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Green Extraction, Characterization, Applications and Antimicrobial Analysis of Natural Dye from *Phyllanthus emblica*

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Global consciousness towards the organic value of eco-friendly products has attracted the interest of people towards the use of naturally dyed textiles and organic mordants. The present study has been focused not only on exploring the bio-colourant activity of *Phyllanthus emblica* (Amala) but also on the application of natural mordant for textile dyeing and analysis of its medicinal properties. It has been discovered from the investigation that biomordants like extract of *Aloe vera*'s as well as extract of mango's bark extract were able to evince their characteristic colour ameliorate behaviour close to synthetic ones. Besides, the disparity in absorbance band in ultraviolet spectroscopy, distinction in functional groups and differences in surface morphology of two extracted dyes were observed that provided information on colour variation in the cotton fabrics. An eagle gray shade and brown-hued on the cotton fabric were noticed from water and ethanol extracted dyes, respectively. Further, it is confirmed that the natural dyes contain bioactive phytochemicals like tannins, phenols and flavonoids that provide a significant antibacterial activity which will help it to be beneficially utilized in protective medical clothing.

Keywords: Natural dyes, *Phyllanthus emblica*, Natural mordants, Phytochemicals, Antimicrobial.

INTRODUCTION

Natural colourant has been utilized and exploited not only in the colouration of textiles but also for food ingredients, leather and cosmetics since antiquity [1]. But its use has been declined with the development of synthetic dye as artificial dyes are produced in cheaper ways with moderate to excellent fastness. Lately because of worldwide concern over the use of eco-friendly and biodegradable materials, the use of chemical dyes (azo and benzidine) is banned because of its toxic, carcinogenic and allergic effect [2]. This fact has motivated the revitalization of natural dye because of its biodegradability and eco-friendly nature [2]. Dyes are intense colouring organic substances used to impart colours to textile fabrics and sometimes to leather, wood, bone and other materials [3].

Dyes obtained from natural sources are renewable and sustainable bioresource products known to have a minimum environmental impact [1]. The substantive nature of natural dyes *i.e.* limitation on colour yield, insufficient shades and inadequate fastness property prompted a search for ideal mordant, the chemicals, which increase natural dye uptake by textile fiber [4]. However, studies have suggested that not all the mordants used are energy efficient and non-polluting thereby, it is essential to inspect bio-friendly natural mordants and check their capability and sustainability for better fastness properties against the noxious synthetic mordant. Similarly, the natural dyes are colour-fast with fibers; therefore, the use of mordant, which increases the dye fastness to the fabric is necessary [5]. Plant based mordants such as pomegranate rind, juniper needles, *Aloe vera*, mango bark, *etc*. [4] are eco-friendly and can be used as natural mordants.

Certain organic dyes and colourants not only been used to colour the textile but have been proven to provide ultraviolet (UV) protection, insect repellent functions [1] and medicinal healing qualities, *e.g.* turmeric, the brightest of naturally occurring yellow dyes is a powerful antiseptic which revitalizes the skin, while indigo gives a cooling sensation. *Punica granatum* L. and many other natural dyes such as walnut and alkanet are reported as potent antibacterial and antifungal agents [6]. The medicinal potential of the plants lies in bioactive phytochemical constituents such as alkaloids, phenolic compounds, saponins, terpenoids, flavonoids, essential oils, *etc*. The presence of different protective bioactive phytochemicals makes natural dye a remarkable anti-inflammation, antibacterial, antioxidant, anti-allergic, antiviral as well as anticarcinogenic agent [7].

Exhibiting interest towards the environment and healthy prospect, the bark of *Phyllanthus emblica* as a dye yielding plant belonging to phyllanaceae family locally called Amala in Nepal and commonly called Indian gooseberry was examined. It is an antioxidative plant containing tannins whose fruit renders valuable antiscorbutic in fresh as well as in dry condition and is employed for treatment of anemia and jaundice [8]. Chemical constituents, anti-collagenase, anti elastage, quantification of gallic acid from fruits, antioxidant activity as well as anti-microbial activity of *Phyllanthus emblica* has been studied by various researchers for different medicinal properties but dye extraction from the bark of the plant has not been explored so far [9-11]. Therefore, this research is inclined to achieve the legitimate dyeing of textile by eco-friendly extraction of natural dye using ethanol and water as a solvent with the assistance of different natural (mango's extract and *Aloe vera*'s extract) and synthetic mordant ($CuSO_4$). The study is also focused on to characterize aqueous and ethanol dye extract of the plant by various advanced analytical techniques as well as to determine and compare the phytochemical and antimicrobial activity of each of the extracts.

