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Extraction of Dye from *Castanopsis indica* for Its Use in Textile Dyeing and Medicinal Purpose with Natural Mordant

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Abstract

The environment-friendly natural dyes recently rejuvenated due to the mutagenic and sensibilizing manner of many synthetic dyes. An attempt has been made to test the inherent bio colorant property and medicinal characteristics of the bark of *Castanopsis indica* (English name; chestnut and local name; *Katus*) via green technique using water and ethanol as solvent. Dyeing of fabric was accomplished under optimized conditions with the application of natural (such as *Aloe vera*, mango's bark extract), and commercial $CuSO_4$ mordant. The extract was tested for antimicrobial properties, phytochemical screening, and characterized by ultraviolet-visible (UV-Vis) and Fourier transform infrared (FTIR) spectroscopy. Most importantly, phenols specifically flavonoids and tannins were found to be present among various phytoconstituents which are responsible to provide colors to the fabric in each dye extract. Besides, two of the extracted dye is demonstrated to be active against fungal strains *Candida albicans* (*C. albicans*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) to bacterial pathogens. The results revealed the coloring of fabric with water extracted *C. indica* dye with organic and synthetic mordant is to be bright colored compared to dull color from ethanol extract.

Keywords: *Castanopsis indica*, natural dyes, natural mordant, phytoconstituents

Introduction

Dyeing is the process of imparting hues and tints to yarns, fabric, leather, cosmetics, and other materials [1]. Bio-colorant obtained from natural sources are renewable and sustainable bio-resource products, with the minimum environmental impact that are cutting down the use of high-priced metal complex sensitizers as well as replacing expensive chemical synthesis processes through simple extraction methods [2]. Recently, because of the worldwide concern over the use of eco-friendly and bio-degradable colorant along with rigorous environmental standards imposed by many countries in response to noxious and hypersensitive reactions associated with synthetic dyes, interests in the use of natural dyes has been growing rapidly [3,4]. Natural dyes are more favored due to their nonhazardous and wide applicability in pharmaceuticals, food, cosmetics, leather, and other art of dyeing [5]. Besides, limitation on color yield and poor fastness properties of natural dyes prompted a search for ideal mordants that create an affinity between the fiber and the dyestuffs and intensifies the property of natural dye uptake by textile fibers [6]. Some common mordants are an alum, chrome, stannous chloride, copper sulfate, ferrous sulfate, and so forth. Although these metallic mordants contribute to the increased colorfastness property of natural dye and have their benefits, most of them are virulent. Thus, to reduce its effect, natural mordants are recommended, as they can provide fabrics with similar characteristics to those provided by synthetic mordants [7].

The dye-yielding plants that are used for coloration purposes are often sorted with medicinal properties and are shown to acquire strong anti-microbial activity. Plant pigments such as anthocyanins and carotenoids have been scientifically validated to possess antioxidant and anti-inflammatory

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benefits [8,9]. The medicinal values of plants are almost related to the bioactive phytochemical constituents such as alkaloids, flavonoids, essential oils, tannins, terpenoids, saponins, phenolic compounds, etc. that produce definite physiological action on the human body. The presence of different protective phytochemicals makes organic dyes a remarkable anti-inflammation, antibacterial, antioxidant, antiallergic, antiviral as well as anti-carcinogenic in nature [10].

Showing concern towards nature as well as medicinal value, *Castanopsis indica* (*C. indica*) from the Fabaceae family, commonly known as Katus (Nepali), and Indian chestnut tree (English) which is found throughout the Northeast Himalayan region of Nepal. India, Bhutan, Thailand, Vietnam, and Bangladesh were also famous for the extraction of natural dye [11]. The plant has proved to show a great therapeutic effect and is used to cure chest pain, skin disease, and stomach disorder [10]. Previously antioxidant activity, phytochemical screening, total flavonoid, and phenolic content were determined from this plant [12]. Antitumor and free radical scavenging activity of *C. indica* were also investigated in the literature [11,13]. But textile dyeing from this plant has not been studied scientifically; therefore an effort has been made in coloring fabric from natural dye extracted utilizing ethanol and water as a solvent, both solvents being eco-friendly. This study focused on the use of natural mordants (*aloe vera* leaf extract, mango bark extract) as well as synthetic ones ($CuSO_4$) to compare the effective shades provided by two of them. Along with this, the study also aimed to subject extracted dye to phytochemical analysis, anti-microbial tests and characterize it by various advanced analytical techniques to achieve the medicinal worth of the plant.

Experimental

Plant material collection and dye extraction

The bark of the chestnut tree was collected from the forest of Gulmi district of Nepal and it was authenticated as *Castanopsis indica* (Roxb. ex Lindl.) by the National Herbarium and Plant Laboratories, Godawari, Lalitpur and Botany Department, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal. It was cleaned, rinsed with water, shade-dried for a week, and ground into the powdered form by a mechanical grinder. 50g of powder was subjected to Soxhlet extractor with solvent, water, and ethanol separately to sample ratio 1:10 for 4-5 hours. The solution is concentrated on a rotary evaporator followed by hot air oven-dried for about 36-48 hrs. The dry form of dye was weighed and the yield was calculated regarding the dried plant materials initially taken.

