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Investigations on the Cytotoxicity, Neurotoxicity and Dyeing Performances of Natural Dye Extracted from *Caulerpa lentillifera* and *Sargassum* sp. Seaweeds

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Abstract: Nowadays, the demand for natural dyes is increasing due to the fact that they are less toxic and more environmental friendly. In this study, natural dyes from *C. lentillifera* and *Sargassum* sp. seaweeds were extracted using boiling water extraction methods. Exhaustion dyeing was then performed on silk fabrics at 85°C for 60 minutes with simultaneous addition of mordant and dye in the dyebath. The dyed samples were then measured using spectrophotometer to analyse the L*a*b* values and K/S values of the shades obtained. The ability to withstand washing, perspiration, rubbing/crocking and light of the dyed fabric was compared. Cytotoxicity and neurotoxicity tests were performed on the natural dyes in the form of liquid and dyed silk fabrics. Primary cells from mouse embryonic cells and cell line from SH-SY5Y were used to investigate the cytotoxicity test. Neuro-like cells obtained from retinoic acid treated SH-SY5Y was used to conduct neurotoxicity test. MTS assay method was carried out to the entire cells to evaluate the toxicity of the dye. The results showed that the extracted dye is toxic free and the fastness properties of the dyed silk fabric gave ratings from good to excellent except light fastness which was rated as poor.

Keywords: *Caulerpa lentillifera, Sargassun* sp., Cytotoxicity, Neurotoxicitity, Extraction, Fastness Properties

1. Introduction

Textile manufacturing used a wide range of chemicals and most of them are harmful to the environment, to the people working in textile processing and potentially to consumers. Different levels of toxicity are produced at different textile processing stages. However, there are limited data available on the biological effects of the treated textiles with these chemicals. Klemola *et al*. [1] cited a statement from Sundquist [2] which stated that textile dyes form a major group of textile chemicals and comprise of over 8,000 different compounds with almost 40,000 commercial names. According to Samanta and Agarwal [3], Ali *et al.* [4] and Iqbal and Ashiq [5], there are approximately 10,000 different dyes and pigments produced globally in which the production capacity per year is over 700,000 tons. Statistically, during the dyeing process, up to 15% of the dyes and pigments produced are lost in the effluent and released into the environment [6].

The most common occupational diseases which have been traced among the workers in the textile industry are allergic reactions and irritation to the skin and respiratory tract [7], [8] Schneider *et al.* [9] and Mathur and Bhatnagar [10] claimed that some textile dyes have been assessed for potential mutagenicity. On the other hand, Sharma and Sobtin [11] found that some textile dyes have potential genotoxicity.

Extensive researches have been conducted from time to time in order to reduce the effluent of textile processing. There are two main resolutions that can be emphasized from

the researches i.e. build highly effective and sufficient effluent treatment plants as well as to produce and utilize eco-friendly dyes and chemicals. Hence, the use of natural dyes are emerging globally due to the fact that they are environmental friendly, less toxic, less allergic and biodegradable in comparison with synthetic dyes.

Seaweeds are promising plants of the millennium because it is a renewable and sustainable source which can be harvested at 6 to 8 weeks after the cultivation [12], [13]. Furthermore, in 2012 the production of seaweed in Malaysia is 23,940 metric tonnes and expected to boost production up to 35,000 metric tonnes in the year of 2013 [14]. Arad and Yaron [15] and Prasanna *et al.* [16] stated that algae has a wide variety of natural pigments like chlorophyll, carotenoids and phycobiliproteins, which exhibit colours ranging from green, yellow, brown and red. Algae pigments have great commercial value as natural colorants in nutraceutical, cosmetics and pharmaceutical industry as well as their health benefits [17], [18]. On the other hand, Muhammad Ismail *et al.* [19] have successfully extracted and applied the dyes on silk and bamboo fabrics.

