DISCOVERY

58(323), November 2022

Extraction of eco-friendly natural dye from claitoria plant and its application in textile by economic methods

To Cite:
Abd El-Samie FS, Ali NF, Abd El-Salam IS, Bayad Gh Y. Extraction of eco-friendly natural dye from claitoria plant and its application in textile by economic methods. DISCOVERY 2022; 58(323):1204-1212

Abd El-Samie FS[1], Ali NF[2], Abd El-Salam IS[3], Bayad Gh Y[4]

Author Affiliation:
[1]Agronomy Department, Faculty of Agriculture, Fayoum University, Egypt
[2]Dyeing and Printing Departement, National Research Center, Egypt
[3]Chemistry of Natural and Microbial Products Dept National Research Centre, Egypt
[4]Art Education Department, Faculty of Specific Education, Fayoum University, Egypt

Peer-Review History
Received: 16 August 2022
Reviewed & Revised: 19/August/2022 to 04/October/2022
Accepted: 08 October 2022
Published: November 2022

Peer-Review Model
External peer-review was done through double-blind method.

ABSTRACT

Clitoria plant which grows widely and wildly in Sudan as a forage crop its seeds were obtained from the Sudanese Ministry of Agriculture in order to study on extracting a natural dye from its flowers for the first time in Egypt . Where, natural dyes from plants have been given much interest in recent years due to the threat and harmful effects arised by synthetic dyes and environmental awareness created by researchers. In conjunction with the increasing public awareness of the infectious diseases. Several researches are developed hygienic fabrics by using natural dyes as substitute of synthetic dyes. The current investigation was carried out by application of natural dye produced from clitoria plant for dyeing wool fibers for studying its effects on wool quality and antimicrobial resistance. Antimicrobial activity is investigated against a broad range of microorganisms including bacteria, yeast and fungi. By using innovation technique to save energy and time. The obtmizing conditions as concentration, temperatut, pH and the time of dyeing were studied. The fastness properties were also studied. The fibers dyed with the investigated dye, and the antimicrobial activity was recorded. The wool materials showed antimicrobial effects by killing and/or suppressing growth of a broad spectrum of microbes such as Bacillus subtilus, Escherichia coli, Candida albicans and Aspergillus niger. The results recorded good color strength (K/S) and fastness properties. Application was reflected positively on the zones of growth inhibition of wool fibers E. Coli gave the highest diameter of the inhibition zone. On the hand, the applied pigment and the nano forms showed minimum effect on *C. A*libican and A niger.

Keywords: Clitoria plant; natural dye; textile

1. INTRODUCTION

Clitoria ternatea L commonly known as butterfly pea belongig to the family Fabaceae and sub-family Papilionaceae is a perennial leguminous twiner, which originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalized (Barik et al., 2007). Clitoria ternatea L is a highly palatable for age legume generally preferred by live stock over other legumes. It exhibits excellent regrowth after cutting or grazing within short period and produce high yields.

DISCOVERY SCIENTIFIC SOCIETY

Also extracts of the flowers of Clitoria ternatea L can serve as a natural blue colorant, tend to be convenient to use, and possess a longer shelf life than comparable plant-based colorants (Azima et al., 2017). Egypt suffers from an acute shortage of forage crops during summer season, especially legume crops. In this respect, clitoria might be very promising summer legume for age crop in the future especially in the newly reclaimed soils. Hence, it does not compete with the main strategic summer crops such as maize, rice and cotton in the limited arable land in Nile valley and Delta. Therefore, the principal objective of the ongoing study was to maximize the productivity of clitoria crop by obtaining a product other than fodder yield, which is the natural dye yield from flower petals. Natural dyes as naturally derived colorants and antimicrobial agents have more advantages over synthetic dyes in that they exhibit good biodegradability and compatibility with the environment (Zhou et al., 2015).

