaria conoides.*

2. Extraction of Algal pigments
2.1. Extraction of phycocyanin from *Spirulina* and fucoxanthin *Turbinaria conoides* were performed through three different ways viz., freeze thawing, mechanical shaking and soxhlet extraction

2.1.1. Freeze thawing
The dried powders of *Spirulina* and *Turbinaria* were mixed separately with distilled water in the ratio of 1:25 and were frozen at -21˚C for 24 hours and then thawed at room temperature. The extract was centrifuged at 5000 rpm for 15 mins. The extraction was also carried with phosphate buffer (0.1M).

2.1.2. Mechanical Shaking at room temperature:
The dried powders of *Spirulina* and *Turbinaria* were mixed separately with distilled water in the ratio of 1:25 in a mechanical shaker at 150 rpm at room temperature for 24 hours. The extract was centrifuged at 5000 rpm for 15 mins. The extraction was also carried with phosphate buffer (0.1M).

2.1.3. Soxhlet Extraction:
The dried powders (5 grams) of *Spirulina* and *Turbinaria* were loaded separately in thimble and operated for 5 cycles with 3 different solvents viz., acetone, methanol and chloroform with their respective boiling temperature. The extracted solvents were recovered using rotary evaporator.

2.2. Phycoerythrin from *Halymenia* was extracted by following the above mentioned procedures (freeze thawing and cold maceration).
2.1.2. Cold maceration was done using chilled distilled water and phosphate buffer (0.1M) as extractants in the ratio of 1:5 and was blended in a blender for two minutes. Extracts were filtered using a muslin cloth and the filtrates were centrifuged for 15 minutes at 5000 rpm and at 4˚C.

2.1.3. Freeze thawing
The fresh *Halymenia* sample was mixed with distilled water in the ratio of 1:5 and was frozen at -21˚C for 24 hours and then thawed at room temperature. The extract was centrifuged at 5000 rpm for 15 mins at 4˚C. The extraction was also carried with phosphate buffer (0.1M).

3. Spectrophotometric analysis of algal extracts
The quantitative estimation of algal pigments were carried out by recording the absorbance maxima (λ max) using UV-Vis spectrophotometer from 200-800 nm (Varian Cary 300 Bio UV-Vis).

4. *Phytochemical tests*
The following phytochemical tests viz., Mayer's test (Alkaloids), Benedict's test (Carbohydrates), foam test (Saponins), Ninhydrin test (Proteins &Amino acids), Salkowski test (Steroids), Ferric Chloride test (Tannins & Phenols) were performed to detect to detect the mentioned compounds in the extracts.

4. Dyeing experiments

4.1. Mordanting

A mordant is a substance used to fix dye to fabrics. Mordant forms a coordination complex with the dye that aids in the attachment of dye/pigment onto the fabric. Mordants used in this research weretannic acid, alum, mango kernel residue, fresh lemon juice and aloe vera gel. Three percentage of mordant over the weight of fabric (owf) was used for 3×4 inches of white cloth. Material liquor ratio (MLR) followed for mordanting was 1:100. Water was mixed with respective mordant, heated up to 70˚C, temperature was brought down to 40˚C and wet fabric was carefully added to mordant solution and stirred continuously for 60 mins. Mordanted fabric was rinsed with tap water and dried under shade for further dyeing process.

4.2. Dyeing

Dyeing experiment was carried out in the water bath with continuous stirring. Dye solution was prepared by mixing each of the extract (phycocyanin, fucoxanthin and phycoerythrin) with distilled water in the ratio 1:1.The mordanted fabrics were immersed in the closed container consisting of dye solution. Dyeing was carried out at 9±2˚C and another one at RT (27˚C±2) for 24 hrs. After 24 hours, they were taken out of the container and let to dry at room temperature. One half of the dyed fabric was rinsed with water and the other half was immersed in industrial dye fix solution for 30 seconds and observations were recorded.

5. Antimicrobial assay

Evaluation of antimicrobial properties of the dyes, phycocyanin from *Spirulina*, fucoxanthin from *Turbinariaconoides* and phycoerythrin from *Halymenia* were tested for their antimicrobial activity against *Klebsiella spp*, *Proteus vulgaris*, *Shigella spp*, *Staphylococcus aureus* and *Pseudomonas spp*. Disc diffusion method was followed for the antimicrobial assay. Whatman No. 1 filter paper discs were employed to load the dye sample in varying concentration (60mg/ml, 30mg/ml and 15mg/ml). Amoxicillin (30mg/ml) was used as the positive control and the solvent in which the pigment was extracted was used as a negative control. Sterility of the algal extracts were tested and confirmed by spreading 100µl of each extract onto nutrient agar plates and incubating the plates at 37˚C for 4 hours. The absence of microbial colonies confirmed the sterility of the extracts. Extract (50µl) were loaded onto the disc using a micropipette and then left to dry. Nutrient agar plates were prepared followed by placing the dried discs onto the surface of the agar medium coated with pathogen culture. The petriplates were sealed and incubated at 37˚C for 24 hours and observations were recorded.