2. Methodology

2.1. Materials and Reagents

Caulerpa lentillifera and *Sargassum* sp. seaweeds were collected from Bum-Bum Island, Semporna, Sabah as shown in Figures 1 and 2 respectively. Hundred percent (100%) plain weave silk fabric was used as the substrate. Two percent (2%) of metallic salts of ferrous sulphate (iron) and potassium aluminium sulphate (alum) as well as vinegar were used as mordants for each different dyebaths.

Fig. 1. *C. lentillifera Seaweed.*

Fig. 2. *Sargassum sp.Seaweed.*

2.2. Dye Extractions

Boiling water extraction method was used to boil *C. lentillifera* and *Sargassum* sp. seaweed in distilled water for 60 minutes with a liquor ratio of 1:20 (weight of material in gram: amount of water in mL).

2.3. Isolation of Mouse Embryonic Fibroblasts (MEFs)

A pregnant mouse was sacrificed by cervical dislocation. The uterine horns was dissected out, rinsed in 70% (v/v) ethanol and placed into a falcon tube containing phosphate buffered saline (PBS) without $Ca^{2+}Mg^{2+}$ [20]. The embryo was separated from its placenta and embryonic sac. Then, it was washed in PBS and all embryos were placed in a clean Petri dish. The embryo was harvested from the mice at approximately day 11 post-coitus, washed three times with 1 x phosphate buffered saline (PBS) and placed into individual 15 ml falcon tube. 500 µl of collagenase type 4 (~66 U/ml) was added to each embryo followed by incubation at 37°C until digested [21]. Then it was incubated for 15 minutes at 37°C. The cells were dissociated by pipetting up and down thoroughly every 5 minutes after incubation [20].

2.4. Medium Preparation

The medium for cell growth was prepared. A 450 ml of Dulbecco's Eagle's Medium (DMEM) solution was prepared as a medium for the primary cell culture. Whilst, for the cell line culture, a minimum Essential Medium Eagle (EMEM) solution was prepared as a medium.

2.5. Primary Cell Preparation

Mouse embryonic cells were incubated at 37°C for 30 minutes with fibroblast isolation enzyme (with papain). The cells were washed (2 times) in Hanks` Balanced Salt Solution (HBSS). Then, the cells were disrupted with Dulbecco's Modified Eagle Medium (DMEM), a culture medium. The cell yield and viability were determined from cell suspensions isolated from single mouse embryo [22]. The culture medium, DMEM, supplemented with 10% fetal bovine serum (FBS) and 1× l-glutamine, penicillin and streptomycin solution in a temperature controlled at 37°C in 5% CO_2 incubator.

2.6. Cell line Preparation

The cells of SH-SY5Y were grown and maintained in T-75 flask by using maintenance medium Essential Medium Eagle (EMEM) to a cell density of 80% prior to infection. The SH-SY5Y is a cell line which was derived from human's bone marrow from 4 years female patient that suffer neuroblastoma disease [23]. These cells often used *in vitro* models for neurological function, differentiation test and other scientific research. EMEM supplemented with 10% FBS and 200mM glutamine with media solution for the cells culture [24]. The cell line was seeded into 35 mm tissue-culture dishes (1 x 10^5 cells/2ml) and incubated

for 4 days at 37°C in humidified 5% of CO_2 incubator [25].

2.7. MTS Assay

The cells were seeded into 96-well plates at a density of $1x10^4$ per well (100 µl). 10 µl of the MTS reagent was added into each well and cells were incubated at 37°C for 3 hours. The absorbance was detected at 490 nm with a Microplate Reader (VersaMAx, Molecular Devices). All the experiments were repeated three times [26].

2.8. Dyeing of Silk Fabric

Silk was dyed with extracted colorant using exhaustion dyeing technique. Two percent (2%) of colorant in the form of liquid based on weight of fabric were used to dye silk fabric using a liquor ratio of 1:20. Two percent (2%) of each mordant was used to fix the colorant onto the fabric. Dyeing and mordanting were carried out simultaneously in one bath. The dyeing process was performed at 85°C for 60 minutes. After the dyeing cycle was completed, the dyed fabrics were washed, rinsed with tap water and then left to dry.

