

# Extraction, Characterization, and Antimicrobial Evaluation of Plant-Based Natural Dyes from *Rosa* spp., *Allium cepa*, and *Curcuma longa* for Sustainable Textile Applications



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## ABSTRACT

The ecological and health aspects of synthetic dyes have brought back interest in the use of natural colorants that are plant based. In the research, *Rosa* spp. petals, *Allium cepa* peel onions and *Curcuma longa* rhizomes were extracted to natural dyes and tested on their textile applicability and functionality. They used aqueous extraction, dyed, cotton and silk fabrics using alum as a natural mordant by pre-simultane-, and post-mordanting. The color stability to washing and rubbing was determined on the basis of ISO standards. Fourier Transform Infrared (FTIR) spectroscopy was used to carry out functional characterization. Agar well diffusion was used to determine antimicrobial activity against *Staphylococcus aureus* and pH sensitivity of the dyes was also studied. The findings indicated good to moderate color fastness especially in mordanted samples, and silk had high dye affinity than cotton. The presences of phenolic, flavonoid, anthocyanin, and curcuminoid functional groups were confirmed by FTIR spectra. Each dye was antimicrobial and had a change in color with pH. The paper identifies the potential of these natural dyes as environmentally friendly multifunctional substitutes of synthetic dyes in textiles, in order to have sustainable applications.

**Keywords:** Antimicrobial activity, Color fastness, FTIR, Natural dyes, Plant pigments, Sustainable textiles.

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## Introduction

Increasing interest in synthetic dyes is causing a resurgence of scientific interest in naturally occurring dyes based on plants. Such synthetic dyes as those used in textile, food, cosmetic, and pharmaceutical production are usually non-biodegradable and produce toxic by-products contaminating water and soil during production and use, which is a significant cause of environmental pollution [1]. As an alternative, natural dyes are biodegradable, renewable, and tend to be less toxic, which is why they are appealing to be included in the context of sustainable development and green technology [2].

The natural pigments found in plants are those that create a large variety of colors and biological processes. These pigments can generally be divided into chlorophylls, carotenoids, anthocyanins, and betalains, which produce different colors, i.e. green, yellow-orange-red, red-blue-purple, and red, respectively [3]. Removal of these pigments on the plant matrices is a very important process, which defines the yield of the dye, stability and utility of the dye in different industrial fields. Traditional extraction procedures, including Soxhlet extraction, have been in use but recent innovations have brought non-traditional approaches to extraction, such as supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric field treatment, and enzyme-assisted extraction which present individual benefits in terms of efficiency and less

solvent use [4], [5].

The extraction of pigment depends on various factors which include plant species, solvent, pH, temperature, extraction time, and agitation. The choice of solvents is also extremely important, with ethanol being proven to be more effective than water in extracting anthocyanin in hibiscus and rose petals, especially when the solvents are acidified to stabilize the flavylium ion structure [6]. It has also been reported that microwave-assisted and ultrasound-assisted extraction methods can better recover pigments and retain antioxidant activity and low solvent consumption [7]. In carotenoid extraction, hexane and acetone, which are traditional solvents, are effective; however, deep eutectic solvents and ethyl lactate, which are greener solvents, are being used to replace them [8]. Additional pre-processing such as grinding, freezing, and agitation have also been demonstrated to have a huge impact on enhancing pigment release in plant tissues [9].

Stability of pigments is one of the major concerns about practical use after extraction. PH, temperature, light exposure and oxygen are some of the factors that have a strong effect on pigment degradation. An example is anthocyanins which have color change observed to be affected by changes in pH and are highly vulnerable to thermal and photodegradation. Acylation and glycosylation are structural changes that improve the stability of pigments, and this supports the role of molecular structure in pigments maintenance [10].

As a result, the natural dyes should be characterized chemically to comprehend their structure-function relations.

This is done using spectroscopic and chromatographic methods. The most frequent ones are UV-Vis spectroscopy to measure absorbance maxima and pigment concentration, and Fourier Transform Infrared spectroscopy to give data on functional groups and bonding nature of molecules in the dye. Nuclear Magnetic Resonance spectroscopy also makes it possible to confirm the structure and purity of molecules. Separating, identifying, and quantifying individual pigment components within complex extracts by chromatographic methods such as thin-layer chromatography and high-performance liquid chromatography also provide consistency and appropriateness to be used [11].