Qualitative phytochemical screening

The phytochemical screenings of alkaloids, phenols, terpenoids, tannins, glycosides, saponins, and flavonoids were carried out as suggested by Harborne [14], Bate-Smith [15], and Santosh and Vaithiyanam [10].

Evaluation of the antimicrobial activity of natural dye

The test solutions of concentration 0.5 g/mL were prepared by dissolving ethanol extract of *Castanopsis indica* (ECI) and water extract of *Castanopsis indica* (WCI) dye in their respective water and ethanol solvent [10]. Antibacterial activity of the dyes was tested against gram-positive bacteria, *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis,* gram-negative bacteria, *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella enterica subsp. Enteric pathovar. Typhi (S. Typhi)* and *Shigella dysentrae (S. dysentrae)* and fungal strains *Candida albicans (C. albicans)* and *Saccharomyces cerevisiae (S. cerevisiae).* All the microbial culture was obtained from the biological section, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The pure bacterial cultures were maintained on nutrient agar medium (NA plates) and fungal strains in saturated dextrose agar (SDA) culture slants at 4 ºC [16]. Wells of 6 mm diameter were made using sterile cork borer into Muller Hinton Agar (MHA) containing the bacterial inoculums and Muller Hinton agar with glucose methylene blue (MHA.GMB) containing fungal inoculums. Four wells were filled with test solutions and another two wells with water and ethanol as the negative control. After incubation of MHA plates for 24 hrs at 35±2 °C and MHA.GBA plates at 25±2°C for 24-48 hrs, zone of inhibition (ZOI) around the wells were calculated [16]. The minimum inhibitory concentration (MIC) was determined by observing the visible growth of the test microorganism in two-fold serial diluted antimicrobial substances in a broth culture medium. The minimum microbicidal concentration (MMC) was determined by sub-culturing the MICs cultures on suitable agar plates [17].

Optimization of concentration

Solutions of varying concentrations (0.2-1.4 g/mL) from the dyes were prepared and pure bleached cotton fabrics (plain weave 8x5 cm^2, 0.27 g/cm^2, 0.01 mm thickness) were separately dyed by exhaustion method [17] with all the solution on dye bath for 1 hr. The dye bath was allowed to cool and fabrics were taken out. Percentage absorption of cotton fabrics was calculated based on the difference in before-dyeing and after-dyeing. Considering the highest value of percentage absorption, optimum dyeing concentration for coloring cotton fabrics with WCI and ECI dyes was determined [19].

Optimization of pH

The pH range from 2.0 to 6.0 was adopted for the dyeing of cotton fabrics where dye solution of optimum concentration was used in WCI and ECI dye extract. The optimum pH was taken based on the maximum percentage absorption [19].

Optimization of time

The optimum time for dyeing of cotton fabrics with each of the WCI and ECI dyes was determined by trialing varying dyeing time ranging from 15 to 90 min under constant optimized dye concentration and pH. The dye solution giving the maximum absorption percentage is taken as optimum time [19].

Dyeing and mordanting of textile

Dyeing of cotton fabrics was carried out with optimum dyeing concentration, pH, and time while dyeing temperature for WCI and ECI colorant was maintained at 90-95 °C and 60-70 °C accordingly. Before dyeing process, cotton fabrics were immersed in hot water to facilitate uniform penetration of the dye molecules which were then washed with cold water and dried in air at room temperature. The slightly modified post mordanting techniques from Zerin and Foisal, 2010 [20] were adopted for mordanting dyed cotton fabrics where 3% of natural (mango's bark extract, *Aloe-vera* leaves extract) and commercial blue vitriol ($CuSO_4$) mordants were used in 1:20 liquor ratio of fabric to a mordant solution. Then mordanting of dyed cotton fabric was accomplished at 80 °C temperature for 60 minutes.

Spectroscopic analysis

The ultraviolet-visible (UV-vis), and Fourier transform infrared (FTIR) absorption spectra of the samples were recorded individually using T80$^+$ UV/vis spectrometer over the range of 190-600 nm and Fourier transforms infrared (FTIR: SHIMADZU, Japan using KBr pellets method, the wavelength range of 500-4000 cm^{-1}) spectroscopic methods.

Results and Discussion

Percentage Yield and qualitative phytochemical screening of extracted dyes

In this study, it was observed that yield of the coloring matter varied depending upon the extraction procedure. The percentage yield of the ethanol extract (12.85%) for *Castanopsis indica* was found to be slightly higher than the water extract (12.14%). The variation in the yield of extracted dye is due to the difference in the polarity of the solvents [21].