EXPERIMENTAL

Plant material collection and extract preparation: The bark of gooseberry was collected from the forest of Gulmi district of Nepal which was authenticated as *Phyllanthus emblica* by the National Herbarium and Plant Laboratories, Godawari, Lalitpur and its herbarium species were deposited at Botany Department, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal. The barks were cleaned, shaded dried and homogeneously pulverized by a mechanical grinder. The obtained powder was subjected to a Soxhlet extractor with a weighed quantity of sample (50-100 g) and measured volume of solvent, ethanol and water separately in 1:10 MLR ratio for 4-5 h. The volume of the solution was then concentrated on a rotary evaporator and oven dried for about 36-48 h. Thus dried powdered form of dye is weighed and its percentage yields were calculated regarding the dried plant materials initially taken.

Qualitative phytochemical screening of dyes: The phytochemical compounds present in extract were analyzed as alkaloid, flavonoids, tannin, glycosides, saponin, terpenoid and phenols with the help of phytochemical screening test using standard methods [12-14].

Optimization of dyeing concentrations, pH and time: Solutions of varying concentrations (0.2-1.0 g/mL) from the water extract of *P. emblica* bark (WPEB) dye and ethanolic extract of *P. emblica* bark (EPEB) bark were prepared and pure bleached cotton fabrics (plain weave 8 cm × 5 cm, 0.27 g/cm^2, 0.01 mm thickness) were separately dyed by dye exhaustion method [15] with all the solution on dye bath for 1 h. The dye bath was allowed to cool and fabrics were taken out.

For optimum pH, varying pH ranging from 2.0 to 6.0 was adopted for dyeing of cotton fabrics using WPEB and EPEB dyes. The optimum time for dyeing of cotton fabrics was chosen from 15 to 90 min under optimized dye concentration and pH [15]. The absorbance of dye solutions before and after dyeing was recorded at the optimum wavelength. The dye solution giving the maximum percentage of dye absorption or dye exhaustion value was taken as optimum dye concentration, pH and time [16].

Dyeing and mordanting of cotton fabrics: Cotton fabrics were dyed by WPEB and EPEB dyes separately following optimum dye concentration, pH and time. Before the dyeing process, the sample fabrics were immersed in hot water to facilitate uniform penetration of the dye molecules. The dyeing temperature for cotton fabrics is maintained at 80 ºC. After dyeing, all dyed fabrics were washed with cold water and dried in air at room temperature. Post mordanting techniques were adopted for mordanting cotton fabrics for which 3% of natural (mango's bark extract, *Aloe vera*'s leaves extract), as well as commercial blue vitrol ($CuSO_4$) mordant, were used in 1:20 liquor ratio of fabric to the mordant solution. Then mordanting of dyed cotton fabric was accomplished at 80 ºC for 60 min, which is a slightly modified method from that of Zerin and Faisal [17].

UV-visible spectroscopic analysis: The ultraviolet-visible (UV-VIS) absorption spectra of WPEB and EPEB dyes were recorded using a T80$^+$ UV-Vis spectrometer over the range of 190-700 nm in the natural product laboratory of Department of Plant Resources, Government of Nepal, Kathmandu, Nepal.

FT-IR spectroscopic and SEM analysis: The Fourier transform infrared (FT-IR) spectra and surface morphology on scanning electron microscopy (SEM) of both the samples were recorded from Department of Chemistry, Korean Center for Artificial Photosynthesis (KCAP), Sogang University, Seoul, South Korea.

Antimicrobial activity of natural dyes: Test solutions were prepared by dissolving 0.5 g of WPEB and EPEB dyes in their respective water and ethanol solvents [14]. Antibacterial activity was tested against Gram-positive bacteria, *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis* and Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonellaenterica* subsp. *Enteric pathovar*, *S. typhi* and *Shigella dysentrae* and two fungal strains, *Candida albicans* and *Saccharomyces cerevesiae*. All the microbial culture was obtained from the biological section, Department of Plant Resources, Kathmandu, Nepal. The pure bacterial cultures were maintained on nutrient agar medium whereas fungal strains were maintained in saturated dextrose agar (SDA) culture slants at 4 ºC [18]. A 6 mm diameter wells on Muller- Hinton agar (MHA) plates containing bacterial strains and Muller Hinton agar with glucose methylene blue (MHA.GMB) plates containing fungal ones were punched with a sterile cork borer. Four wells were filled with test solutions of natural dye and two wells with water and ethanol as the negative control. After incubation of MHA petri-discs for 24 h at 35 ± 2 ºC and MHA.

GMB petri-discs at 25 ± 2 ºC for 24-48 h, zone of inhibition (ZOI) around the wells were measured [19]. The minimum inhibitory concentration (MIC) was determined by observing the visible growth of the test microorganism in two-fold serial diluted antibacterial substances in the broth culture medium. The minimum bacterial concentration (MBC) was determined by subculturing the MICs cultures on suitable agar plates [19].