Onion peels (*Allium cepa*), turmeric rhizomes (*Curcuma longa*) and rose petals (*Rosa* spp.) are of special interest as they have high pigment content and are readily available. Quercetin and kaempferol are flavonoids that can be found in onion peels as an agricultural waste that is abundant in flavonoids giving them a yellow-brown color and possess high antioxidant properties [12]. The varieties of red onions also have anthocyanins that play a role in producing a strong colour and enhancing the affinity of fibre to mordant [13]. The yellow-orange color of turmeric is explained by the presence of curcuminoids, in the form of curcumin, which has the antioxidant, anti-inflammatory, and antimicrobial effects [14]. The presence of anthocyanins such as cyanidin and pelargonidin glycosides provides rose petals with their red color and causes the stability of the anthocyanidin to increase due to the glycosylation and co-pigmentation processes [15].

The versatile quality of these pigments applies to the fact that they can be used in various ways besides being eco-friendly, which makes plant-based natural dyes one of the promising alternatives to synthetic dyes. Not only do they decrease the load of pollution but also favour waste valorization and sustainable industrial operations [16]. In that regard, the current paper is devoted to the extraction, characterization, and functional assessment of natural dyes of the plants *Rosa* spp., *Allium cepa* and *Curcuma longa* to be used in sustainable textile processes.

## Materials and Methods

**Plant materials:** Natural dye extraction was done using three sources of plants; *Rosa* spp. (red rose petals), *Allium cepa* (onion peels), and *Curcuma longa* (turmeric rhizomes). Fresh and unprocessed hybrid tea roses with no wilting or fungal infection were bought in a local florist and the petals were cut and washed by hand and using distilled water. The best onion bulbs were taken that had no visible damage; the outer dry peels were scraped off with the help of sterile forceps and washed in distilled water. Mature rhizomes of turmeric were purchased in a local market with no mould and fungal contamination and washed, sliced and ground into fine powder using a laboratory blender.

**Textile substrates and mordant:** The substrates used in dyeing were plain silk and polycrystalline cotton (2 m each). Alum (potassium aluminium sulfate) was used as a natural mordant because it is low toxicity, and it is environmentally friendly as well as does not reduce dye fixation as well as color vibrancy.

**Chemicals and reagents:** The whole study involved the use of analytical-grade sodium hydroxide, hydrochloric acid, and distilled water.

Microbiological media were Mueller-Hinton agar and Luria-Bertani broth (HiMedia, India). As positive antibiotic controls tetracycline discs were used. The test organism was *Staphylococcus* species that were used to do the evaluation of antimicrobials.

**Instrumentation:** Key equipments used were autoclave, laminar airflow chamber, water bath, potentiometric pH meter, Fourier Transform Infrared spectrometer, launderometer and crockmeter. Laboratory glassware and accessories were used routinely.

**Extraction of natural dyes:** All plant materials were extracted using water. Rose petals (90 g dry weight) were added into 1500 mL of distilled water and be heated in a water bath at 80 °C. Whatman filter paper was used to filter the extract which was stored to be analyzed further. The extraction process of the onion peels (20.24 g) was performed with 500 mL of distilled water at 70 °C for 80 min and then filtered. Similarly, a 500 mL distilled water was used to extract turmeric powder (28.80 g) at 80 °C in 1 h, followed by its filtration. The extracts were all kept in amber containers to avoid photodegradation.

**Fabric preparation:** Silk and cotton fabrics were cut into 16 x 16 cm pieces and destarched in 1% solution of sodium hydroxide solution during 30 min to eliminate surface impurities and sizing agents. The fabrics were then diligently washed using distilled water and dried using air before dyeing.

**Dyeing and mordanting processes:** Pre-mordanting, simultaneous mordanting and post-mordanting were used for dyeing purpose. The concentration of alum was kept at 15-20% fabric weight. In the case of pre-mordanting, the fabrics were impregnated with alum solution and allowed to dry overnight then dyed. In simultaneous mordanting, the dye bath was placed in the dyeing bath at the start of the dyeing process and alum was added. In the case of post-mordanting, coloured fabrics were dipped into alum solution overnight. Fabrics that were non-mordanted were dyed individually to determine the contribution of mordanting. To avoid uneven fading of the dyed samples, they were dried in a well-ventilated place under indirect sunlight.

Table 1: List of types of mordanting done for samples

Sample mordant name	Sample mordant type
M1 a	Simultaneous mordanting of silk sample
M1 b	Simultaneous mordanting of cotton
M2 a	Pre, Simultaneous and Post mordanting of silk
M2 b	Pre, Simultaneous and Post mordanting of cotton
M3 a	Non mordanting of silk
M3 b	Non mordanting of cotton

**Assay of antimicrobial activity:** The agar well diffusion technique was used to determine antimicrobial activity of dye extracts and dyed fabrics against *Staphylococcus* species. Mueller-Hinton agar plates were inoculated with bacterial suspension that was recently grown. Dye extracts were loaded in wells and tetracycline discs used as positive controls. The plates were incubated at 37 °C and inhibition zones were measured and documented.