Results

1. Extraction of phycocyanin

Pigment extraction from *Spirulina platensis* was performed through three different ways viz., freeze thawing, mechanical shaking & soxhlet extraction with distilled water and phosphate buffer (0.1M). Pycocyanin was efficiently extracted with freeze thawing and mechanical shaking method (Fig. 1) where as soxhlet extraction method did not support pigment extraction. The Mount of pigment extracted through freeze thawing and mechanical shaking methods with phosphate buffer (0.1M) and distilled water, extracted about 4±0.2 mg mL^{-1} of phycocyanin (C-Phycocyanin) pigment (Fig. 2).

Maximum fucoxanthin from *Turbinaria conoides* was successfully extracted by freeze thawing method (phosphate buffer and distilled water) (Fig.3) and mechanical shaking with phosphate buffer (Fig.4). Phycoerythrin from was successfully extracted with cold maceration method using phosphate buffer as an extractant (Fig.5). Phycoerythrin was also extracted using distilled water, but phosphate buffer gave more intense colour compared to the water macerated one (Fig.6).

2. Phytochemical test

The phytochemical tests were performed to qualitatively analyze the bioactive compounds in the algal extracts. Extract from *Spirulina platensis* recorded positive for the presence of proteins, amino acids tannins and phenols (Table 1), whereas extracts of other two algae species did not exhibit positive for any of the tests.

Table 1: Phytochemical test results

Phytochemical tests	*Spirulina platensis*	*Turbinaria conoides*	*Halymenia dilatata*
Ninhydrin test	+	-	-
$FeCl_2$ test	+	-	-
Mayer's test	-	-	-
Benedict's test	-	-	-
Salkowski test	-	-	-
Saponins test	-	-	-

3. Antimicrobial assay

Phycocyanin, fucoxanthin and phycoerythrin dyes were tested for their antibacterial activity. Eventually, zone of inhibition observed were not significant enough to report for antibacterial activity against selected bacterial pathogens.

Fig. 1: Phycocyanin - Freeze thawing with (a) Phosphate buffer (0.1M); (b) Distilled water

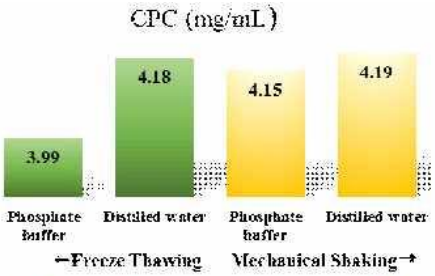

Fig. 2: Phycocyanin concentration in different extractant

Fig. 3: Fucoxanthin - Freeze thawing with (a) Distilled water; (b) Phosphate buffer (0.1M)

Fig. 4: Fucoxanthin – Mechanical shaking with Phosphate buffer (0.1M)

Fig. 5: Phycoerythrin - Cold Maceration with Phosphate buffer (0.1M)

Fig. 6: Phycoerythrin - Freeze thawing with Distilled water

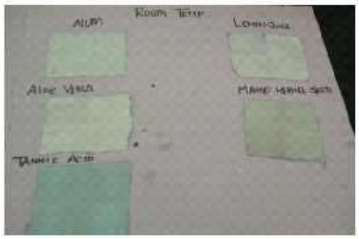

Fig. 7: Phycocyanin dyed fabric at room temperature (27±2°C) with natural mordants

4. *Dyeing*

Dyeing experiments were carried out with phycocyanin and fucoxanthin. Dyeing was performed at room temperature (27±2˚C) and at cold condition (9±2˚C). Among all the mordants used (alum, tannic acid, lemon juice, aloe vera and mango kernel seed powder), fabric treated with tannic acid could uptake and dye in blue colour better than other mordants at cold condition (9±2˚C) (Fig. 8-10). Rest of the mordants dyed phycocyanin in pale blue shade. Room temperature dyeing was not successful as the dye uptake was very less (Fig. 7). Similarly, fucoxanthin dyeing recorded good dye uptake which tannic acid as a mordant. Other mordants also supported dye uptake but not significantly when compared to tannic acid. Surprisingly, fucoxanthin dyed better in room temperature than in cold temperature (Fig.11).