RESULTS AND DISCUSSION

Percentage yield and qualitative phytochemical screening of extracted dyes: It was observed that yield of the colouring matter varied depending upon the extraction solvent. The percentage yield of the water extract was found maximum for *Phyllanthus emblica* (9.33 %) in contrast to ethanolic extract (6.56 %). The variation in the yield may be related to the polarity of the solvent used in the extraction procedure [20].

Qualitative phytochemical screening of WPEB and EPEB natural dye extracts chosen in this study has shown the presence of flavonoids, phenolic compounds, terpenoids, glycosides, saponins and tannins while alkaloids are absent in both dye colours (Table-1). Water extracted dye possessed maximum bioactive components concerning ethanol extracted dye. Differences in the polarity of the extraction solvents have caused a variation in the level of extraction of bioactive compounds [21]. The presence of flavonoids, tannins and phenolic compound indicates a good dyeing characteristic of the extracted colourant. Plant tannins are known to possess antibacterial, antiviral and antitumor activity while the presence of saponins in plants acts as an antioxidant, anti-inflammatory and weight loss agent. For instance, flavonoids present in the plant is considered as natural biological modifier as it can modify the body's reaction to allergies [7].

TABLE-1
PHYTOCHEMICAL SCREENING OF NATURAL DYES

Phytochemicals	EPEB dye	WPEB dye
Alkaloids	–	–
Flavonoids	+	+
Terpenoids	–	+
Phenolic compounds	+	+
Tannins	+	+
Glycosides	–	+
Saponin	–	+

Note: + Indicate Presence, – Indicate absence.

Optimization of dye concentrations, pH and time: The maximum percentage absorption for optimum dye concentration value for dyeing cotton fabrics was determined for WPEB and EPEB dyes whose outcomes are presented in Table-2. Here, the maximum percentage absorption for EPEB dye was found to be at 0.6 g/mL (35%) concentration and for WPEB dye it was found to be at 0.4 g/mL (80.12%). Further increase in concentration did not increase the dye exhaustion value. This may be a result of an increase in the driving force of the concentration gradient with the increase in the initial dye concentration [22]. The decrease in colour strength and adsorption capacity on woolen yarn on further increase in concentration is reported by Mohammed *et al.* [22] and Ali *et al.* [23], respectively.

The required optimum pH for dyeing using WPEB dye was found to be 3 and that for EPEB dye was found to be 4 (Table-3). This result designates the benefit of the acidic medium to make different chromophores active that is present in both WPEB and EPEB dyes. Also, penetration of solvent is improved through disruption of plant tissues in acidic conditions for imparting colour to the fabrics [24]. Analogous results were reported by Kayodé *et al.* [25] that upgraded the extractability of the native colourants from sorghum leaf by adding HCl to

TABLE-2
OPTIMIZATION OF DYE CONCENTRATIONS OF WATER (WPEB) AND ETHANOL (EPEB) EXTRACTS OF DYE FROM *Phyllanthus emblica* FOR DYEING COTTON FABRICS

S. N.	For WPEB				For EPEB			
	Dye concentration (g/mL)	Absorption (before-dyeing)	Absorption (after-dyeing)	Dye exhaustion value or absorption (%)	Dye concentration (g/mL)	Absorption (before-dyeing)	Absorption (after-dyeing)	Dye exhaustion value or absorption (%)
1	0.2	0.435	0.351	19.31	0.2	0.004	0.003	25.00
2	0.4	0.473	0.094	80.12	0.4	0.019	0.014	26.31
3	0.6	0.515	0.490	4.85	0.6	0.028	0.018	35.00
4	0.8	0.481	0.237	50.72	0.8	0.035	0.029	17.00
5	1.0	0.738	0.470	36.31	1.0	0.041	0.035	14.63

TABLE-3
OPTIMIZING pH OF WATER (WPEB) AND ETHANOL (EPEB) EXTRACTS OF DYE FROM PHYLLANTHUS EMBLICA FOR DYEING COTTON FABRICS

S. N.	For WPEB					For EPEB				
	Dye conc. (g/mL)	pH	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)	Dye conc. (g/mL)	pH	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)
1	0.4	2	0.014	0.002	85.71	0.6	2	0.583	0.098	83
2	0.4	3	0.017	0.008	52.94	0.6	3	0.571	0.089	84.41
3	0.4	4	0.019	0.011	42.10	0.6	4	0.581	0.089	84.68
4	0.4	5	0.018	0.007	61.11	0.6	5	0.271	0.057	78.96
5	0.4	6	0.021	0.011	47.61	0.6	6	0.250	0.042	83.2

the ethanol/water solvent extraction system. Zhou *et al.* [24] also reported the use of acidic conditions *i.e.* 5% (v/v) HCl that significantly improved colour extraction in all conditions of temperature and time [24].