Recent studies in textile proceesing interested in using new techniques in dyeing textile to decrease energy and water consumed. The use of microwave in textile industry is one way for this purpose (Ali and El-Mohamedy 2011). The advantages of microwaves microwave heating technique, is the use much less liquid, they can save dyes and leave no waste of dye comparing to conventional methods. Also the less power consumption leading to production of desired shades, and fast dyeing (Singh 2000; Hebeish et al., 2012). Natural dyes help in protecting the environment and reduce pollution by industries (Ali and El-Mohamedy 2011; Gao and Cranston 2008). Recent studies towards application of natural dyes in textile have been developed due to the result of stringent environmental standards caused by environmental board and pollution control board of different countries related to toxic and allergic reactions associated with synthetic dyes. Recently, the ability of using natural dyes in dyeing textile as UV-protection and anti-microbial has been investigated. Some natural dyes require the use of mordents, such as aluminum, iron, chromium, copper and tin, to increase color fastness to light and washing. In this study we use chitosan instead of mordents. Textile materials are subjected to attack by microbes because they provide the basic requirements for growth of microbes (Thiry 2001; Avadi et al., 2004).

For example (keratin) and silk as natural fibers is made of protein, which provide moisture, oxygen, nutrients and temperature for bacterial growth and multiplication. This results in odors, infection, product d Avadi, eterioration allergic responses, consequently causes related diseases (El-Khatib et al., 2014; Ali et al., 2015). In this study we use high-quality and eco-friendly natural dye extracted from plant as claitoria. Natural dyes as naturally derived colorants and antimicrobial agents have more advantages over synthetic dyes in that they exhibit good biodegradability and compatibility with the environment (Divya Lekshmi and Ravi 2013). Research has shown that synthetic dyes are suspected of releasing harmful chemicals that are considered to be a high pollutant in both water and l and which would be allergic, carcinogenic and detrimental to human health. Clitoria ternatea L. known by several common names including butterfly pea or blue pea, is a plant species belonging to the Fabaceae family. The flower petal of Clitoria ternatea L is commonly used as a natural food colorant and healthy beverages around the world. Clitoria ternatea L.(Figure 1), a well-known plant drug in possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties (Mukherjee et al., 2008). The most studied and the main colorant pigment of flower Clitoria ternatea L reported are anthocyanin confirmed by HPLC and other spectral analysis (Patil et al., 2009) molecular structures are given in Figure 2.

Figure 1 Flower of Clitoria ternatea L

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

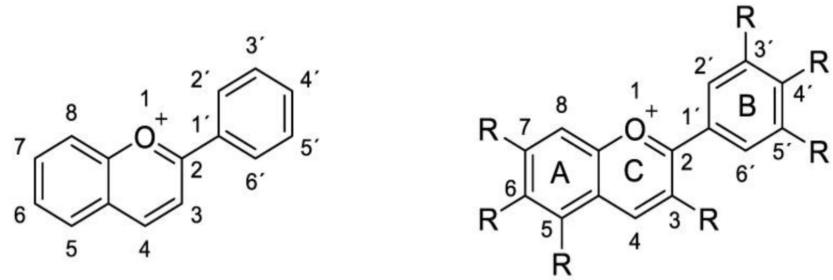

Figure 2 Chemical structure of antocyanine pigment (R_ H,OH)

2. MATERIALS AND METHODS

Cultural practices

A field experiment was carried out at the Experimental Farm, Faculty of Agriculture, at (Dar El-Ramed), Fayoum University, Fayoum governorate, Egypt, during the summer season 2022. The total area of the experiment is 350 m^2 to prepare an appropriate seedbed, the land was plowed three times then leveled by laser equipment, ridged and divided into experimental plots with an area of 42 m^2 (1/100 fed) and consisted of seven rows 60 cm apart and 10 m long, the total number of experimental plots is eight. Seeds of clitoria (Clitoria ternatea L cv Baladi) were obtained from the Sudanese Ministry of Agriculture. Then, it is soaked in tap water for three days before sowing with changing water 2 to 4 times daily in order to quickly germinate in the field. Immediately prior to planting clitoria seeds were inoculated with Rhizobium sp of the cowpea group according to (Abreu et al., 2014) to fix atmospheric nitrogen. Uniform 3 to 5 seeds of clitoria were hand sown in each hill. The plants were thinned to two plants per hill before the first irrigation. Clitoria plants were clipped three consective cutting . The 1st cutting was performed at the age of 60 days after sowing while the 2nd and 3rd cuttings were performed after 45 days from cut of previous growth period also all cuttings were cut at a uniform stubble height of approximately 10 cm above ground (Mahala et al., 2012). The flowers of Clitoria ternatea L was picked in three pickings, first picking was done before the 1st cutting, second picking was done before the 2nd cutting and third picking was done before the 3rd cutting. The flowers of Clitoria ternatea L was cleaned and dried in an oven at a low temperature in order to remove all the moisture content in the flower. Then, the dried flower petals was crushed into small pieces, about 1 mm to increase the surface area.