**pH sensitivity analysis:** The pH-sensitive characteristics of dye extracts were determined by altering the pH of dye solutions to 2, 4, 6, 8, and 10 using 0.1N hydrochloric acid and 0.1 N sodium hydroxide.

The visual recording of the color change was done with the help of untreated dye solution as control.

**Fourier Transform Infrared spectroscopy:** The FTIR analysis was conducted to determine functional groups in the dye extracts and dye-mordant complexes. The spectra were taken between 4000- 400 cm<sup>-1</sup>. Molecular vibrations of the phenolic, flavonoid, anthocyanin and curcuminoid structures were evaluated by using FTIR spectroscopy [17].

**Color fastness evaluation:** The fastness of colors to washing was determined with the help of launderometer with standard ECE detergent at 40 °C and 60 °C temperatures in a 1:50 Fabric/liquor ratio and 30-45 minutes. The changes of color and staining at 40 °C and 60 °C were evaluated with the help of standard grayscale ratings according to the ISO 105 A02 and ISO 105 A03 methods. The fastness of color to rubbing was measured on a crock meter at normal pressure and at wet and dry rubbing.

## Results and Discussion

### Yield of Extraction and Visual Properties of Natural Dyes

Water extraction of plant-based dyes of *Rosa* spp., *Allium cepa* and *Curcuma longa* produced highly coloured solutions of dye, which demonstrates that water-soluble pigments are effectively extracted. The removal of rose petals led to the production of about 800 mL of a dark red extract, typical of solutions with a high concentration of anthocyanin. Likewise, about 800 mL of a deep reddish-brown extract was obtained when onion peel was extracted, which is due to the presence of flavonoids including quercetin and its derivatives. By contrast, a lower volume of extract (around 450 mL) of yellow-orange color was obtained in turmeric extraction, which is in line with average aqueous solubility of curcuminoids. The visualized difference in the extraction volume and intensity of color can be explained by the difference in pigment chemistry, solubility, and matrix composition. Glycosylated structures of anthocyanins and flavonoids can be more readily extracted by water compared to curcuminoids, which are rather hydrophobic, and hence they have low extraction levels in aqueous systems.

### Mordanting Effects on Dye Uptake and Fabric Coloration Effect

#### Simultaneous Mordanting

Concurrent mordanting on alum gave different color effects on the silk and cotton fabrics depending on the source of dye. Rose dye gave a greyish-pink color, onion peel dye gave a greenish-yellow color and turmeric dye gave a bright yellow color. During dyeing, the presence of alum was found to coordinate the dye molecules and the functional groups of the fibers and the fixation of the dye was increased.

#### Pre-, Simultaneous-, and Post-Mordanting

Co-pre-, co-simultaneous-, and co-post-mordanting gave all dyes much darker and more homogeneous colors. The fabrics dyed in rose-dye were darker, more of greyish pink, onion peel-dye fabrics were also darker in their yellowish-green shade, and the fabrics dyed with turmeric were also darker in colour. The enhancement is contributed by constant complexation of alum ions and hydroxyl or carbonyl group on the dye molecules which enhances the interaction of dyes with fibers.

### Non-Mordanted Dyeing

The fabrics that had not been mordanted had relatively light and duller colors. Pale pink was obtained with rose dye, dull parchment-like colour with onion peel dye, and a bright and less stable yellow with turmeric dye. These findings affirm the critical nature of mordants in enhancing the substantivity of dye and depth of shade to a significant extent to cellulosic fibers.