The qualitative phytochemical screening of dyes from *C. indica* from both water and ethanol solvents revealed the presence of terpenoids, tannins, flavonoids, phenolic compounds, and saponins presented in Table 1. Shrestha *et al.,* 2020 also depicted the presence of parallel compounds in methanol and hexane extracts of C. indica [22]. Glycosides were found to be present only in water extracted dye while alkaloids were absent in each dye extract. The presence of flavonoids, tannins, and phenolic compound indicates a good dyeing characteristic of all dyes. Plants containing glycosides are known to exert a beneficial action on the immune system, while saponin is known to have the property of precipitating and coagulating red blood cells [10]. The tannin-containing plants are used to treat inflammations of the mouth and throat and slightly injured skins whereas flavonoids are potent water-soluble antioxidants and free radical scavengers that have strong anticancer activity [10].

TABLE 1. Phytochemical screening of dye extract obtained from the bark of *Castanopsis indica* in water, and ethanol

Phytochemicals	WCI dye	ECI dye
Alkaloids	-	-
Flavonoids	±	±
Terpenoids	+	+
Phenolic comp.	±	±
Tannins	+	+
Glycosides	+	-
Saponin	+	+

Note: + Indicate Presence, - Indicate absence, ± Indicate may or may not

Evaluation of the antibacterial activity of the extract

The antibacterial activity of the natural dyes was established by the agar diffusion method and their potency was determined by measuring the diameter of the zone of inhibition (ZOI). The measurable ZOI and minimum microbicidal concentration (MMC) is illustrated in table 2 and 3 and figure 2 respectively. The, *B. subtilis. P. aeroginosa* and *S. typhii* were found to be resistant to water extracts whereas ethanol extracts revealed inappreciable ZOI against *P. aeroginsa* (6.12 mm) and *S. typhii* (6.52 mm). As an antimicrobial test of *C. indica* has not been carried out yet in any of the kinds of literature available, its activity is evaluated with other crude plant extracts. In the literature study of antimicrobial activity of stem extracts of *Tamarindus indica Lin*, analogous activity is represented where ethanol extracts showed inhibitory and MMC against bacterial pathogens [23]. Also Ishak et al., 2003 reported no antibacterial activity in water extracts comparable to this analysis from barks of C*assia alata* [24].

It is evident from the study that water extracted dye exhibited higher ZOI against fungus *S. cerevisiae* (25.99 mm) in contradiction to ethanol extract (20.79 mm) while ECI showed higher ZOI against *C. albicans* (10.41 mm) comparative to water extract (7.21 mm). Besides, the antimicrobial activity of *Tamarindus indica.* It was able to perform significant effects against bacterial strains; it was unable to display any measurable zones as well as MMC against fungus, *C. albicans* [23]. The result depicted in our investigation is in contradiction to the literature as ethanol extracts revealed higher activity against fungal pathogen to bacterial one. The differences in antifungal and antibacterial activity are because of the difference in polarity and solubility of the extracting solvents as well as disparities in the phytochemical composition and variance in the cell membrane structures of different bacterial and fungal strains [25,26].

The present study manifested the potent anti-*S. cerevisiae* (6.25 mg/mL MMC) and anti-*C. albicans* activity (12.25 mg/mL MMC) by ethanol extracted dye in contrast to WCI that needed more than 50 mg/mL concentration for *C. albicans* and only 12.50 mg/mL MMC for *S. cerevisiae*. *C. albicans* remains the most common infection-causing fungus: about 45% of clinical fungal infections are caused by *C. albicans* [25]. The literature study of *Tribulus terrestris L* also depicted similar ethanol extract potency in opposition to *C. albicans* to water extract [27]. The antifungal activity may be attributed to various chemicals detected in its extracts [27]. The low MMC values of *C. albigans* and *S. cerevisiae* is an indication of the efficacy of the plant extracts while higher MMC values for other bacteria is an indication that either the plant extracts are less effective on bacteria or that the organism has the potential of developing antibiotic resistance [23].

The lowest concentration of antimicrobial agents is the inhibition of the growth of organisms as detected by visible turbidity is the MIC. The results for MIC were interpreted based on the fact that growth occurs in the negative control and any other tubes in which the concentration of extract is not sufficient to inhibit the growth. The tubes shown in figure1 with minimum concentration of extract in which the growth was completely checked was noted as the MIC of the plant extract which is regarded as a representative for evaluation of minimum fungicidal concentration (MFC) of water extract of *Castanopsis indica* dye against test bacteria *P. aureginosa* whose activity is represented in figure 3.