Consequences of the optimized time revealed that the cotton fabric must be immersed for about 90 min (Table-4) for WPEB dye to penetrate the textile by chromophores present in it according to the description given by Saxena and Raja [26]. Moreover, for EPEB it should be dipped to about 45 min (Table-4) that indicates the fact that the required chromophores present in EPEB dye are very active as it can impart colour on the textile in just 45 min. A further increase in time resulted in a decrease in percentage absorption which may be attributed to decomposition of colouring component or desorption of dye molecule from fabric into dye bath during longer dyeing time at longer time exposure [22,27]. A comparable result was attributed to optimizing alkaline extraction of natural dye from heena leaves [15].

Dyeing of cotton fabrics and its mordanting: An initial dye concentration of 0.4 g/mL and 0.6 g/mL, pH of 2 and 4, dyeing time of 90 and 45 min at 90 ºC and 60-70 ºC correspondingly were used as optimized conditions for dyeing of cotton fabrics using WPEB and EPEB dyes, respectively. Mordanting with natural dye of dyed fabrics with WPEB and EPEB dyes produced various attractive shades on cotton fabrics under the influence of various mordants are shown in Fig. 1.

Fig. 1 showed that the dye extracted using water displayed eagle gray shade while ethanol extracted dye unveiled light brown coloured hue. Under simple water bath extraction conditions, the optimized extraction time and temperature for WPEB dye were 90 ºC and 90 min whereas for EPEB dye is 60-70 ºC and 45 min. Since high temperature decreases solvent viscosity, increases kinetic energy and enables more penetration into the matrix of fibers that enhances dye/colourant extraction [24] the variability in the tone of WPEB and EPEB dye must be related to temperature and dyeing time parameters. Besides longer dyeing time and temperature cause the insoluble impurities to absorb on fabric and dissimilarity in colourant shade was obtained [23].

Along with that, natural mordants like extract of mango's bark and *Aloe vera* were also able to fix the natural dye. The visual comparison of natural mordants with metallic mordants revealed that natural mordants can also be used for dyeing purposes as they also manifested brighter and similar colour shades. Mordant from the bark of the mango tree exhibited darker shades almost in both the fabrics than mordant from *Aloe vera*. Except in the case of water extract of *Phyllanthus emblica* (WPEB) dye, *Aloe vera* displayed the best result as it fixed the black colour produce by WPEB dye and imparted a dense black hue.

UV visible spectroscopic analysis: The UV-Vis spectrum of *P. emblica* in ethanol solution (Fig. 2) is characterized by

TABLE-4
OPTIMIZATION OF DYEING TIME OF WATER (WPEB) AND ETHANOL (EPEB) EXTRACTS OF DYE FROM *Phyllanthus emblica* FOR DYEING COTTON FABRICS

S. N.	For WPEB						For EPEB					
	Dye conc. (g/mL)	pH	Dyeing time (min)	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)	Dye conc. (g/mL)	pH	Dyeing time (min)	Absorbance (before-dyeing)	Absorbance (after-dyeing)	Absorbance (%)
1	0.4	2	15	1.369	1.184	13.51	0.6	4	15	0.749	0.449	40.05
2	0.4	2	30	1.182	0.778	34.17	0.6	4	30	0.755	0.408	45.96
3	0.4	2	45	1.260	0.549	56.42	0.6	4	45	0.737	0.275	62.68
4	0.4	2	60	1.265	0.534	57.78	0.6	4	60	0.749	0.336	55.14
5	0.4	2	75	1.389	0.537	61.33	0.6	4	75	0.700	0.324	53.71
6	0.4	2	90	1.421	0.531	62.63	0.6	4	90	0.731	0.469	35.84

Fig. 1. Shades of dyed cotton fabric of WPEB and EPEB dye under the influence of various mordant

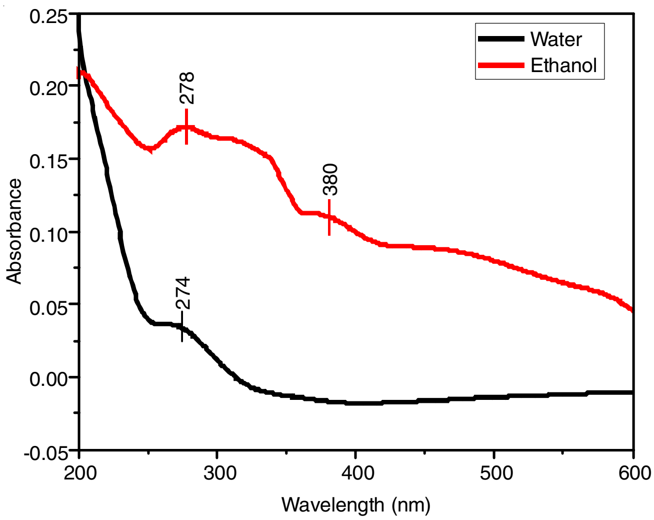


Fig. 2. UV-visible spectrum of water extract of *P. emblica* (WPEB) dye and ethanol extract of *P. emblica* (EPEB) dye