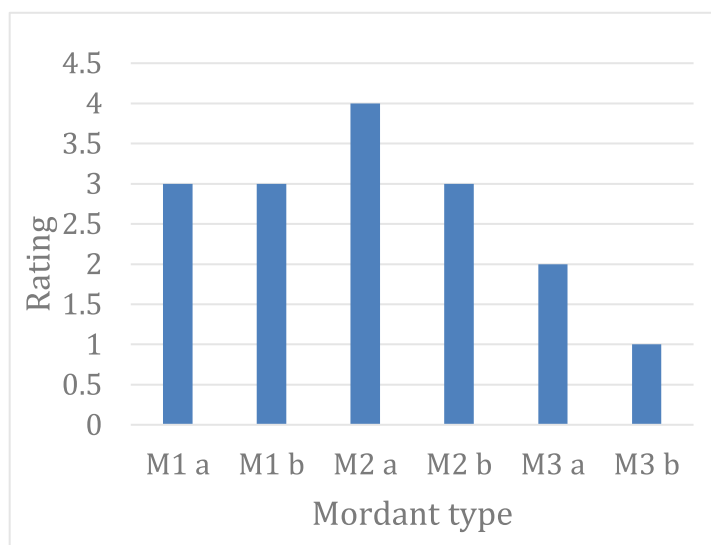
### Color Fastness to Washing

The washing fastness was tested according to the ISO 105 A03 criteria. In general, mordanted samples were found to have better fastness than non-mordanted fabrics of all dyes and fabric types.

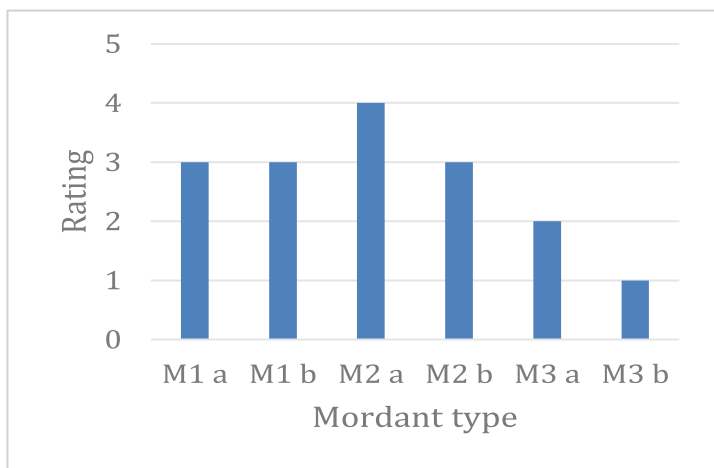
**Turmeric-Dyed Samples:** Silk fabrics that had undergone a combination of pre-, simultaneous-, and post-mordanting had the best washing fastness. Simultaneously and combined mordanting of cotton fabrics exhibited moderate fastness, and non-mordanted silk and cotton exhibited serious loss of color (Fig 1). It is possible that an increase of performance on silk is because of the presence of stronger hydrogen bonding interactions and coordination between curcuminoids and protein fibers.

**Onion Peel-Dyed Samples:** Sample peel-dyed silk peels that had been mordanted when combined with peel were the fastest and then peel-dyed cotton samples were also mordanted simultaneously. The existence of a flavonoid like quercetin with various hydroxyl groups facilitates binding with alum-mordanted fibers. Weak resistance to the washing of non-mordanted samples proved that it is essential to apply a mordant.

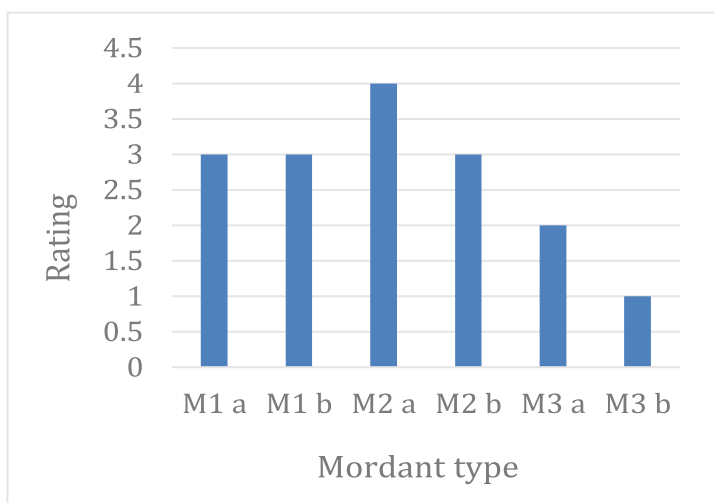
**Rose-Dyed Samples:** Silk fabrics dyed in rose using mixed mordanting had the best washing fastness. Simultaneously mordanted cotton and silk samples were observed to be moderately fastened and those that were non-mordanted had poor fastness. The sensitivity of anthocyanins to washing is a pH-dependent stability and therefore, mordanting is essential to the stability of anthocyanin. Overall, the results of washing fastness testing demonstrate that alum-mordanted natural dyes have a potential to be used in the fashion of sustainable textiles, as their effects on the durability of natural fibers are good to moderate (Fig 1).