Fig. 8: Phycocyanin dyed fabric at cold condition (9±2˚C) with natural mordants

Fig. 9: Phycocyanin dyed fabric at cold condition (9±2˚C) with natural mordants

Fig. 10: Phycocyanin dyed fabric at cold condition (9±2˚C) with chemical mordants

Fig. 11: Fucoxanthin dyed fabric at cold condition (9±2˚C) with natural mordants

Discussion

Phycobiliproteins are accessory photosynthetic pigments that participate in an extremely efficient energy transfer chain in photosynthesis (Roman *et al*., 2002) responsible for about 50% of light capitation from cyanobacteria and red algae (Williams *et al*., 1980). C-phycocyanin (C-PC) extracted from cyanobacteria such as *Spirulina platensis* has wide range of application in industries such as food, cosmetic and textile as natural blue dye (Moraes *et al*., 2011). During the present work, phycocyanin was efficiently extracted from dry *Spirulina* powder using freeze thawing and mechanical shaking (0.1 M phosphate buffer and distilled water). Acker and McGann (2003) opine that freeze thawing method works better as freezing of cell causes intracellular ice formation that damages the cell thereby resulting in finer extraction of pigments.

Much of the brown algal extracts have been proven to have antioxidant (Kuda *et al*., 2005) and antimicrobial properties (Ely *et al*., 2004). The major antioxidant compounds in brown algae are fucoxanthin, astaxanthins, carotenoids and polyphenols (Yoshi *et al*., 2000). During the present study the fucoxanthin was extracted from *Turbenaria* using freeze thawing (Horng *et al*., 1998, Shanmugam *et al*., 1998) and mechanical shaking with phosphate buffer and water. Soxhlet extraction with acetone, chloroform and methanol was found to extract only the chlorophylls with visibly no brown pigments. Mechanical shaking and freeze thawing methods of extraction were tried using phosphate buffer and water as solvents. The freeze thawing method of extraction with water was found to be the best method of extracting fucoxanthin based on the spectrophotometric analysis of the extract. This extract was used for further analysis.

Macroalgae contain phycoerythrin and carotenoids which represent valuable pigments for the textile finishing industry. Phycoerythrin is a water soluble, light harvesting protein which is specialized in the energy transfer chain (Liu *et al*., 2005). During the present work, cold maceration was used with phosphate buffer as well as water as

solvents. The pigment extract with the former was found to exhibit a bright pink colour as compared to the other. Freeze thawing method (with phosphate buffer and water) also gave similar results as cold maceration in terms of intensity of the colour.

All the three above pigment viz., phycocyanin, fucoxanthin and phycoerythrin were tested qualitatively to check the presence of certain compounds of which only extract from Spirulina recorded positive for the presence of proteins, amino acids, tannins and phenols. The other two
pigments were not found to produce any positive for phytochemical analysis. Anti-bacterial assays using all the three pigment extracts were found to exhibit only trace activity against selected bacterial pathogen, indicating that high concentration may be required for gross antimicrobial activity. Since phycocyanin, fucoxanthin and phycoerythrin could be easily extracted with water and the extract method is simple, these could be considered for further dyeing processes. Dyeing experiments were performed on cotton fabric with selected mordants (alum, tannic acid, aloe vera, mango kernel powder and lemon juice). Since phycocyanin was sensitive to higher temperature, dyeing practice methodology was carried at room temperature (27˚C±2) and at cold condition (9±2˚C). The cold dyed and normal dyed fabrics of phycocyanin did not show any difference and they possessed same adherence and retention of colour. Tannic acid mordanted fabric showed a darker and better colour uptake, than rest of the mordants. Non mordanted fabric recorded less colour uptake of the algal dye and loss of colour upon rinsing with water. Addition of other medicinal plant extracts (Nitha *et al*., 2012) as adjuncts while dyeing would enhance the colour as well as the antimicrobial property of the fabric dyed.

Conclusion

The algal pigments could be effectively used as natural dyes for the fabrics in the place of synthetic dyes since the extraction methods are economically feasible, eco-friendly and do not cause any health hazards. However more work needs to be carried out on areas such as the antimicrobial properties of different algal pigments, colour characterization, colour fastness and dyeing efficiency.

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