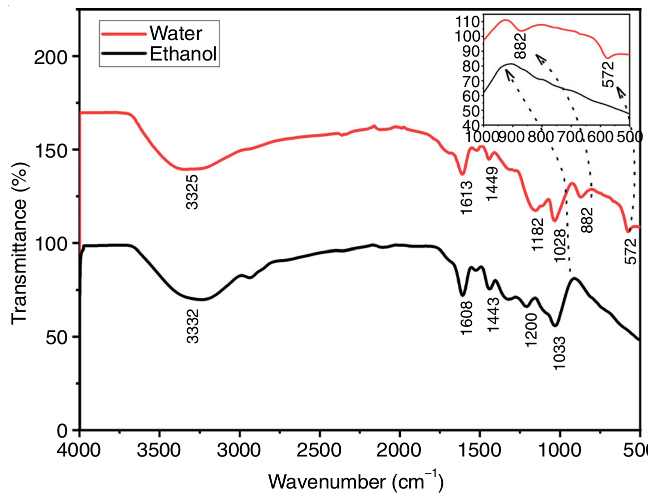


Fig. 3. FTIR spectrum of water extract of *P. emblica* (WPEB) dye and ethanol extract of *P. emblica* (EPEB) dye

two major absorption bands with maxima at 278 nm and 380 nm while in aqueous solution by only one absorption maxima at 274 nm. As all the typical flavonoids have an absorption maximum at around 250-285 and 320-385 nm [28], each extract suggested the presence of the phenolic molecule in general and flavonoids compounds in particular. For natural dyes, the absorbance spectra especially indicate different peaks for mixed colourants available in their extract [29]. The colour differences in water and ethanol dyed sample might be related to the absorbance of mixed colourants given by different groups of flavonoids as various groups are responsible for the production of various colourants. Flavones, flavonols are the main chromophores responsible for yellow to brown colour and anthocyanins for red to purple and blue colour [30]. Furthermore, various kinds of literature help to confirm that these absorptions spectra are responsible for the observed colour. A similar peak of the UV spectrum of powdered natural dye is demonstrated in eco-friendly dyeing of woolen yarn by *Terminalia chebula* [22]. Also, the ethanolic extract of *Phyllanthus emblica* showed analogous maximum UV absorption peak at 276 nm and 360 nm [31].

Ethanol extracted dye exhibited a somewhat proper and strong absorption band against water extracted dye. As the nature of the solvent has a significant effect on the extraction capacity, the difference in the absorbance values among the extracted dye solutions of water and ethanol is a result of the difference in the dissolution power and polarity of respective solvents [24].

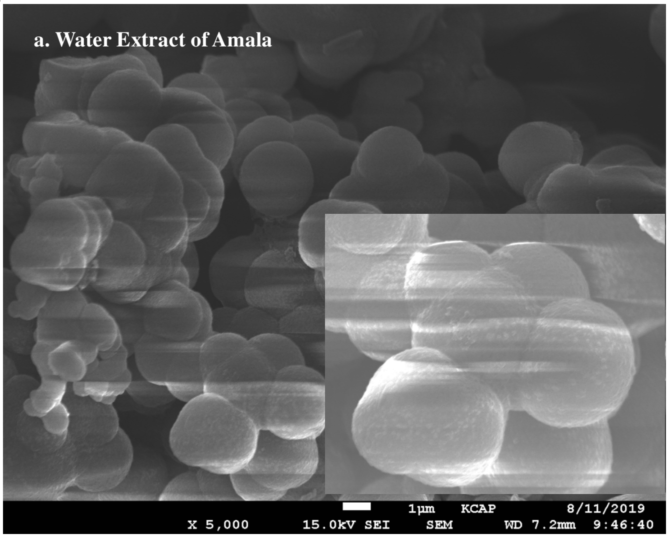
FT-IR analysis: FT-IR analysis showed the presence of various functional groups in ethanol and water extract of *P. emblica* (Fig. 3). The bands at 3325 and 3332 cm^{-1} indicate broad and strong -OH stretching vibration of benzene rings of tannin/flavonoid bearing intermolecular H bonded framework and methyl groups whereas absorption bands at 1608 and 1613 cm^{-1} illustrates stretching vibrations of aromatic C=C. The C-O-C stretching of the ethereal group and pyranose ring structures appeared at 1033 and 1028 cm^{-1}. The transmittance band at around 1443 and 1449 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 aromatics, respectively. The band approximately at 1200 and 1182 cm^{-1} is related to phenol, C-O stretch. The spectral peak at 882 cm^{-1} is associated with aromatic ring vibration *i.e.* C-H bond whereas peaks from 720-590 cm^{-1} spectral peak belong to out of a plane bend or wagging vibration of O-H [32].