Materials

Wool fibers Mill scoured 100% wool fibers used for this study weresupplied from Misr Co. (El Mehalla El-Kobra, Egypt for spinning and weaving). The fibers werewashed in a bath containing 2g/l non-ionic detergent (Nonidet) at 40°C to remove any impurities and then thoroughly washed with water and then dried by air at room temperature. Wool fibers 10/2 fibers supplied by El Mahalla company-Egypt. Neem oil extract was purchased from EL Gmhoriacompany, Egypt. Chitosan high molecular weight (210,000), Poly (D-glucosamine), was purchased from ROTH, Germany. Dyestuff: Algal Pigments Scientific classification: Kingdom: Protesta Division: Chlorophyta Phylum: Charophyta Class: Zygnematophyceae Family: Zygnemataceae Order: Zygnematales Genus: Spirogyra.

Apparatus

Microwave

The microwave equipment used in this experiment was the Samsung M 245, with an output of 1,550 watts operating at 2450 MHz.

Dye extraction

The flowers of Clitoria ternatea L was cleaned and dried in an oven at a low temperature in order to remove all the moisture content in the flower. Then, the flower was ground into small pieces, about 1mm, to increase the surface area. Then, the blue natural dye was obtained by using ratio 0.12 g/ml to the ratio of 1.2 g of the flower to 10 ml of water (Kanchana et al., 2013). The dye was separated using centerfuge at 4 ^{o}C, 10000 rpm for 15 minutes. The solution was filtered to collect the natural dye. By using Whatman filter paper and pH was adjusted by HCl and NaOH if needed. The resulting extract was used for the further experiment (Makasana et al., 2017).

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Dyeing procedure

Dyeing wool fibers using the microwave method

Dyeing was carried out using 2 g/l of a dye extracted from clitoria Ternatea plant blue dye with a liquor ratio 1: 50, the wool fibers were dyed using microwave at pH (5) time for 5 minutes. The dyed fibers were washed using warm water and then cold water, Washing was carried out using 5g/l non-ionic detergent at 50ºC for 30 minutes, consequently washed and dried in air at room temperature (Ali et al., 2014).

Dyeing wool fibers using conventional heating

Dyeing yakes place at different concentratios of dye with liquor ratio 30: 1, wool fibers were dyed using conventional heating at different pH values (3-9) for different durations (30-60 min.) and at different temperatures (50-90 ºC) (Ramachandran et al., 2004). The dyed fibers were washed with cold water at liquor ratio 60: 1 using 3 g/L non-ionic detergent (Hostapal CV, Clariant) at 50 ºC for 30 min., consequently washed and then dried at room temperature.

Measurements

Measurements of color strength

Color strength was measured using An Ultra Scan PRO spectrophotometer. The reflectance of the samples, and then, the K/S was assessed spectrophotometrically at wavelengths: λ_{max} 385 nm). The K/S of dyed wool fibers was evaluated.CIELAB coordinates (L* a* b*) of undyed and dyed wool fibers were determined using an Ultra Scan PRO spectrophotometer (Hunter Lab) with a D65 illuminant and 108 standard observers.

Fastness properties

According to ISO standard methods. The specific tests were ISO 105X12 (1987), ISO 106-C06 (1989), ISO 105-E04 (1989), and ISO 105-B02 (1989), corresponding to colorfastness to rubbing, washing, perspiration and light, respectively. The color changes of the samples were assessed against an accurate Grayscale.