2.9. Colour Assessments

The dyed fabrics were assessed for colour fastness to washing, perspiration, rubbing/crocking and light in accordance to MS ISO standards as tabulated in Table 1.

Table 1. *Standard Methods Used for Colourfastness Assessments.*

Colour-fastness	Standard Methods	Equipments
Washing	MS ISO 105-C01-1966	Auto-wash
	MS ISO 105-A05-2003	Change in Colour
	MS ISO 105-A04-2003	Staining
Perspiration	MS ISO 105-E04-1996	Perspirometer
	MS ISO 105-A05-2003	Change in Colour
	MS ISO 105-A04-2003	Staining
Rubbing/ Crocking	MS ISO 105-X12-2001	Crockmeter
	MS ISO 105-A04-2003	Staining
Light	MS ISO 105-B02-2001	Light Fastness Tester

The percent reflectance (%R) and L* a* b* values of the dyed fabric were measured using HunterLab LabScan XE (LSXE) spectrophotometer and analysed using HunterLab EasyMatchQC software within the visible spectrum of 400 nm -700 nm. The K/S values (colour strength) of the dyed fabric were then calculated according to Kubelka-Munk equation as shown in Equation 1. The higher the K/S value, the more is the dye-uptake, resulting in better color strength.

$$K/S = (1-R)^2/2R \quad (1)$$

Where:

K = absorption coefficient, depending on the concentration of colorant

S = scattering coefficient, caused by the dyed substrate

R = reflectance of the colored sample

3. Results and Discussion

3.1. Toxicity Tests

The *C. lentillifera* and *Sargassum* sp. extracts and dyed silk fabric were subjected to cytotoxicity test using theprimary cell from mouse embryonic cells and cell line from SH-SY5Y. Neurotoxicity test was performed on SH-SY5Y treated with retinoic acid.

Primary cells from Mouse Embryonic Fibroblasts (MEFs) cell treated with seaweed extracts in the form of liquid and dyed silk fabric were tested with cytotoxicity. Figure 3 shows the percent cell viability after being exposed for 24 hours to *C. lentillifera* and *Sargassum* sp. extracts.

The highest percentage of cell viability (522.38%) was obtained from 5% extract of *Sargassum* sp. and the lowest percent cell viability was obtained from 1% *Sargassum* sp. extract (156.41%) as shown in Figure 3. Generally, *Sargassum sp*. extracts showed higher percentage of cell viability in comparison with *C. lentillifera* extracts. According to the MTS reading, most of the samples stimulate the cell growth rapidly.

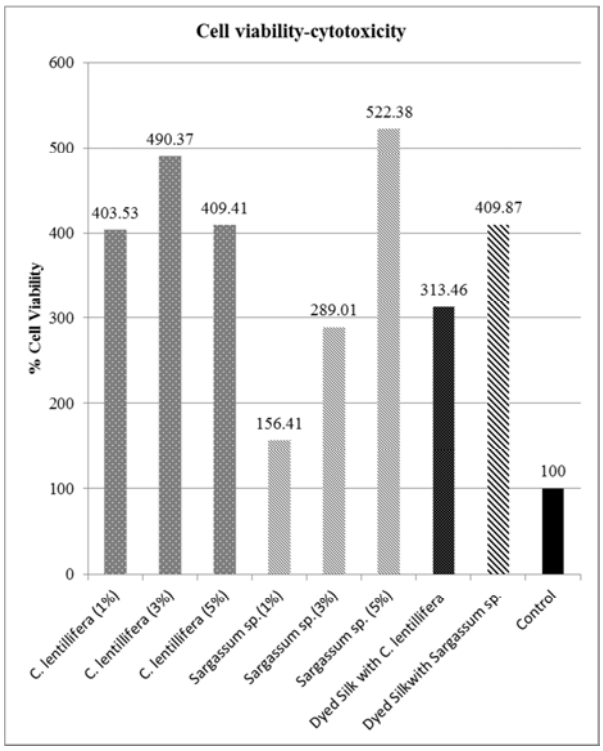

Fig. 3. *Percentage of Cell Viability for Primary Cells from Mouse Embryonic Fibroblasts (MEFs) on Cytotoxicity Test.*

Figure 4 shows the percent cell viability of SH-SY5Y cell lines which regard to cytotoxicity test after being exposed for 24 hours to *C. lentillifera* and *Sargassum* sp. extracts. Dyed silk fabric with *Sargassum* sp. extract showed the highest percentage of cell viability (192.67%).