On correlating the obtained results with the studied spectra as of FTIR of natural dyes in different kinds of literature [24,33] these spectral patterns are indicative of the presence of phenolics constituents, most possibly flavonoids as colourants in both the dyes. Slight peak dissimilarity and sharp and strong peaks of ethanol against weak peaks in water extracted colourant are observed in the above spectra [34]. The differences can be related to different classes of flavonoids.

SEM analysis: The surface morphology of natural dyes from water and ethanol extracts is presented in Fig. 4. Water extracted dye showed somewhat irregular oval bulky groups attached while ethanol extracts revealed heterogenous irregular curved or bean-like appearance. The distinction result in the surface morphology of each extract might be related to the dissimilar molecular composition of the extracts. The variability of colours in their tone in each dyed fabric can also be connected to this result.

Antimicrobial activity of natural dyes: The antifungal and antibacterial activity of the natural dyes were established by the agar diffusion method and their potency was determined by measuring the diameter of growth of inhibition (ZOI). The zone of inhibition and minimum microbicidal concentrations of natural dyes against microbial pathogens are shown in Tables 5 and 6, respectively.

The water extracted dye of *P. emblica* showed 12.81 mm ZOI against *P. aeruginosa* followed by *B. subtilis* (11.96 mm) and *S. typhi* (11.80 mm) whereas no activity was revealed by ethanol extract against these bacteria except for *P. aeroginosa* (6.48 mm). The result of Nahor and Ahmed [35] for *Phyllanthus emblica* extracts is in good accordance with our analysis as water extracts revealed higher antimicrobial properties compared to ethanol. The natural dye extracts from *Quercus infectoria* (13 mm from 10 mg concentration) and *Acacia catechu* (13



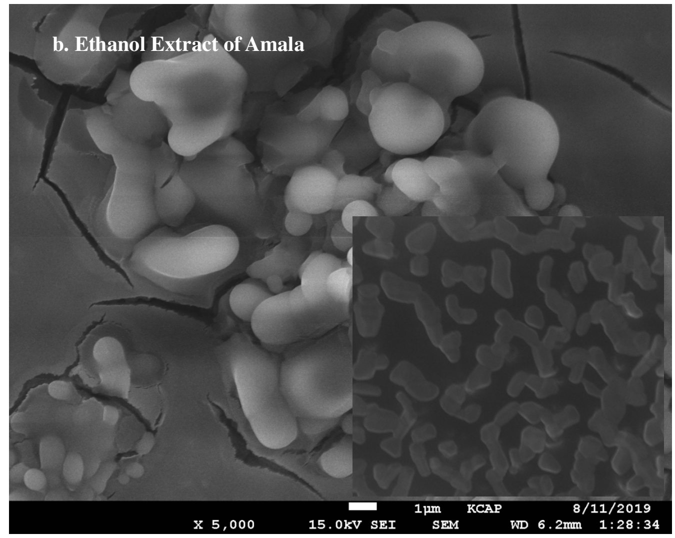


Fig. 4. Surface morphology of water extract (WPEB) dye and ethanol extract (EPEB) dye of bark of *Phyllanthus emblica*

TABLE-5
ZONE OF INHIBITIONS (ZOIs) OF NATURAL DYES AGAINST TEST ORGANISMS

Natural dyes	Diameter of zone of inhibition (mm)				
	B. subtilis	*P. aeruginosa*	*S. typhi*	*C. albicans*	*S. cerevisiae*
Ethanol extract of *Phyllanthus emblica* (EPEB)	0	6.48	0	0	6.42
Water extract of *Phyllanthus emblica* (WPEB)	11.96	12.81	11.80	7.12	0

TABLE-6
MINIMUM MICROBICIDAL CONCENTRATION (MMC) OF NATURAL DYES AGAINST TEST ORGANISMS

Natural dyes	Minimum microbicidal concentration (MMC) (mg/mL)				
	B. subtilis	*P. aureginosa*	*S. typhi*	*C. albicans*	*S. cerevisiae*
Ethanol extract of *Phyllanthus emblica* (EPEB)	ND	> 50	ND	0	> 50
Water extract of *Phyllanthus emblica* (WPEB)	12.50	6.25	25	> 50	0

mm from 40 mg concentration) also displayed comparable ZOI against *P. aeroginosa* and *B. subtilis* to this investigation [36]. For fungal pathogens, the WPEB dye exhibited 7.12 mm ZOI against *C. albicans* yet no antifungal activity against *S. cerevisiae* while EPEB dye showed nil activity against *C. albicans* but a measurable zone of inhibition against *S cerevisiae* (6.42 mm). Besides, literature revealed that even the water extracted dyes of *Punica granatum*, *Eucalyptus globulus* demonstrated antifungal activity against *S. cerevisiae* [37]. The disparities in the phytochemical composition of various extracts and variance in the cell membrane structures of different bacterial and fungal strains might be the reason behind the difference in antimicrobial activity [24].