(a)



(c)

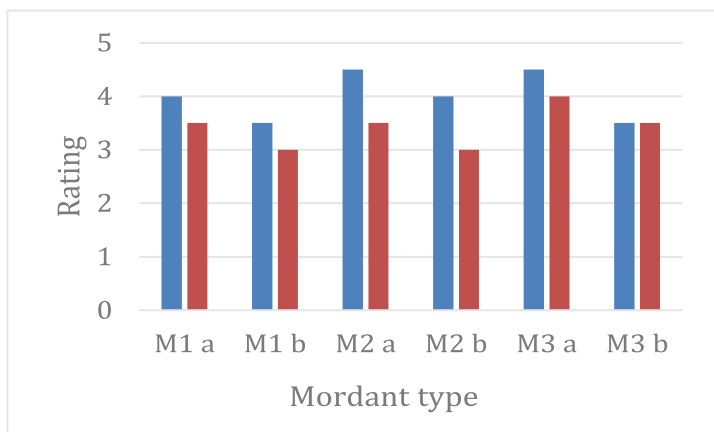


(b)

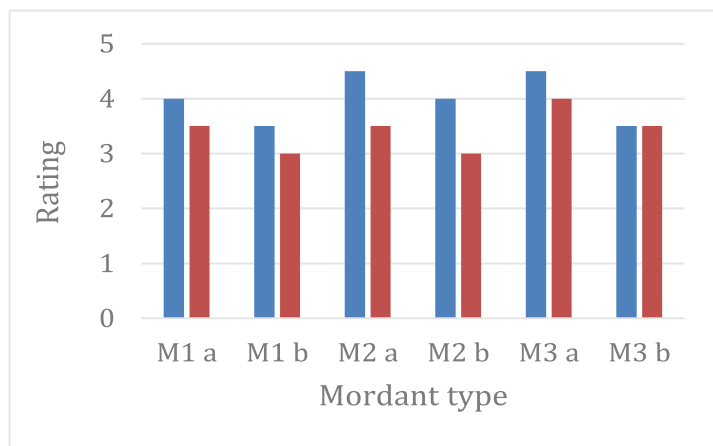
Figure 1: Graphical representation of colour fastness of rose, onion peel and turmeric by washing

**Color Fastness to Rubbing**

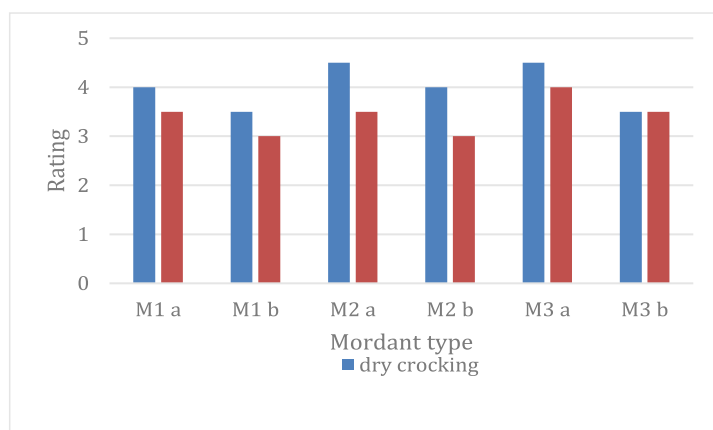
Fastness tests involving rubbing showed that all the mordanted samples had better to excellent dry rubbing fastness and additional fair to good wet rubbing fastness (Fig 2). Rose and onion peels worked well in dry rubbing particularly, which is probably because of its high surface adhesion and dye-fiber adhesion. Interestingly, the non-mordanted samples had also good dry and wet rubbing performance, which could be attributed to the limited dye penetration and dye retention on the surface, which causes a low level of dye transfer during the friction process.



(a)



(b)



(c)

Figure 2: Graphical representation of colour fastness of rose, onion peel and turmeric by rubbing