Fig. 1. Determination of minimum inhibitory concentrations (MIC) of water extracted dye of *Castanopsis indica* against *S. cerevisiae* by two-fold serial dilution method

TABLE 2. Zone of inhibition (ZOIs) of dye extract against test organisms

Natural dyes	**Zone of inhibition (ZOI) in mm**				
	C. albicans	***S. cerevisiae***	***B. subtilis***	***P. aeruginosa***	***S. typhi***
WCI (water extract from the bark of *Castanopsis indica*)	7.21	25.69	0	0	0
ECI (ethanol extract from bark of *Castanopsis indica*)	10.41	20.79	0	6.12	6.52

TABLE 3. Minimum microbicidal concentrations (MMC) of natural dyes against test organisms

Natural dyes	**Minimum microbicidal concentrations (MMC) in mg/mL**				
	B. subtilis	***P. aureginosa***	***S. typhi***	***C. albicans***	***S. cerevisiae***
Water extract of *Castanopsis indica* (WCI)	ND	ND	ND	>50	12.50
Ethanol extract of *Castanopsis indica* (ECI)	ND	>50	>50	12.25	6.25

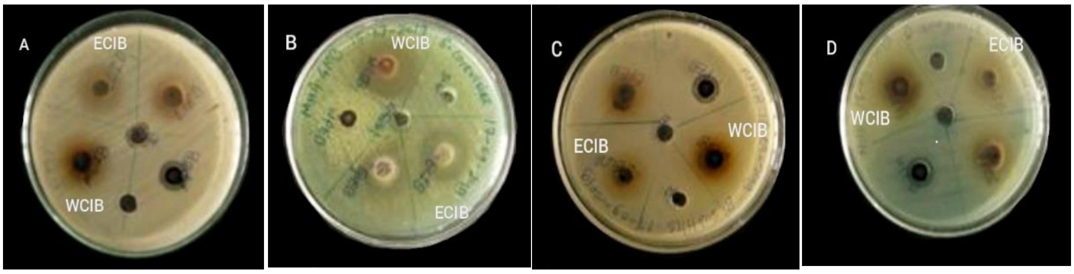

Fig. 2. ZOIs of water and ethanol extracts of *Castanopis indica* against a) *Bacillus subtilis,* b) *Pseudomonas aeruginosa,* c) *Salmonella typhi* and d) *Saccharomyces cerevisiae*

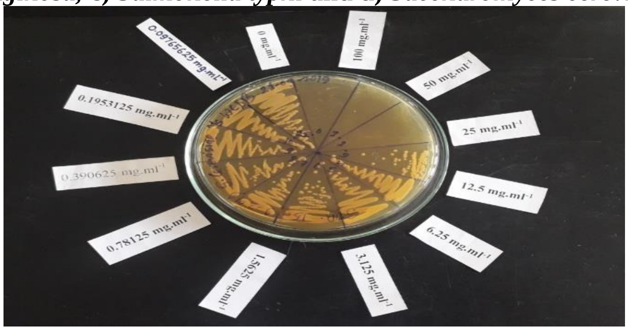

Fig. 3. Determination of MFC of water extracts of bark of *Castanopsis indica* against *S. cerevisiae* by two-fold serial dilution method

The most important thing noticed from the antimicrobial study was that both water and ethanol extracted dye of *Castanopsis indica* was more effective against fungus as compared to bacteria. Besides, the presence of phenols and flavonoids in both ethanol and water extracts as verified by phytochemical as well as FTIR and UV analysis is the reason behind the inhibition of bacteria and fungal growth [28].

Optimization of concentration

Considering the highest value of percentage of absorbance, the optimum concentration for dyeing cotton fabrics of WCI and ECI dyes are presented in table 4 where 0.8 g/mL and 0.4 g/mL is found to be the optimized dye concentration for water and ethanol extracts of *C. indica* respectively for dyeing cotton fabrics. Further, an increase in dye concentration in both the solvents does not increase the percentage adsorption capacity significantly. This may be a result of an increase in the driving force of the concentration gradient with the increase in the initial dye concentration [29]. A comparable effect of concentration on the adsorption capacity of dye in the woolen yarn is reported in Shabbira et al., 2016 [29].

TABLE 4. Optimization of dye concentration of water extracts of *Castanopsis indica* (WCI) and ethanol extracts of *Castanopsis indica* (ECI) for dyeing cotton fabrics

Dye concentration (g/mL)	**Absorbance (before-dyeing)**	**Absorbance (after-dyeing)**	**Dye-exhaustion value or Absorbance (%)**
For WCI			
0.2	0.139	0.047	66.187
0.4	0.141	0.103	26.95
0.6	0.145	0.06	58.62
0.8	0.159	0.025	**84.27**
1.0	0.161	0.082	49.068
For ECI			
0.2	0.002	0.001	50.00
0.4	0.039	0.002	**69.00**
0.6	0.070	0.046	34.28
0.8	0.663	0.648	2.262
1	0.304	0.286	5.92

Optimization of pH

The optimum pH value for dyeing cotton fabrics using WCI and ECI dyes were determined by evaluating the highest value of percentage absorption and results are expressed in table 5. The maximum absorption for WCI and ECI dye is found to be at pH 2 and 3 respectively. This suggests that an acidic medium is required for the activation of different chromophores present in WCI and ECI dyes for dyeing of cotton fabric. Ahmed et al., 2020, also proclaimed the use of the acidic medium (pH = 3-4.5) for higher color strength of dyed fabric [30]. Further, Rather et al., 2020 reported on the use of 5% (v/v) HCl that significantly improved color extraction in all conditions of temperature and time [26].