Water extract of *Phyllanthus emblica* (WPEB) dye displayed the potent anti-*B. subtilis, P. aeruginosa* and *S. typhi* activity recorded as 12.50 mg/mL, 6.25 mg/mL and 25 mg/mL still zero potencies by ethanol extracted dye against test organisms except for *P. aeruginosa* (> 50 mg/mL). Apart from present study, the water extract of *P. emblica* revealed only 0.06 mg/mL MBC against *S. typhi* and 0.59 mg/mL MBC against *B. subtilis*. Even the ethanol extract of *P. emblica* in Nahor and Ahmed [35] illustrated MBC against different test organisms. The low MMC values of bacterial pathogens by water extracts is an indication of the efficacy of the plant extracts while the higher MMC values for fungus and bacterial strains by ethanol extracts is an indication that either the plant extracts are less effective on fungal strains or that the organism has the potential of developing antibiotic resistance [38].

The lowest concentration of antimicrobial agent 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 with a 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 bacterial concentration (MBC) of water extract of *Phyllanthus emblica* (WPEB) dye against test bacteria *P. aureginosa*.

Therefore, from the evaluation of functional properties of natural dyes, it can be concluded that the water extract of *Phyllanthus emblica* dye can be used as an antibacterial agent to control some bacterial pathogens but seems to be ineffective against the fungal strains chosen in this study.