**Antimicrobial Effect of Dye Extract and Dyed Fabrics**

The antimicrobial effect of natural dye extracts and dyed textile samples against *Staphylococcus aureus* was tested with the use of agar well diffusion method (Table 1). The tetracycline standard antibiotic showed a significant zone of inhibition, which ensured the sensitivity of the test organism and the accuracy of the assay. Comparatively, each of the three plant extracts of dyes had a measurable activity but weakly inhibitory. Rose extract was the most responsive dye extract in terms of antimicrobial reaction, and turmeric and onion peel extracts were the other responsive extracts. The inhibition is observed, which is due to the presence of bioactive phytochemicals like anthocyanins, curcuminoids, and flavonoids, which have been known to disrupt cell membranes of the bacteria, interfere with protein synthesis, and cause oxidative stress in the microbial cells [18, 19]. Although the negative control was relatively smaller in comparison with the inhibitory areas, the findings indicate the intrinsic antibacterial prospects of the sources of natural dyes. These findings were consistent with and corroborated by numerous researchers who have investigated medicinal plants and their products for antimicrobial potential and drug discovery against diverse microorganisms [20-26]. There was a significant decline in antimicrobial activity after fixation of dye on silk and cotton fabrics (Table 1). Sample dyes of both dyed silk and dyed cotton had negligible or trace inhibition circles and turmeric-dyed fabrics had slight residual activity.

This decrease can probably be explained by the fact that while mordanting and dyeing the active compounds are immobilized in the fibre matrix preventing their diffusion into the surrounding medium. The same trends are indicated in naturally dyed textiles where there is a high level of dye-fibre interaction, which increases the color fastness but reduces the release of antimicrobial constituents.

Although antimicrobial response in dyed fabrics has decreased, the fact that there is trace activity is an indication that some types of bioactive compounds are still attached to the textile surface. This property can be effective in lowering microbial adherence as opposed to having bactericidal effects, and this is especially true about the application of this property to textiles, including clothing, medical fabrics, and hygiene products. Its results suggest that though natural dyes cannot be regarded as an alternative to traditional antimicrobial substances, they provide an added protective effect to natural fibres.

Table 1: Antimicrobial activity of dye extracts and dyed fabrics against *Staphylococcus aureus*

Sample category	Sample	Concentration (µg)	Incubation time (h)	Zone of inhibition (cm) (Mean ± SD)	Relative inhibition (%)*	Activity level
Positive control	Tetracycline (agar diffusion)	30	24	2.60 ± 0.05	100	Strong
Dye extracts	Rose extract	40	24	0.10 ± 0.01	3.8	Mild
	Onion peel extract	40	24	0.05 ± 0.01	1.9	Mild
	Turmeric extract	40	24	0.09 ± 0.01	3.5	Mild
Dyed silk fabric	Silk (Rose dyed)	40	24	0.00	0	None
	Silk (Onion peel dyed)	40	24	0.00	0	None
	Silk (Turmeric dyed)	40	24	0.01 ± 0.01	0.8	Trace
Dyed cotton fabric	Cotton (Rose dyed)	40	24	0.00	0	None
	Cotton (Onion peel dyed)	40	24	0.00	0	None
	Cotton (Turmeric dyed)	40	24	0.01 ± 0.01	0.8	Trace

\*Relative inhibition calculated with respect to tetracycline control.

### Natural Dyes Sensitivity to pH

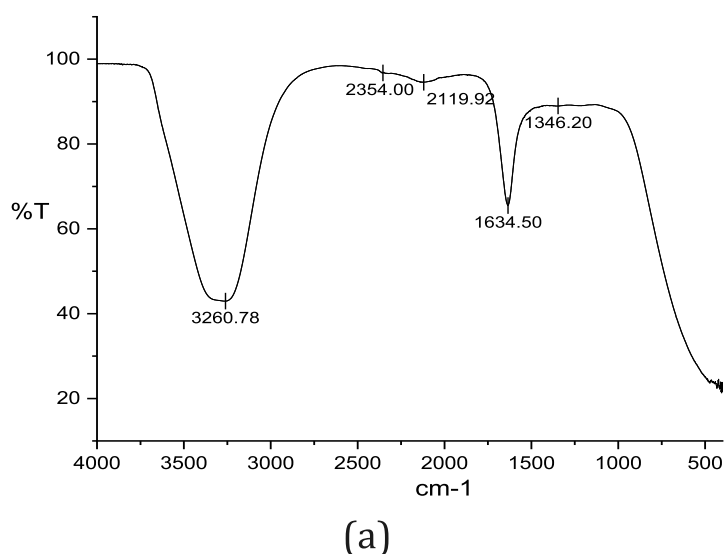
The test of pH indicator showed that the dyes had a distinct pH-dependent behavior. The color of Rose extract changed into cherry red, with acidic conditions to blackish red with alkalinity, and expected anthocyanin chemistry. The pH dependence of onion peel dye showed a change in cherry red to greenish black, which was caused by the structural changes in flavonoid. When kept in an acidic environment, turmeric dye did not fade but changed brown at pH above 9.5, which is the degradation of curcumin under alkaline conditions. These findings point to the possibility of using the dyes in natural pH detection and the need to control pH during dyeing (Fig 3).