TABLE 5. Optimization of pH of water and ethanol extracts of dye from *Castanopsis indica* and dyeing cotton fabrics

Dye conc. (g/mL)	pH	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)
For WCI				
0.8	**2**	0.124	0.003	**97.58**
0.8	3	0.011	0.008	27.27
0.8	4	0.013	0.007	46.15
0.8	5	0.015	0.013	13.33
0.8	6	0.010	0.001	90.00
For ECI				
0.4	2	0.029	0.016	44.62
0.4	**3**	0.049	0.024	**51.12**
0.4	4	0.027	0.023	14.81
0.4	5	0.021	0.014	33.33
0.4	6	0.164	0.088	16.34

Optimization of dyeing time

Analyzing the results from table 6, the absorbance value for the WCI dye was found to be maximum at 90 minutes (54.86 %). This value designates that more than an hour is needed for the dye to activate the chromophores present in the dye to penetrate the cotton fabric for imparting color according to the literature reported by Saxena and Raja, 2014 [31]. As the time of dyeing increases slowly, progressively more colorant attaches to the fabric, thus creating higher absorbance until dye exhaustion attains equilibrium [19].

TABLE 6. Optimization of dyeing time of water and ethanol extracts of *Castanopsis indica* on cotton fibers

Dye conc. (g/mL)	pH	Dyeing time (min)	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)
For WCI					
0.8	2	15	1.337	1.171	12.41
0.8	2	30	1.501	1.251	16.67
0.8	2	45	1.335	1.031	22.77
0.8	2	60	1.563	1.074	31.28
0.8	2	75	1.524	1.130	25.85
0.8	2	**90**	1.511	0.682	**54.86**
For ECI					
0.4	3	15	1.256	1.121	10.74
0.4	3	30	1.277	1.100	13.86
0.4	3	45	1.339	1.121	16.23
0.4	3	**60**	1.280	1.043	**18.52**
0.4	3	75	1.349	1.115	17.34
0.4	3	90	1.278	1.116	12.67

But maximum absorption value for ECI dye is found to be at 60 mins (18.52%) which means the chromophores of ECI dye can penetrate the cotton fabric in just an hour to impart color. After 60 minutes the dye exhaustion value decreases for ECI dye. The decline in color strength is attributed to the shift in the equilibrium of the coloring component from the fabric into a dye bath and also insoluble impurities have the opportunity to sorb on fabric during longer dyeing time [18,32]. The corresponding effect of dyeing time is attributed to the optimized alkaline extraction of natural dye [18] and antimicrobial activity of wool fabric dyed with natural dye [32].

Dyeing and mordanting of fabric

An initial dye concentration of 0.8 g/mL and 0.4 g/mL, pH of 2 and 3, and dyeing time of 90 and 60 minutes for 90-95 and 60-70 °C were used as optimized conditions for dyeing of cotton fabrics using WCI and ECI dye respectively. After dyeing, all dyed fabrics were rinsed with cold water and dried in air at room temperature followed by post mordanting process. Mordanting of fabrics colored with WCI and ECI dyes produced various attractive shades on cotton fabrics under the influence of various mordants under the study was shown in figure 4.

Name of plants	**Obtained dye color**	**Color with mordants** **A- mango's extract, B- *Aloe vera* extract, C- $CuSO_4$**		
Water extract of bark of *Castanopsis indica* (WCI)			c 	
Ethanol extract of bark of *Castanopsis indica* (ECI)				b  c a

Fig. 4. Shades of dyed cotton fabric of WCI and ECI under the influence of various mordant

From the visual observation of dyed fabrics, it can be analyzed that dyeing of fabric using water extract gave brighter brownish color shade comparatively to dull shade provided by ethanol extract. The dyeing resulting with ethanol extracts obtained with less temperature (60 °C) and less dyeing time (60 minutes) had poor uniformity with more dullness in shade, while that obtained at 90 °C gave uniform and bright shade results. This phenomenon can be attributed to better solubility of color component and better penetration of the dyeing component as higher temperature and time is believed to boost the swelling of fiber and diffusion of dye inside the fabric [18,30].

The natural mordants like extract of Mango's bark and *Aloe-vera's* leaf extract are also able to fix the natural dye similar to synthetic dye as depicted in figure 4. The visual comparison of natural mordants and metallic mordants revealed that natural mordants also can be used for dyeing purposes as they also give better and brighter shades. Mordant from the bark of the mango tree revealed darker shades than mordant from *aloe-vera* in both dyes.