Whereas, 1% *C. lentillifera* extract showed the lowest cell viability which is 84.84%. Again, *Sargassum* sp. extracts gave higher percentage of cell viability compared to *C. lentillifera* extracts.

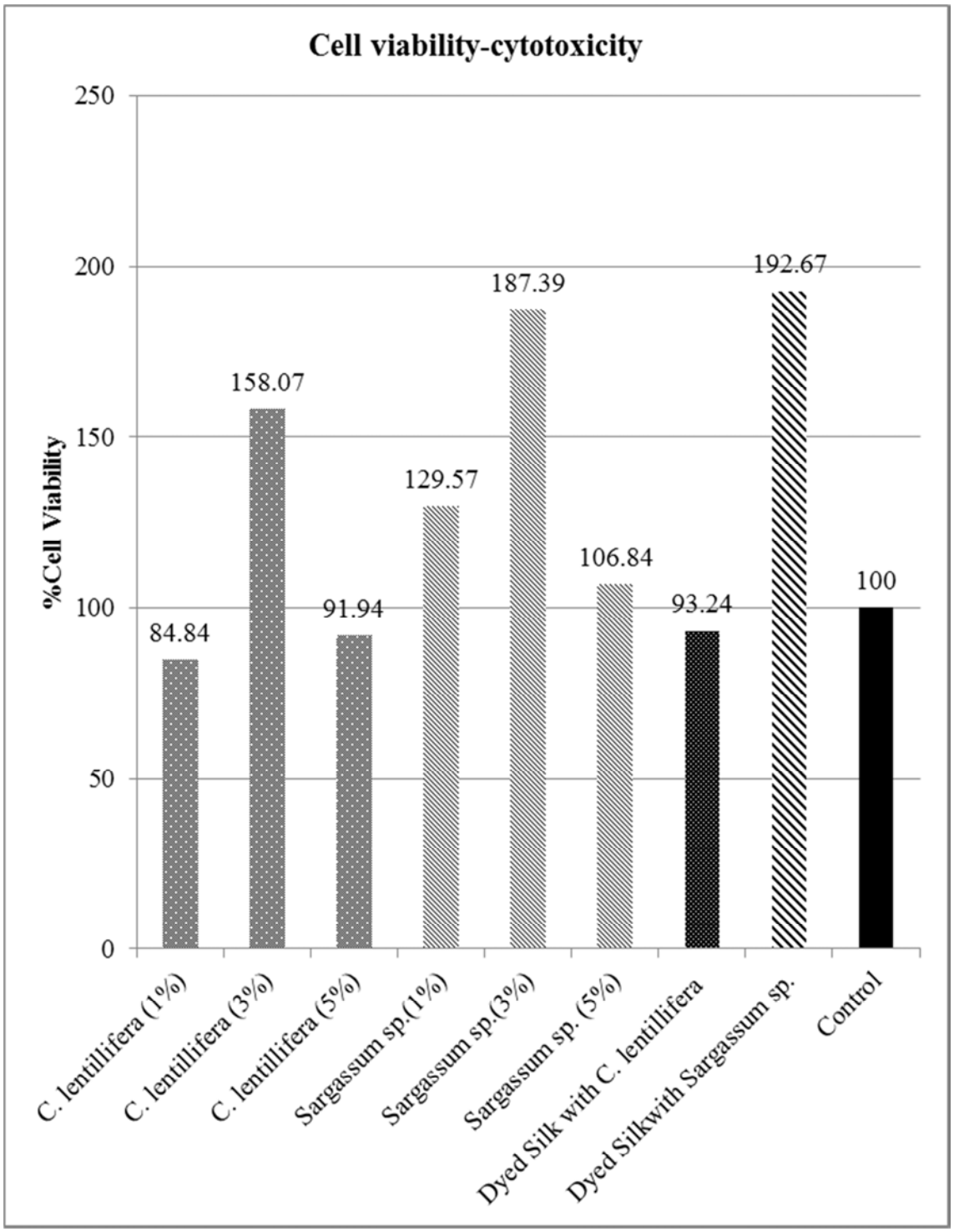

Fig. 4. *Percentage of cell viability for SH-SY5Y cell lines on Cytotoxicity Test.*

Figure 5 shows the percentage of cell viability of neurotoxicity test of cell line from SH-SY5Y treated with retinoic acid (neuro-like cells) after being treated with *C. lentillifera* and *Sargassum* sp. extracts for 24 hours. The results generally showed that the cells were able to grow during the tissue culture. This indicates the present of *C. lentillifera* and *Sargassum* sp. did not produce any toxic effect to the cell and suspended the cell growth *in vitro*. The dyed silk fabric with *Sargassum* sp. extract showed the highest percentage of cell viability (138.28%) followed by 5% of *Sargassum* sp. extract which illustrated at 128.99% of cell viability. The lowest percentage of cell viability was obtained from 1% of *C. lentillifera* extract (101.92%). In average, *Sargassum* sp. extracts produced higher percentage of cell viability in comparison with *C. lentillifera* extracts.