Figure 3: pH indicator test for dye extracted from rose, onion peel and turmeric at pH 2, 4, 6, 8 and 10.

### FTIR Examination of Dye Extraction and Dye-Mordant Complexes

FTIR spectra of every dye extract proved an existence of both phenolic and aromatic functional groups. The O-H stretching band of Rose extract was broad at 3260 cm<sup>-1</sup> and had anthocyanins and flavonoid phenolic hydroxyl groups and a distinct band at 1634 cm<sup>-1</sup> indicating conjugated C=C or C=O groups which produced pigmentation. The presence of quercetin and other flavonoids in the extract was confirmed by the presence of O-H and conjugated carbonyl bands in onion peel extract. The turmeric extract exhibited typical absorption at 1632 cm<sup>-1</sup>, which is representative of the b-diketone form of curcumin. FTIR spectra of dye samples treated with alum showed other peaks at 1090-1100 cm<sup>-1</sup> which were attributed to sulphate or phosphate groups of alum (Table 2). These peaks affirm chemical interactions between alum and dye molecules, and this explains the increased stability, the intensity of color and the permanence of alum-mordanted fabric (Fig 4).



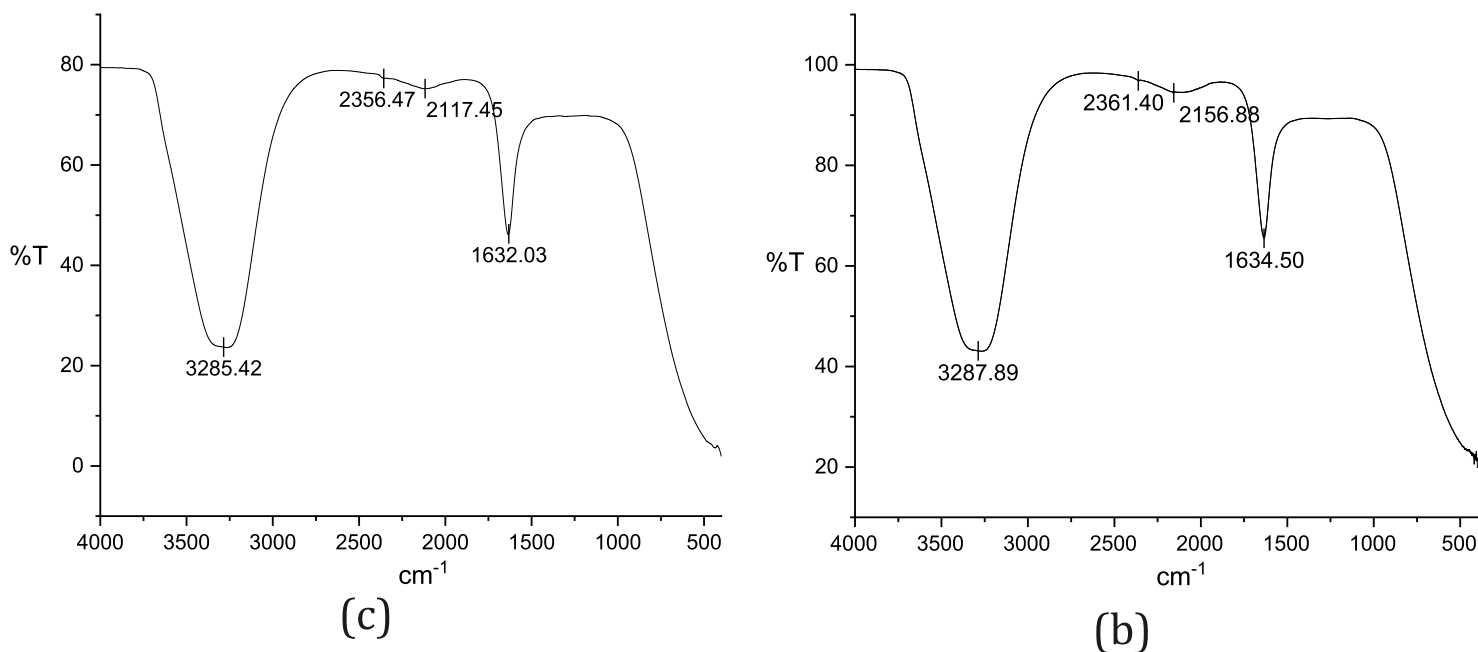


Figure 4: Graphical representation of FTIR of rose, onion peel and turmeric sample