FTIR characterization of natural dye

Numerous peaks of water and ethanol extracts of *C. indica* are shown by the FT-IR spectroscopy which is represented in figure 5. The peak at 1024 cm^{-1} representing C-O-C stretching of an ethereal group and pyranose ring structures. The band at 3264 cm^{-1} and 3251 cm^{-1} is related to the hydroxyl group, 2935 cm^{-1} is attributed to ethanol or corresponds to C-H stretching of methyl ($-CH_3$) group [33]. The band at 1446 cm^{-1} and 1454 cm^{-1} represented CH_3, CH_2, flavonoids, and aromatic rings, where the vibrations would be the bending (δ) vibration of C-H and stretching vibration of aromatic, 1024 cm^{-1} peak is related to secondary alcohols or C-O stretching ester group. The band approximately at 1207 cm^{-1} is related to phenol, C-O stretch, and 1726 cm^{-1} illustrates stretching vibrations of -C=O group of flavonoid and tannin respectively [26]. This group (-C=O) conjugates with the ring double bonds giving a -C=C stretching vibration at 1608-1610 cm^{-1}. The transmittance band near 1317 cm^{-1} and 1319 cm^{-1} corresponds to the C-O of phenols. These spectral patterns of the extracted dyes are in fine agreement with the previous literature of IR of tannins and flavonoids [26,34,35].

The comparison of water extracted dye and ethanol extracted dye with the powdered form of the bark of Indian Chestnut tree as well as with each other exhibited slight differences in peak and sharpness. The result might be related to the differences in the polarity of the solvent used in the extracting procedure as well as metabolites varies due to the geographical distribution of plant habitat.

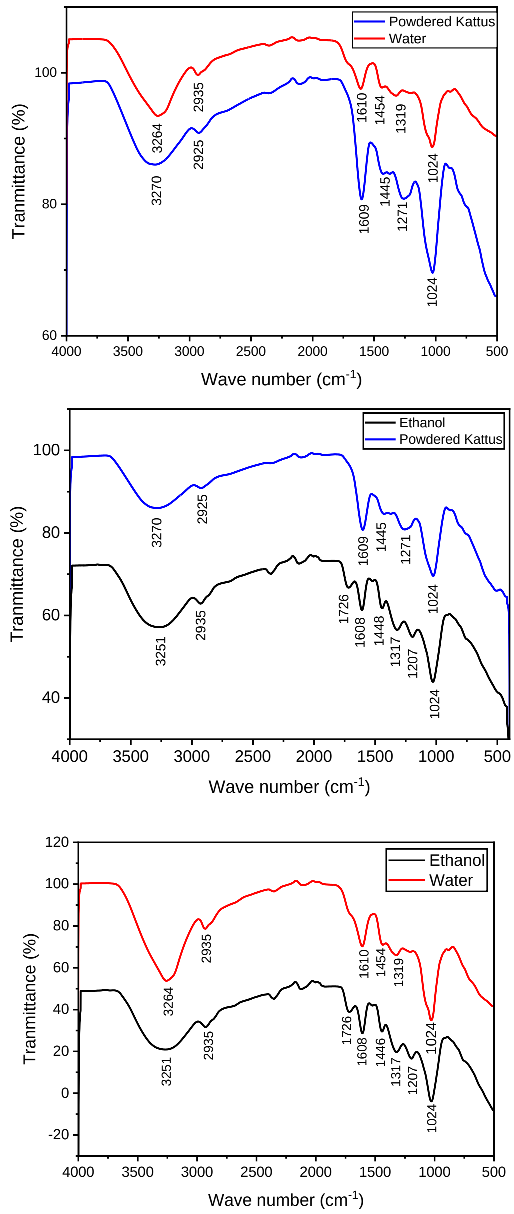

Fig. 5. Comparison of FTIR spectrum of water extract (WCI) dye and ethanol extract (ECI) with powdered Katus and with each other dye of bark of *Castanopsis indica*

UV visible spectroscopy analysis

The UV spectra of WCI dye and ECI dye (Figure 6) showed UV absorption maxima at 329 nm and 275 nm respectively. The difference in the absorbance values among the extracted dye solutions is a result of the difference in the dissolution power and polarity of respective solvents [26]. The peaks in the region of 275 to 288 represent the presence of tannin components [26]. As all the typical flavonoids have an absorption maximum at around 250-285 nm and 320-385 nm [36], the absorption maxima obtained is a characteristic spectral pattern of flavonoids. Results suggest the presence of the phenolic molecule in general and flavonoids compounds in particular in each extracted natural dyes. Various kinds of literature aided to give the conclusion that these absorptions spectra are responsible for the observed color of natural dye.

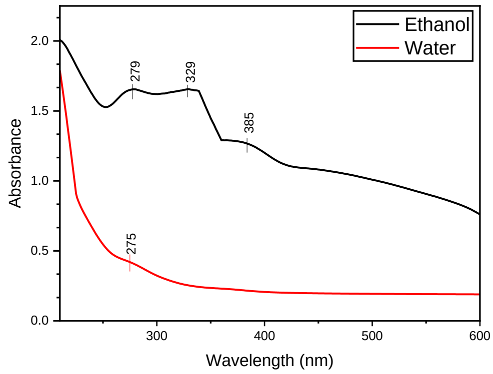

Fig. 6. UV spectrum of natural dye from bark of Castonopsis indica water extract (WCI) dye and *Castanopsis indica* ethanol extract (ECI) dye

The approximately similar peak of the UV spectrum of powdered natural dye is demonstrated in the eco-friendly dyeing of woolen yarn by *Terminalia chebula* [29]. Also, the ethanolic extract of *Phyllanthus emblica* conveyed analogous maximum UV absorption peak at 276 nm and 360 nm to this research [37].