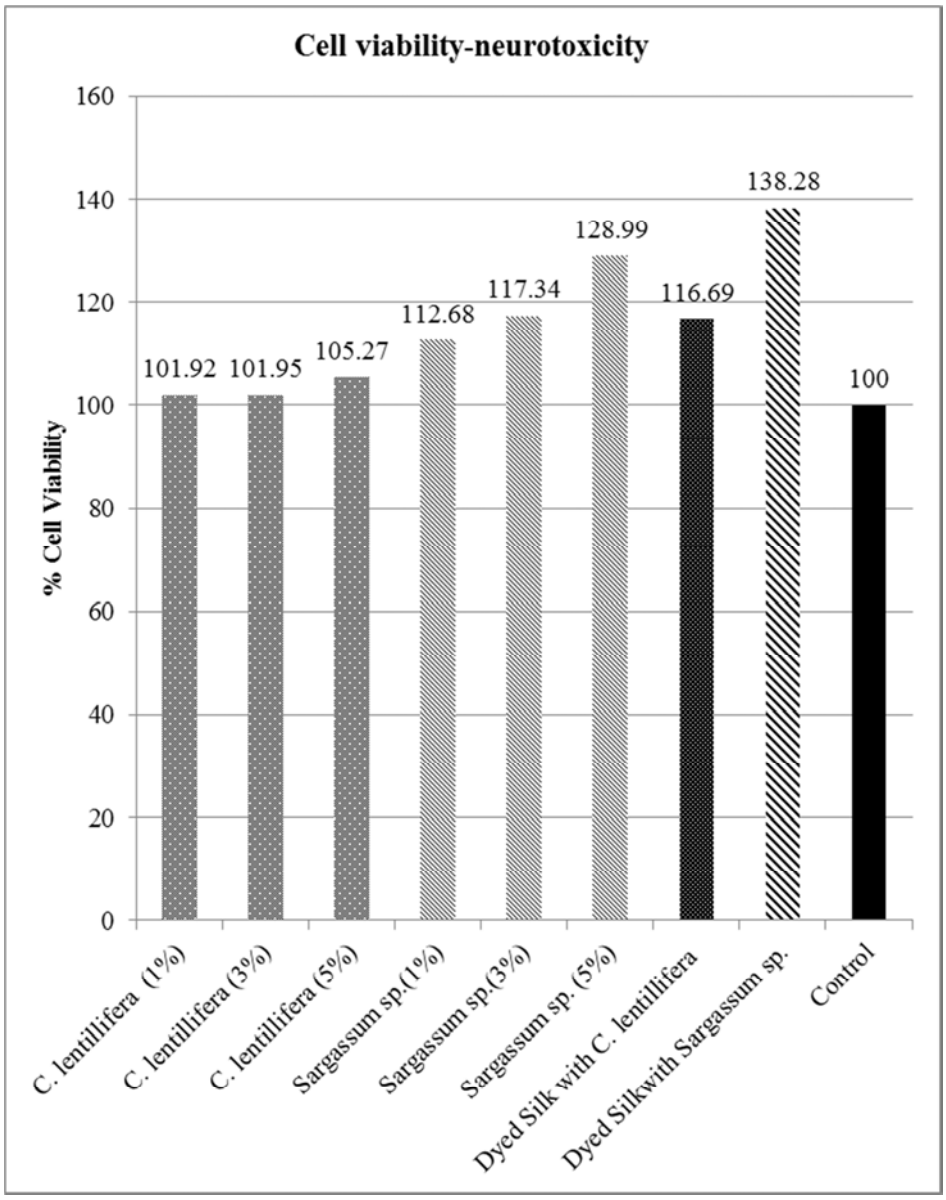

Fig. 5. *Percentage of cell viability for SH-SY5Y cell lines treated with retinoic acid on Neurotoxicity Test.*

3.2. Colorimetric Coordinates of the Dyed Fabric

The L*a*b* values of dyed silk fabrics dyed with *C. lentillifera* and *Sargassum* sp. extracts from boiling water extraction is tabulated in Table 2. The L* values indicate perceived lightness or darkness. Value of 0 indicates black and 100 indicates white. The values of a* indicate red (+a) and green (-a) while b* indicates yellow (+b) and blue (-b).

Table 2. *L* a* b* Values of C. Lentillifera and Sargassum sp.*

Seaweed Species	Mordant	L*	a*	b*
C. lentillifera	No Mordant	74.49	-2.09	18.07
	Vinegar	77.52	-1.75	18.67
	Alum	81.5	-1.19	18.12
	Iron	57.18	5.14	21.33
Sargassum sp.	No Mordant	78.61	-3.23	18.36
	Vinegar	79.46	-3.34	17.51
	Alum	77.85	23.95	20.54
	Iron	68.73	1.76	20.14

Generally, the L* values for all dyed fabrics were reduced (which indicates darker shades) when treated with iron as mordant. The darkest shades were obtained from *C. lentillifera* treated with iron which L* value coordinated at 57.18. On the other hand, the lightest shade (higher L* values) was obtained from dyed silk fabrics treated with alum (81.5).

3.3. Colour Strength

The colour strength (K/S) values for all dyed samples are presented in Figure 6. The highest K/S values were obtained from silk dyed with *C. lentillifera* extract. Iron is a mordant which contributes significantly in enhancing the K/S values of the dyed fabrics. Remarkably, unmordanted dyed fabrics gave slightly higher K/S values in comparison with vinegar and alum-mordanted dyed fabrics for both *C. lentillifera* and red strain of *Sargassum* sp.