Testing antimicrobial activity measurement

Nutrient agar medium (g/L) consists of peptone 5.0 g, beef extract 1.5 g, yeast extract 1.5 g, NaCl 5.0 g, and agar 20 g at pH 7.5 which was prepared and autoclaved at 121 °C for 20 min. Sterilized petriplates were prepared with an equal thickness of nutrient agar. Test organisms involved gram negative bacteria (Escherichia coli) and gram positive bacteria (Bacillus subtilis) and fungi Candida albicans, Aspergillus niger were grown overnight at 37 °C, 120 rpm in 2 ml nutrient broth. This broth was used for seeding the agar plates. Wool samples were placed on top of the seeded medium under sterile conditions. After overnight incubation at 37 °C, the zones of inhibition were measured. In the second set of experiments, untreated wool samples were tested (Ali and Abd-Elsalam, 2022).

3. RESULTS AND DISCUSSION

Effect of dye concentration

In the present experiment, the effect of different dye concentrations of 2,4,6,7 and 8 g/l was investigated. The results indicated that the maximum color strength value was obtained at concentration of 8g/l after which a reduced color strength which noticed in Table 1 and Figure 6 on the other hand, at low concentration showed that remarkable lowered in color strength.

Different concentrations of extracted dye were done by microwave method. It can be concluded that the increase in extracted dye concentration used, there was decreased in L* values and thus the color of the samples got darker. As the dye concentration increased, a* and b* values increased in the positive direction. The color of dyed wool fibers turned to reddish blue color and became darker with increased dye concentration from 2 to 8 g/ L. Microwave dyeing considers the dielectric and the thermal characters of matter. The dielectric property refers to the intrinsic electrical properties that is affected the dyeing by dipolar rotation of the dye and influences the microwave field upon the dipoles. The dye solution consists of two components that are polar in microwave field at high frequency. It influenced the vibration energy in the water molecules and the dye molecules. The color strength of dyed wool fibers was affected by concentrations. The dye under investigation has high affinity for the fibers. Result showed that the color strength of dyed fibers with the dye under investigation gave the highest value of color strength (K/S) at conc 8g/L. The color strength also was affected by dye bath pH. The dye had high affinity for the fibers in acidic medium. In acidic medium, the cationized amino groups can be adsorbed an anionic dye molecules by the electrostatic attraction. The color strength

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

of dyed wool fibers is dyed by microwave that affected by duration of time. The color strength of wool fibers dyed with the dye under investigation gave the largest values of (K/S) after 5 min which similar to research work of (Abd-Elsalam and Ali 2020).

The measurement of chromaticity values such as L*, a*, b* was used to evaluate the dyed wool obtained by microwave technique. The chromaticity values shown in Tables 1 were for dyed fibers by chlitoria natural dye. The value of a* which represent the redness effect for dyed fibers exhibited highest value at conc. 8gl. This indicates that, the dyed fibers was reddish blue. Table 1 also show that, the colorimetric data (L*, a* and b*) of different fibers dyed with different dye concentration. From these data we can be concluded that increasing of the dye concentration, accompanied by decreasing of L* values and thus color of samples got darker. By growing the dye concentration, a* and b* values increased in the positive direction (Ali et al., 2019). The color of dyed wool fibers turned to more reddish blue color and became darker with increasing the dye concentration from 2 to 8%.

Table 1 Effect of conc. of the dye extracted from clitoria plant on the color strength (K/S) and the color differences for wool fibers. Effect of time of dyeing on the color strength (K/S) and the color differences for wool fibers.

Conc. %	K/S	L*	a*	b*	C*	H	Δ E
20	10.32	66	19.92	9.09	21.90	24.53	42.93
40	6.52	51.0	31.52	14.58	34.73	24.82	52.58
60	15.82	50.53	35.7	15.8	35.74	25.7	55.78
80	16.34	42.5	36.7	16.8	37.73	28.5	55.58

The results indicated that the color strength of dyed wool fibers dyed by microwave affected by duration of time (1-5 min) as seen in Table 1. The dye under investigation was high affinity done for the fibers. The results showed that the time increased the dyeing efficiency increased and reached to the maximum at 5min.