Conclusion

The analysis has demonstrated that efficient and green extraction of natural dye from *Castanopsis indica* is possible through Soxhlet extraction methods using water and ethanol as bio-friendly solvents. The use of extracted dye through optimized concentration, pH, and time confirmed it to be a potential source of the dye as it was able to impart dull and bright colored brownish shade. *Aloe vera* and mango extract were successively able to intensify the bio-colorant activity. FTIR and UV absorbance characterization techniques proved flavonoids and tannins to be potent coloring functional groups to the cotton fabrics. The consequences of this work offer a significant reference for tint industries where naturally dyed brown colored shade is scarce in textile dyeing. Further value is added as it is confirmed from the study that the dye contains bioactive phytochemicals like tannins, phenols, and flavonoids as well as provide significant antifungal activity for its use in the biomedical textile sector.

The research can not only be limited to textile dyeing but also can be applied to the dyeing of foods, cosmetics, and leathers. Therefore, colorfastness properties, color strength, and antimicrobial properties of not only extracted dye but also dyed and undyed fabrics must be investigated to confirm their resistivity against microorganisms.

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References

1. Bhuyan R., Saikia C.N. and Das K.K., Extraction and identification of color components from the bark of *Mimusops elengi* and *Terminalia arjuna* and evaluation of their dyeing characteristics on wool. *Indian Journal of Fibre Textiles and Research,* **29**, 470-476 (2004).
2. Verma S. and Gupta G., Natural dyes and its application: a brief review. *International Journal of Research and Analytical Reviews,* **4**, 57-60 (2017).
3. Geetha and Sumathy V., Extraction of natural dyes from plants. *International Journal of Chemistry and Pharmaceutical Sciences,* **1**, 502-509 (2013).
4. Megally M., Elshemy N.S., Haggag K., and Hashem A.I., Development and optimization of a non-traditional extraction process of yellow colorant from safflower petals as a natural colorant. *Egyptian Journal of Chemistry*, **62** (2), 231-245 (2019).
5. Bhandari N.L., Pokhrel B., Bhandari U., Bhattarai S., Devkota A. and Bhandari G., An overview of research on plant-based natural dyes in Nepal: scope and challenges. *Journal of Agriculture and Natural Resources,* **3**(2), 45-66 (2020).
6. Singh S. and Singh D.R., Application of natural mordants on textiles. *International Journal of Applied Home Science,* **5**, 252-260 (2018).
7. Uddin M., Effect of different mordants on silk fabrics dyed with onion outer skin extracts. *Journal of Textiles,* **14**, 1-8 (2014).
8. Wangatia L., Tadesse K., and Moyo S., Mango bark mordant for dyeing the cotton with natural dye: fully eco-friendly natural dyeing. *International Journal of Textiles Science*, **4**, 36-41 (2015).
9. Wang Y.W., Yuen C.W., Ku S.K. and Lam H.L., Selected application of nanotechnology in textiles. *Autex Research Journal,* **6**, 111-118 (2006).
10. Santhosh T. and Vaithiyanathan T., Phytochemical screening and antibacterial activity of natural dye from *Beta vulgaris* L. *International Journal of Pharmaceuticals and Biological Science,* **8**, 741-746 (2018).
11. Dolai N., Karmakar I., Kumar R.B.S., Bala A., Mazumder U.K. and Haldar P.K., Antitumor potential of *Castanopsis indica* (Roxb. ex Lindl.) A. DC. leaf extract, against Ehrlich's ascites carcinoma cell. *Indian Journal of Experimental Biology,* **50**, 359-365 (2012).
12. Chhetri S.B. and Khatri D., Phytochemical screening, total phenolic, and flavonoid content and antioxidant activity of selected Nepalese plants. *World Journal of Pharmacy and Pharmaceutical Science,* **6**(12), 951-968 (2017).
13. Dolai N., Karmakar I., Kumar S., Kar B., Bala A. and Haldar P.K., Freee radical scavenging activity of *Castanopsis indica* in mediating hepatoprotective activity of carbon tetrachloride intoxicated rats. *Asian Pacific Journal of Tropical Biomedicine*, **2**(1), S243-S251 (2012).
14. Harborne J., *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 2nd ed, Chapman and Hall, New York, (1984).
15. Bate-Smith E.C., Phenolic constituents of plants and their taxonomic significance. I. dicotyledons. *Journal of the Linnean Society of London, Botany,* **58**, 95-173 (1962).
16. Perez C., Paul M., and Bazerque P., An antibiotic assay by the agar well diffusion method. *Acta Biologiaeet Medicine Experimentaalis,* **15**, 113-115 (1990).
17. Forbes B., Sahm D. and Weissfeld A., *Bailey & Scott's Diagnostic Microbiology*. 12th ed, Mosby Elseviver, Missouri, U.S.A, (2007).
18. Ali S., Hussain T. and Nawaz R., Optimization of alkaline extraction of natural dye from henna leaves and its dyeing on cotton by exhaust method. *Journal of Cleaner Production,* **17**, 61-66 (2009).
19. Mahilrajan S., Nandakumar J., Kailayalingam R., and SriVijeindran S., Optimization of dyeing condition and it's dyeing on palmyra (*Borassus flabellifer*) leaves. *IOSR Journal of Environmental Science Toxicology and Food Technology,* **8**, 47-52 (2014).
20. Zerin I. and Foisal A., Mordanting process on cotton dyeing with *Acacia Catechu. 3rd Textile Research Conference (TRC),* Dhaka, Bangladesh, 4-6 (2010).
21. Benmehdi H., Fellah K., Amrouche A., Memmou F., Melainine H., Dalile H., and Siata W., Phytochemical study, antioxidant activity and kinetic behavior of flavonoids fractions isolated from *Prunus persica* L. leaves. *Asian Journal of Chemistry*, **29**(1),