Conclusion

The present investigation unveiled a green and environment friendly approach for the establishment of eco-friendly hues on fabrics from organic dyes extracted using two non-polluting solvents ethanol and water from *Phyllanthus emblica*. The dyed cotton fabric under optimized conditions exhibited acceptable colouring properties by the use of natural mordant (mango's bark and *Aloe vera* leaves) as that of artificial mordant ($CuSO_4$) on visual observations. The UV-vis, as well as FTIR spectroscopic analysis, manifested slight differences in absorption band between ethanol (278 nm) and water (270 nm) and transmittance band in IR evaluation. Both bands are the features of flavonoids but different classes of flavonoids which are morphologically differentiated in SEM imaging. All the characterized values and differences in optimized time and temperature parameters guide towards the colour variation created by ethanol (brown) and water (eagle gray) extracted dyes. In addition to this, phytochemical and antimicrobial analysis proved *P. emblica* to be an excellent medicinal important plant due to the presence of protective phytochemicals such as phenols, tannins and flavonoids in each extract. Moreover, water extracted dye comparatively to ethanol possessed the highest antimicrobial activity against bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*) and fungi (*Candida albicans*).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A.K. Samanta and P. Agarwal, *Indian J. Fibre Text. Res.*, **34**, 384 (2009).
2. L.J. Rather, S. Akhter, R.A. Padder, Q.P. Hassan, M. Hussain, M.A. Khan and F. Mohammad, *Dyes Pigments*, **139**, 812 (2017); https://doi.org/10.1016/j.dyepig.2017.01.018
3. O. Abdel-Kareem, *Res. J. Text. Apparel*, **16**, 79 (2012); https://doi.org/10.1108/RJTA-16-04-2012-B009
4. S. Singh and D.R. Singh, *Int. J. Appl. Home Sci*., **5**, 252 (2018).
5. N.L. Bhandari, K. Bist, J. Ghimire, S. Chaudhary, D.P. Pandey and R. Adhikari, *J. Inst. Sci. Technol.*, **25**, 30 (2020); https://doi.org/10.3126/jist.v25i1.29421
6. B. Chengaiah, K.M. Rao, K.M. Kumar, M. Alagusundaram and C. Chetty, *Int. J. PharmaTech. Res.*, **2**, 144 (2010);
7. S. Gowri and K. Vasantha, *Int. J. PharmaTech. Res.*, **2**, 1569 (2010).
8. A. Sharma, *Indian J. Appl. Res.*, **9**, 1 (2019).
9. S. Pientaweeratch, V. Panapisal and A. Tansirikongkol, *Pharm. Biol.*, **54**, 1865 (2016); https://doi.org/10.3109/13880209.2015.1133658
10. A. Sharma and S. Shailajan, *Asian J. Chem.*, **21**, 7111 (2009).
11. M. Xu, H.T. Zhu, R.R. Cheng, D. Wang, C.R. Yang, T. Tanaka, I. Kouno and Y.-J. Zhang, *Nat. Prod. Res.*, **30**, 2726 (2016); https://doi.org/10.1080/14786419.2015.1137573
12. J. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman & Hall: New York, p. 100 (1984).
13. E.C. Bate-Smith, *Bot. J. Linn. Soc.*, **58**, 95 (1962); https://doi.org/10.1111/j.1095-8339.1962.tb00890.x
14. T. Santhosh and T. Vaithiyanathan, *Int. J. Pharm. Biol. Sci*., **8**, 741 (2018);
15. S. Ali, T. Hussain and R. Nawaz, *J. Clean. Prod.*, **17**, 61 (2009); https://doi.org/10.1016/j.jclepro.2008.03.002
16. S. Mahilrajan, J. Nandakumar, R. Kailayalingam and S. Srivijeindran, *IOSR J. Environ. Sci. Tox. Food Technol*., **8**, 47 (2014).
17. I. Zerin and A. Foisal, Mordanting Process on Cotton Dyeing with *Acacia Catechu*, 3rd Textile Research Conference (TRC), Dhaka, Bangladesh (2010).
18. C. Perez, M. Paul and P. Bazerque, *Acta Biol. Med. Exp*, **15**, 113 (1990).
19. B. Forbes, D. Sahm and A. Weissfeld, Bailey & Scott's Diagnostic Microbiology, Mosby Elsevier: Missouri, U.S.A., edn 12 (2007).
20. H. Benmehdi, K. Fellah, A. Amrouche, F. Memmou, H. Malainine, H. Dalile and W. Siata, *Asian J. Chem.*, **29**, 13 (2017); https://doi.org/10.14233/ajchem.2017.20057
21. D.-H. Truong, D.H. Nguyen, N.T.A. Ta, A.V. Bui, T.H. Do and H.C. Nguyen, *J. Food Qual.*, **2019**, 1 (2019); https://doi.org/10.1155/2019/8178294
22. M. Shabbir, L.J. Rather, Shahid-ul-Islam, M.N. Bukhari, M. Shahid, M. Ali Khan and F. Mohammad, *J. Adv. Res.*, **7**, 473 (2016); https://doi.org/10.1016/j.jare.2016.03.006
23. N.F. Ali, R.S.R.E. Mohamedy and E.M. El- Khatib, *Res. J. Text. Apparel*, **15**, 1 (2011); https://doi.org/10.1108/RJTA-15-03-2011-B001
24. L.J. Rather, Q. Zhou, A. Ali, Q.M.R. Haque and Q. Li, *ACS Sustain. Chem.& Eng.*, **8**, 2822 (2020); https://doi.org/10.1021/acssuschemeng.9b06928
25. A.P.P. Kayodé, C.A. Bara, G. Dalodé-Vieira, A.R. Linnemann and M.J.R. Nout, *LWT-Food Sci. Technol.*, **46**, 49 (2012); https://doi.org/10.1016/j.lwt.2011.11.003
26. S. Saxena and A.S.M. Raja, ed.: S. S. Muthu, Natural Dyes: Sources, Chemistry, Application and Sustainability Issues, Textile Science and Clothing Technology, p. 37 (2014); https://doi.org/10.1007/978-981-287-065-0_2
27. S. Adeel, S. Ali, I.A. Bhatia and F. Zsila, *Asian J. Chem.*, **21**, 3493 (2009).
28. S. Kumar and A.K. Pandey, *Scientific World Journal*, **2013**, 1 (2013); https://doi.org/10.1155/2013/162750
29. T. Alemayehu and Z. Teklemariam, *Int. J. Res. Granthaalayah*, **2**, 61 (2014); https://doi.org/10.5281/zenodo.884121
30. O. Deveoglu and R. Karadag, *Int. J. Adv. Eng. Pure Sci.*, **3**, 188 (2019); https://doi.org/10.7240/jeps.476514
31. P. Shukla, P. Shukla and B. Gopalkrishna, *Int. J. Pharm. Sci. Res.*, **3**, 1520 (2012); https://doi.org/10.13040/IJPSR.0975-8232.3(5).1520-22
32. J. Coates, Interpretation of Infrared Spectra, A Practical Approach, In: Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation, J. Wiley & Sons Ltd.: Chichester (2006).
33. H. Hasana and E. Desalegn, *Herb. Med.*, **3**, 1 (2017); https://doi.org/10.21767/2472-0151.100022
34. M.A.M. Al-Alwani, A.B. Mohamad, A.A.H. Kadhum and N.A. Ludin, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **138**, 130 (2014); https://doi.org/10.1016/j.saa.2014.11.018
35. U. Nahor and Z. Ahmed, *IOSR J. Pharm. Biol. Sci.*, **4**, 21 (2012).
36. R. Singh, A. Jain, S. Panwar, D. Gupta and S.K. Khare, *Dyes and Pigm.*, **66**, 99 (2005); https://doi.org/10.1016/j.dyepig.2004.09.005
37. N. Comlekcioglu, A. Aygan, M. Kutlu and Y.Z. Kocabas, *Iranian J. Chem. Chem. Eng.*, **36**, 137 (2017).
38. J.H. Doughari, *Trop. J. Pharm. Res.*, **5**, 597 (2006); https://doi.org/10.4314/tjpr.v5i2.4