Table 2 Effect of timeof dyring for the dye extracted from clitoria plant on the color strength(K/S) and the color differences for wool fibers.

Time(min.)	K/S	L*	a*	b*	C*	H	Δ E
1	10.32	59.20	19.92	9.09	21.890	24.53	41.93
2	13.034	51.11	31.52	14.58	30.73	24.82	50.58
3	14.23	550.3	35.7	15.8	40.74	25.7	60.78
4	14.51	50.7	36.7	16.8	37.73	26.5	57.58
5	24.21	52.48	25.42	42.05	4.25	26	55.5

Figure 3 Samples of dyed wool with the clitoria natural dye by microwave at different time intervals (2,3,4,5 min) consequently.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Figure 4 Samples of fibers dyed with the clitoria natural dye by microwave at different conc (2,4,6,8 g/L) consequently. Effect of pH on the color strength for dyed wool fibers pretreated with nano silver nitrate.

The color strength of wool fibers dyed with natural dye under investigation were affected by pH. The dye is high affinity to the fibers in acidic medium. In acidic medium, the cat ionized amino groups can absorb anionic dye molecules by the electrostatic attraction. Result showed that the color strength of wool fibers dyed with the dye under investigation gave the highest value of color strength (K/S) at pH 5 as presented in Table 3 and Figure 4.

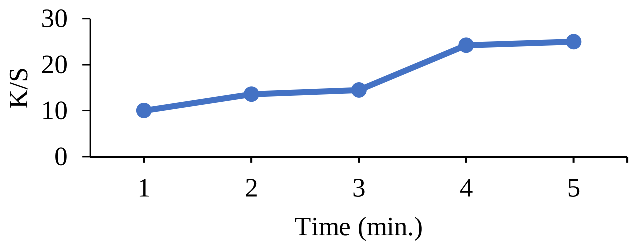

Figure 5 Effect of time on the color strength (K/S) for dyed wool with extracted from clitoria plant.

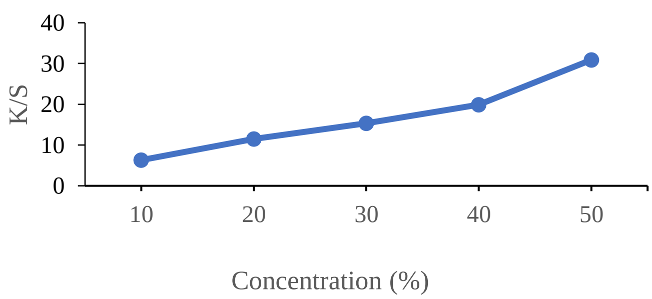

Figure 6 Effect of concentration of the dye extracted from clitoria plant on the color strength (K/S) for wool fibers

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

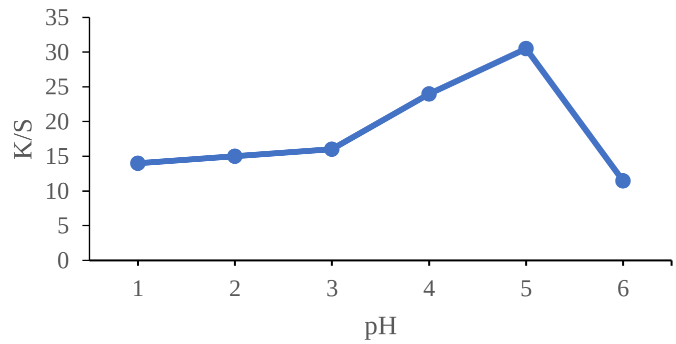

Figure 7 Relation between pH. and (K/S) for dyed fibres with extracted from clitoria plant.