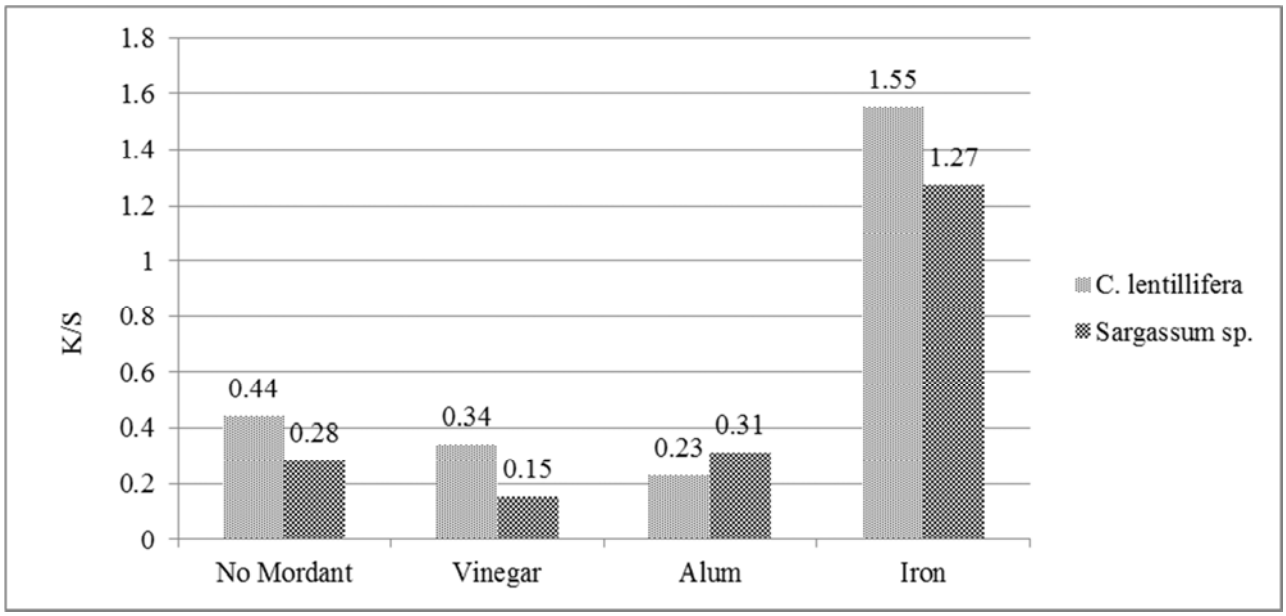

Fig. 6. *K/S Values of the Dyed Silk Fabric.*

3.4. Colourfastness Properties

Table 3 shows the summary of fastness properties assessed from dyed silk fabric. Washing fastness for all dyed samples were rated from 4 to 4/5 for change in colour and staining rated from 4/5 to 5. This rating is considered as good where 5 is the best. The result for fastness properties to perspiration also gave good rating of 4/5 for change in colour and the rating for staining ranging from 4 to 4/5. Same goes to the fastness properties result for rubbing/crocking with rating from 3 to 5 for dry rub and 3/4 to 4/5 for wet rub. Conversely, the fastness properties to light for all dyed samples were poor as rated as 3.

Table 3. *Fastness Properties of the Dyed Silk Fabric.*

Seaweeds	Mordants	Washing			Perspiration			Rubbing/ Crocking		Light
		Change in Colour	Staining		Change in Colour	Staining		Dry	Wet	
			Cotton	Silk		Cotton	Silk			
C. lentillifera	No Mordant	4	4/5	4/5	4/5	4	4	4/5	4	3
	Vinegar	4/5	4/5	4/5	4/5	4	4	4/5	4	3
	Alum	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4	3
	Iron	4	4/5	4/5	4	4/5	4/5	4	4	3
Sargassum sp.	No Mordant	4/5	5	5	4/5	4/5	4	4/5	4/5	4
	Vinegar	4/5	4/5	4/5	4/5	5	4	5	4/5	4
	Alum	4/5	4/5	4/5	4/5	5	4	5	4/5	4
	Iron	4/5	4/5	4/5	4	4/5	4	4	4	4

4. Conclusion

This study investigated the toxicity and the dyeing performances of the natural dyes extracted from *C. lentillifera* and *Sargassum* sp. seaweeds. It can be concluded that *C. lentillifera* and *Sargassum* sp. seaweeds can be exploited as a natural dye source which produces unique and interesting shades with acceptable fastness properties even without mordant. The fastness properties of the dyed silk fabrics gave good to excellent rating except for light fastness properties which can be considered as poor. In terms of cytotoxicity and neurotoxicity tests, the extracted natural dye from *C. lentillifera* and *Sargassum* sp. seaweed were found to be toxic-free because the dye did not interrupt the cell line and primary cell growth in vitro.

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