13-18 (2017).

22. Shrestha P., Bindu M., Prasad A.S., Timilsina H., and Ram B., Total phenolic and flavonoids contents, antioxidant activities, phytochemical and nutritional analysis of *Castanopsis indica* (Indian chestnut). *Sustainable Development*, **10**(2), 175-187 (2020).
23. Doughari J.H., Antimicrobial activity of *Tamarindus indica Linn*. *Tropical Journal of Pharmaceutical Research*, **5**(2), 597-603 (2006**)**.
24. Ishak R., Mutalib A.R., and Ismail E.N., Invitro antimicrobial activity of ethanol and water extracts of *Cassia alata*. *Journal of Ethnopharmacology*, **84**, 1-4 (2003).
25. Gupta N., Haque A., Lattif A., Narayan R.P., Mukhopadhyay G., and Prasad R., Epidemiology and molecular typing of Candida isolates from burn patients. *Mycopatholog*ia, **158**, 397-405 (2004).
26. Rather L.J., Zhou Q., Ali A., Mohd Q., Haquel R. and Li Q., Valorization of natural dyes extracted from mugwort leaves *(Folium artemisia argyi*) for wool fabric dyeing: optimization of extraction and dyeing processes with simultaneous coloration and biofunctionalization. *ACS Sustainable Chemistry & Engineering*, **8**(7), 2822-2834 (2020).
27. Al Bayati F.A. and Almola H.F., Antibacterial and antifungal activities of different parts of *Tribulus terrestris L.* growing in Iraq. *Journal of Zhejiang University Science B*, **9**(2), 154-159 (2008).

28. Abdu O.H., Saeed A.A., and Fdhel T.A., Polyphenols/Flavonoids analysis and antimicrobial activity in Pomegranate peel extracts. *Electronic Journal of the University of Aden for Basic and Applied Sciences*, **1**(1), 14-19 (2020).
29. Shabbira M., Rathera L.J., Islama S., Bukharia M.N., Shahida M., Khanb M.A., and Mohammada F., An eco-friendly dyeing of woolen yarn by *Terminalia chebula* extract with evaluations of kinetic and adsorption characteristics. *Journal of Advanced Research*, **7**, 473-482 (2016).
30. Ahmed N.S.E., Nassar S. H. and El-Shishtawy R.M., Novel green coloration of cotton fabric. part I: bio-mordanting and dyeing characteristics of cotton fabrics with madder, alkanet, rhubarb, and curcumin natural dyes. *Egyptian Journal of Chemistry*, **63** (5), 1605 - 1617 (2020).
31. Saxena S. and Raja A., *Natural Dyes: Sources, Chemistry, Application, and Sustainability Issues*. S.S. Muthu ed, Mumbai, (2014).
32. Ali N.F., EL. Mohamedy R.S.R. and Khatib E.M., Antimicrobial activity of wool fabric dyed with natural dyes. *Research Journal of Textile and Apparel*, **15**(3), 1-10 (2011).
33. Coates J., Interpretation of Infrared Spectra; *A Practical Approach in Encyclopedia of Analytical Chemistry*. RA Meyers ed, J. Wiley & Sons Ltd, Chichester, 8217-8240 (2000).
34. Oliveira R.N., Mancini M.C., Oliveira F.C.S., Passos T.M., Quilty B., Thiré R.M.S., and McGuinness G.B., FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Revista Materia*, **21**(3), 767-779 (2016).
35. Hasana H. and Desalegh E., Characterization and quantification of phenolic compounds from the leaf of *Agarista Salicifolia*. *Herbal Medicine*, **3**, 1-5 (2017).
36. Kumar S. and Pandey A.K., Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, **2013**, 1-16 (2013).

37. Shukla P., Shukla P. and Gopalkrishna B., Isolation and characterization of polyphenolic compound question from *Phyllanthus emblica*. *International Journal of Pharmaceutical Sciences and Research,* **3,** 1520-1522 (2012).