Table 3 The fastness properties of the investigated dye on wool fibers are given

Fiber	K/S	Fastness to rubbing		Wash fastness			Fastness to perspiration						Light
		Wet	Dry	Alt.	Sc	Sw	Alkaline			Acidic			
							Alt	Sc	Sw	Alt	Sc	Sw	
W	24.5	3-4	3	4	3-4	3	3-4	5	4	3-4	4	4	6

C; Cotton, W; Wool, Alt= alteration; Sc= Staining on cotton; Sw =Staining on wool.

Table 4 Color data and K/S of samples at optimum condition for 5 minutes with a concentration of 2g/L and pH 5 at maximum wave length 370. Antimicrobial activty against diferent gram postive and gramnegative bacteria

Sample	K/S	L*	a*	b*	C	H
Blank	0.4	14.12	77.65	0.4	77.65	14.12
Dyed sample	24.5	52	25.42	10.65	29.35	19.73

The results perented in table (3) showed that the lowest inhibition zone was noticed by the non treated samples. On the other hand, the results slaso showed that the majore effects were on gram negative bacteria followed by the moderate effects on gram positive bacteria. Furthermore, the reduced inhibtion was notice on fungi and yeast. The varaition of the antimicriobial activity on the tested microorgaisms results in the variation on cell wall structure of each one. The peptidoglycan layer affect more that the fungi since the later contain more phospholiped inn theier cell menrane which n acts against the piment pentation depends on its lipophobicity.

Table 3 Antimicrobial activity against different gram negative m gram positive, yeast and fungi.

Microorganism	Control (Inhibition Zone cm)	Dyed wool fibers (Inhibition Zone cm)
Bacillus subtilus	1.4	21
B. cereus	1,1	2.2
E.coli	1,3	2.6
Pesudomnas	1,6	2.4

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

aeroginosa		
Candida albicans	1.7	1,8
C. tropicals	1.5	1.7
Aspergillus niger	1.3	1.5

4. CONCLUSION

It concluded that antimicrobial activity was investigated against a broad range of microorganisms including bacteria and fungi. Moreover, dyeing of wool fibers with the extracted dye innovation technique as microwave to save energy and time. The results indicated that reflected positively on the zones of growth inhibition of wool fibers. E coli gave the highest diameter of the inhibition zone. On the hand, the applied dye showed minimum effect on C. alibican and A niger. The effect of dyeing concentration, pH, temperature, and time of dyeing procedure were elucidated. It can be concluded that the increase in extracted dye concentration, there was decreased in L* values, and thus the color of the samples got darker. As the dye concentration increased, a* and b* values increased in the positive direction. The color of dyed wool fibers turned to reddish blue color and became darker with increased dye concentration from 2 to 8 g/ L.

Ethical issues

Not applicable.

Informed consent

Not applicable.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abreu, MLC; Vieira, RAM.; Rocha, NS; Araujo, RP; Gloria, L S; Fernandes, AM.; de Lacerda, PD and Junioe, AG (2014). Clitoria ternatea L as a potential high quality forage legume. Asian Australas J Anim Sci 27 (2) 169-178.
2. Ali NF; El-Mohamedy RSR (2011). Eco-friendly and protective natural dye from red prickly pear (Opuntia Lasiacantha Pfeiffer) plant Journal of Saudi Chemical Society, 15: 257–261.
3. Ali, NF, El-Khatib, EM, El-Mohamedy, RSS, Ramadan, MA (2014). Anrimicrobial activity of silk fabrics dyed with saffron dye using microwave heating. Int J Curr Microbiol App Sci 3 (10): 140-146.
4. Ali, NF; El-Khatib, EM.; El-Mohamedy, RSR (2015). The antimicrobial activity of silk fabrics dyed with natural dye. International Journal of Current Microbiology and Applied Sciences, 4 (6): 1166-1173.
5. Ali, NF and Abd-Elsalam, IS (2022). Improvement of wool dyeing quality and its antimicrobial activity using nano forms of silver. International Journal of Agricultural Technology. 18 (2): 437-446.
6. Ali1, NF, El-Mohamedy, RSR (2011). The antimicrobial activity of pretreated silk fabrics dyed with natural dye. Journal of Saudi Chemical Society, 15 : 257–261.
7. Ali1, NF ; El- Khatib, EM ; El- Mohamedy, RSR ; Nassar, SH and El-Shemy, NS (2019). Dyeing Properties and Color of Wool Fabric Dyeing With Rhubarb as Natural Dye Via Ultrasonic and Conventional Methods Egypt. J Chem Vol 62, No1 pp.
8. Avadi, MR.; Sadeghi, AMM.; Tahzibi, A.; Bayati, KH; Pouladzadeh, M.; Zohuriaan Mehr, M J and Rafiee Tehrani, M (2004). Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. European Polymer Journal, 40 (7): 1355-1361.
9. Azima, AS, Noriham, A, Manshoor, N (2017). Phenolics, antioxidants and color properties of aqueous pigmented plant extracts: Ardisia colorata var. elliptica, Clitoria