Table 2: FTIR result of rose, onion peel and turmeric sample

Sample	Wavenumber (cm <sup>-1</sup> )	Functional Group	Vibrational Mode	Compound Type
Rose	3260.78	O-H stretch (broad)	Hydrogen-bonded O-H stretching	Phenols, alcohols, flavonoids
	2354.00 & 2119.92	C≡C / C≡N stretch (weak)	Alkyne / nitrile stretching	Could be impurities or solvent residues
	1634.50	C=C or C=O stretch	Aromatic ring or conjugated carbonyl	Anthocyanins, flavonoids
	1346.20	C-N stretching or O-H bend	Amine or phenolic O-H bending	Polyphenols, secondary metabolites
Onion peel	3287.89	O-H stretch (broad)	Hydrogen-bonded O-H stretching	Phenols, flavonoids, carbohydrates
	2361.40 & 2156.88	C≡C / C≡N stretch (weak)	Alkyne / nitrile stretching	Minor phytochemicals or impurities
	1634.50	C=C or C=O stretch	Aromatic ring or conjugated carbonyl	Flavonoids, quercetin, polyphenols
Turmeric	3285.42	O-H stretch (broad)	Hydrogen-bonded O-H stretching	Phenols, curcuminoids, flavonoids
	2345.67 & 2117.45	C≡C / C≡N stretch (weak)	Alkyne / nitrile stretching	Minor phytochemicals or impurities
	1632.03	C=C or C=O stretch	Aromatic ring or conjugated carbonyl	Curcuminoids (curcumin), polyphenols

The findings in general indicate that rose petal natural dyes, onion peels and turmeric natural dyes can be successfully extracted by using aqueous solutions and a natural dye applied to a silk and cotton fabric. Mordanting, especially the integrated pre-, simultaneous-, and post-mordanting method correlates highly with boosting the color intensity, washing stability, and stability of the dye in general. FTIR analysis confirms the interaction between dyes and the fiber is based on molecules whereas antimicrobial and pH sensitivity studies indicate other functional advantages of these dyes. The results justify the possibility of using natural dyes that are derived out of plants as an alternative to synthetic dyes in fabrics as being environmentally friendly.

### Conclusion

The mounting environmental issues that come with the synthetic dyes have heightened the demand of the use of sustainable and environmentally friendly dyes in the textile industry. As the current study reveals, natural dyes derived using *Rosa* spp., *Allium cepa*, and *Curcuma longa* may be used to provide useful and eco-safe colourants to natural fibres of textile including silk and cotton. Aqueous extraction procedures produced stable dye solutions and this attests to the viability of using readily available plant materials as well as agricultural by-products in natural dye extraction. The extracted pigments displayed a good performance in dyeing natural fibres and especially with the use of alum as a mordant. Mordanted fabrics had better color depth, greater washing and rubbing fastness and increased overall stability than non-mordanted samples.

These results suggest that the basic characteristics of the dyes are maintained when they are attached to textile fibres, which favours their use over a long period. The antimicrobial property of the dye extracts especially towards *Staphylococcus aureus* further states the functionality of these natural colorants. The antimicrobial activity on colored fabrics was weak, but the preservation of bioactivity indicates that there is a possibility of using textiles as hygienic as well as protective elements.

Another practical benefit of the dyes studied according to the paper is that they respond to pH conditions. The observed color variations at different pH largely in anthocyanin and flavonoid rich dyes imply that they may be used as natural pH indicators. These materials open possibilities of new innovative applications such as smart textiles, pH sensitive wound plastering, wearable health-monitoring clothes, and smart food-packaging materials that can detect spoilage by changing color. Environmentally, extraction and use of natural dyes have great merits as opposed to the synthetic dye systems. The processes utilized in this research have less energy input requirements and have less usage of dangerous chemicals, which lead to a low carbon footprint. Besides, wastes produced in the extraction of natural dyes are biodegradable and non-toxic with minimal effects on soil and aquatic environments. Synthetic dyes production, on the other hand, is based on energy-consuming practices and produces long lasting pollutants like azo compounds, heavy metal, and formaldehyde-leaking agents, which cause environmental pollution in the long run.

Although the extraction techniques that were applied in this research in small scale are not complex and open to replication, the on-large scale application would demand process standardization, optimization, and more human resources to guarantee consistency and efficiency. However, the general results confirm the promise of natural dyes made of plants as sustainable replacements of synthetic dyes. Natural dyes have a promising future in creating a novel avenue to ecologically friendly textile manufacturing as well as creating value-added functional textiles by integrating ecological safety with such practical advantages as antimicrobial properties and sensitivity to pH.

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