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

ternatea, Garcinia mangostana and Syzygium cumini. Journal of Functional Foods, 38, 232-241.

10. Barik, DP; Naik, SK; Mudgal,A and Chand, PK (2007). Rapid plant regeneration through in vitro axillary shoot proliferation of butterfly pea (Clitoria ternatea L)—a twinning legume. In Vitro Cellular and Developmental Biology- Plant, 43 (2): 144-148.
11. Divya Lekshmi RB and Ravi D (2013).Extraction of natural dyes from selected plant sources and its application in fabrics. International Journal of Textile and Fashion Technology, 3 (2): 53-60.
12. El-Khatib, EM.; Ali, NF and Ramadan, MA (2014). Environmentally friendly dyeing of silk fabrics using microwave heating. Int J Curr Microbiol Appl Sci, 3, 757-764.
13. Gao, Y; Cranston, R (2008). Recent advances in antimicrobial treatments of textiles. Textile research journal, 78 (1): 60-72.
14. Hebeish, AA.; Ali, NF and Abd El-Thalouth, J I. (2012). Green strategy for development of antimicrobial printed textile fabrics. Research Journal of Textile and Apparel 16: 77-81.
15. Kanchana, R.; Fernandes, A.; Bhat, B.; Budkule, S; Dessai, S and Mohan, R (2013). Dyeing of textiles with natural dyes-an eco-friendly approach. International Journal of Chem Tech Research, 5 (5): 2102-2109.
16. Mahala, AG; Amasiab, SO; Yousif, MA and Elsadig, A (2012). Effect of plant age on DM yield and nutritive value of some leguminous plants (Cyamopsis tetragonoloba, Lablab purpureus and Clitoria (Clitoria ternatea). International Research Journal of Agricultural Science and Soil Sciencem, 2 (12): 502-508.
17. Makasana, J; Dholakiya, BZ; Gajbhiye, NA and Raju, S (2017). Extractive determination of bioactive flavonoids from butterfly pea (Clitoria ternatea Linn) Research on Chemical Intermediates, 43 (2): 783- 799.
18. Mukherjee, PK.; Kumar, V; Kumar, NS and Heinrich, M. (2008). The Ayurvedic medicine Clitoria ternatea—from traditional use to scientific assessment. Journal of ethnopharmacology, 120 (3): 291-301.
19. Patil, SB; Kondawar, MS; Shah, RR.; Naikwade, NS and Magdum, CS (2009). Use of flower extract of Clitoria ternata L as a compound indicator. Journal of phaemacy Research, 2 (2): 241-242.
20. Singh, O P (2000). Natural dyes: The pros and cons Indian textile journal, 110 (4), 42-47.
21. Suebkhampet, A and Sotthibandhu, P (2012). Effect of using aqueous crude extract from butterfly pea flowers (Clitoria ternatea L) as a dye on animal blood smear staining. Suranaree J Sci Technol, 19 (1): 15-19.
22. Thiry, MC (2001). Small game hunting; antimicrobials take the field. AATCC Rev 1 (11): 11- 17.
23. Zhou, Y; Zhang, J; Tang, R C and Zhang, J (2015). Simultaneous dyeing and functionalization of silk with three natural yellow dyes. Industrial crops and products, 64:224-232.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS