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Research Paper

Extraction And Characterization of Natural Dye from Beetroot Peels (*Beta vulgaris*) For Application in Leather Dyeing

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Abstract— Synthetic dyes are known to be toxic, carcinogenic, mutagenic and non-biodegradable. This has led to increased research by scientists across the globe on natural dyes as viable alternatives. This study has utilized extraction of natural dye using a simple aqueous method which is an economical way of obtaining the dye for application in leather dyeing while utilizing the agricultural waste hence value addition. The dye extract's functional groups were identified using FT-IR analysis, while the concentration of betalain in the dye extract was determined by UV-VIS analysis. Additionally, various tests were conducted to investigate how changes in temperature and pH affect the dye's stability. Characteristic bands were observed in the FT-IR spectra, specifically at 3291.89 for OH stretching, 2884.99 and 2829.06 for C-H stretching, 1634.38 for C=O stretching, 1565.92 for N-H bend, and 1319.07 for the amine bond. The UV-VIS analysis showed a characteristic peak at 535 nm indicating the presence of the red- violet betacyanin which was responsible for the red-violet colour of the extract observed with the maximum absorbance of 0.3. The findings of this investigation indicated that the dye extract obtained from beetroot peels has potential for commercial use in the dyeing of leather.

Keywords— Betalain, beetroot peels, FT-IR, UV-VIS, betacyanin, betaxanthin

1. Introduction

The leather industry is one of those that uses dyes during the manufacturing process to colour the leather and make it appealing to customers. Synthetic dyes are the most commonly used, and due to the rise in environmental and sustainability concerns, extensive research on natural dyes is being conducted in search of a replacement for synthetic colourants. Natural dyes have been used since antiquity, but they were quickly replaced by synthetic dyes, which were discovered in 1856 by William HenryPerkin [1, 2]. Synthetic dyes are simple to make, improve the quality of dyed leather, and come in a wide range of grades, classes, and varieties [3]. It could also be used to dye a variety of textiles or leather. Although it is true that they are less expensive and more technologically advanced than natural dyes, they have several drawbacks that include the usage of raw materials that are not sustainable and pose a risk to the environment during their production. [4]. The availability of these raw materials, such as hydrocarbon resources, has an impact on their productivity [5]. Synthetic dye production also necessitates the use of more energy, which raises the cost. Furthermore, only a small amount of the dye is fixed to the leather during dyeing, and the rest, amounting to about 10–50 % of the dye used, is discarded as waste to the environment, contaminating the water bodies and endangering human health [6]. Moreover, because synthetic dye effluent from the leather industry is non-biodegradable, it is difficult to treat and necessitates the use of complex and time-consuming processes that have a negative impact on the environment [7-9]. This has resulted in an extensive search for a more effective and economically viable dyeing method. As a result, natural dyes have regained prominence in the leather industry in a quest for more sustainable, harmless and environmentally friendly dyeing methods [10]. Natural dyes provide specific properties to the substrate, such as flame retardancy, antibacterial potency, deodorizing properties, and UV radiation resistance, in addition to being environmentally friendly and non-toxic [11]. Moreover, the European Union's tightening regulations concerning the use of synthetic dyes on how synthetic dyes are utilized, like azo dyes, have led to a surge in research exploring alternative sources of natural dyes. [12]. Extensive research is also being carried out to identify feasible, cost-effective, and environmentally friendly methods of extracting natural colourants from natural sources.

In the study reported in this manuscript, we have focused on extracting a natural dye from beetroot peels for application in leather. Our research was motivated by the presence of betalain, a vividly colored water soluble colourant found in beetroot [13] and a quest to utilize the peels that are otherwise considered waste by products. Betalains are compound that contain nitrogen derivatives of the betalamic acid chromophore that always give some fruits and vegetables their red-violet (betacyanin) and yellow (betaxanthins)

colours [14-17]. Due to its medicinal potential, beetroot is grown all over the world and is mostly used in pharmaceutical products [18]. The peels are typically discarded as waste, despite the fact that they can be used to make dyes for the leather, textile, and cosmetic industries. The objective of this research was to explore the potential of beetroot peels, an agricultural waste as a replacement to synthetic colorants in the dyeing of leather. To achieve this goal, the study extracted and analyzed the natural dye from the peels using a straightforward and affordable aqueous method. Beetroot peels were chosen for their reputation as a dependable source of natural dye. This study is is vital in promoting sustainable practices in the leather industry, contributing to the development of green alternatives to synthetic dyes, and adding value to agricultural waste.

2. Related Work

Several researchers from around the world have tested natural dyes derived from various sources on leather, with so far positive results. Mohan *et al* (2020) conducted a study with onion peels to obtain natural dyes using simple aqueous extraction and acetic acid, and the results showed that natural dye extract from beetroot peels could be used in dyeing leather as an alternative to synthetic dye [19]. In another related research Inayat *et al* (2010) utilized aqueous extract from tea, turmeric, eucalyptus and walnut and the leather produced had good fastness properties [20]. One of the research that has been done on beetroot flesh and tried on leather used ultrasound assisted extraction which is an expensive method. The outcomes indicated that the dyed leather possessed favorable fastness properties [21]. In 2016, Velmurugan and colleagues conducted a study investigating the use of natural dye extracted from *Cariopsis tinctoria* flower petals in leather dyeing. The research revealed that the leather dyed using the optimal conditions of cariopsis tinctoria displayed a consistent and vibrant coloration. Furthermore, the fastness properties of the leather were comparable to those of the control leather.[22]. Kurinjimalar *et al* (2022), on the other hand, examined the feasibility of utilizing natural dye extracted from Garcinia Mangostana Linn peels in dyeing leather. The study demonstrated that the dyed leather satisfied the necessary criteria for fastness, organoleptic, and perspiration properties [23]. Selvi and colleagues (2013) researched the utilization of natural dye extracted from the seeds of Bixa Orellana for dyeing of leather and finishing in their exploration of applying natural dyes to leather. The primary objective of the investigation was to create an environmentally friendly material that could serve as a colorant in leather [24]. This study's findings revealed that good rub fastness properties were obtained with improved film adhesion. DEB *et al*. (2017) investigated the potential utilization of lac dye in colouring shoe uppers in another study. The study's findings revealed that the leather had good to excellent fastness properties, with different shades produced by different mordants [25]. Numerous attempts have been undertaken to explore the possibility of using various natural sources for dyeing leather.

Likewise, numerous investigations have explored the potential of using beetroot peels as a sustainable source of betalain for extracting natural dye. Propescu *et al* (2021) also investigated the aqueous extraction method of obtaining natural dye from beetroot peels. The outcomes of the study demonstrated that the dye was suitable for use on protein fibers and exhibited satisfactory fastness properties on the dyed fabric. [26]. Another study was conducted by Zin *et al* (2020) to obtain natural colors from beetroot peels using an aqueous ethanol solvent. The spectrophotometric analysis of the dye indicated the presence of betalain, including Betacyanin and Betaxanthin [27]. Another study by Yaqub *et al*. (2018) investigated the utilization of ultrasonic extraction for obtaining natural dye from Beta vulgaris peels for use on silk fabric. The outcomes of the investigation revealed that the dye extracted from beetroot peels had satisfactory fastness properties on the dyed fabric. [28]. Despite various studies on the extraction of natural dye from beetroot peels, research on its application for leather dyeing remains scarce. Therefore, this study's objective is to investigate the utilization of beetroot peels for natural dye extraction and its application in leather dyeing.

3. Materials and methods

3.1 Materials

Beetroots were purchased at Nyeri Market in Nyeri County, Kenya, for this experiment. The beetroots were properly cleaned with clean water, likely to get rid of any dust, debris, or chemical residues that could affect the extraction process or the quality of the final product. After peeling the beetroots with a sharp knife, the peels were cut into tiny pieces to increase the surface area of the peels, making them more amenable to efficient extraction.The peels were then cleaned with water that was distilled to eradicate impurities and contaminants. The peels were then kept until extraction. This was necessary to prevent the peels from drying out, which could affect the extraction process's efficiency or the quality of the extracted dye. Skytech and Cendico Limited provided the analytical grade chemicals and reagents used in the experiment.

3.2 Methods

3.2.1 Natural dye extraction from beetroot peels

The procedure used for dye extraction from beetroot peels involved the use of a simple aqueous extraction method with slight modifications from previous studies. Approximately 50 g of beetroot peels were weighed and placed in a 500 mL conical flask filled with distilled water. After soaking for 24 hours, the sample was extracted for 45 minutes in a water bath set at 75°C. The dye extract was then cooled, filtered, and concentrated for 4 hours at 75 °C in an oven. The resulting dye was stored in a cool place for future analysis and use. All experiments were performed in triplicate, and the total yield was calculated using the weight of the peels used for extraction. This method was adapted from previous studies with slight modifications [10, 29].

3.2.2 Characterization of betalain dye

3.2.2.1 Effect of temperature on betalain dye

The purpose of this study was to look into the effect of temperature variation on the extracted natural dye from beetroot peels. A range of temperatures from 20 ºC to 100 ºC

was applied to the dye, and any changes were monitored. The dye was prepared by diluting it to 1% concentration and subjected to a wavelength scan ranging between 200-1000 nm using an Ultra Violet – Visible spectrophotometer (UV-VIS). The resulting spectra were analyzed to assess the impact of temperature on the colorant.

3.2.2.2 Effect of pH on betalain dye

In this experiment, the natural dye extracted from beetroot peels was tested for its pH sensitivity. The dye was subjected to a range of pH conditions, which ranged from 2 to 12, and any changes in the dye were monitored closely. The samples of the dye were then taken at pH 2, 7, and 10 for UV-VIS analysis to analyze the impact of pH on the dye extract. The reason for choosing these specific pH levels is that they represent acidic (pH 2), neutral (pH 7), and basic (pH 10) conditions. By testing the dye at these pH levels, the researchers can determine how stable the dye is under different pH conditions, and whether any pH level has a significant impact on the dye's properties.

3.2.2.3 UV-VIS analysis for betalain content

In this procedure, the betalain content of the dye extract was examined using UV-VIS analysis. To begin with, a 1% solution of the dye extract was prepared, and a wavelength scan was performed between 200-1000 nm using a spectro uv-1800 UV-VIS spectrophotometer. The spectrum obtained from the scan was then analyzed to determine the highest absorbance peak, which was used to calculate the betalain content of the dye. The betalain content was calculated using a formula from literature that relates the absorbance of betalain at a specific wavelength to its concentration [30] whereby,

$$\text{Betalain Concentration} = A \times DF \times MW \times 1000 / \varepsilon L \quad (1)$$

Where;
A= Highest absorbance value
DF= Dilution volume
MW= Molecular weight of betalain
Ɛ = Extinction coefficient for betalain
L= Path length of cuvette

3.2.2.4 FTIR Analysis of the dye

Fourier Transform Infrared Spectroscopy (FTIR) was used in this procedure to identify the presence of betalain compounds in the dye extract. To start the process, a small drop of the liquid dye extract was placed on a salt plate (NaCl). The liquid extract was then spread evenly between two plates, which were subsequently placed onto the sample holder of a Jasco FTIR-4700 Spectrometer. The machine then scanned the sample in the 4000-400 cm^{-1} wavelength range. The resulting spectrum was then examined to determine which functional groups were present in the dye extract. The dye extract spectrum was then compared to those available in the literature to determine the presence of betalain compounds in the dye extract. [31].

4. Results and Discussion

4.1 Betalain stability

The stability of betalain in dye extract is affected by a variety of factors, both intrinsic and extrinsic, which must be considered during storage and application.

4.2 Effect of temperature on betalain stability

This test was carried out to determine which temperature is appropriate for storage and application. The dye was subjected to a range of temperature. The dye's colour changed from red-violet to a lighter colour at 82 ºC. This was attributed to the degradation of the dye's compounds [32]. The temperature effect on the dye extract was then investigated using a UV-VIS spectrophotometer. Thermal energy at 82ºC caused the betalain molecule to degrade thermally via a variety of mechanisms, including oxidation, hydrolysis, and condensation reactions [33]. Oxidation can result in the formation of Quinone-like structures, which are less colourful and reduce dye absorption [34]. Hydrolysis reactions can break down the betalain chromophore into smaller fragments, causing the intensity of the absorption spectrum to decrease [32]. Condensation reactions can result in the formation of new compounds, which can reduce the colour properties of betalain [35]. There are several factors that can influence the particular alterations that take place in betalain at a temperature of 82°C, which includes the duration of the exposure and the pH of the solution. When exposed to high temperatures, the colour of betalain at pH 5 may change to greenish-brown, indicating the formation of degradation products [36]. According to the results shown in Figure 1, there was partial degradation of the dye, a wavelength shift, and a decrease in the betalain content in the dye extract. This explains the dye's pale colour when heated to 82ºC. This was caused by the betalain dye's condensation reaction. Because of the degradation of the compounds in the dye, the concentration of betalain decreased by 25.7%, making the dye suitable for application and storage at room temperature.

Temperature is a critical factor to consider, particularly during the dyeing process. A high temperature of approximately 50 ºC is expected to allow for maximum dye penetration on the substrate. Betalain is known to be heat sensitive and degrades at higher temperatures [37]. It has been reported that heating betalain extract reduces the betalain content, which includes betacyanin and betaxanthin.

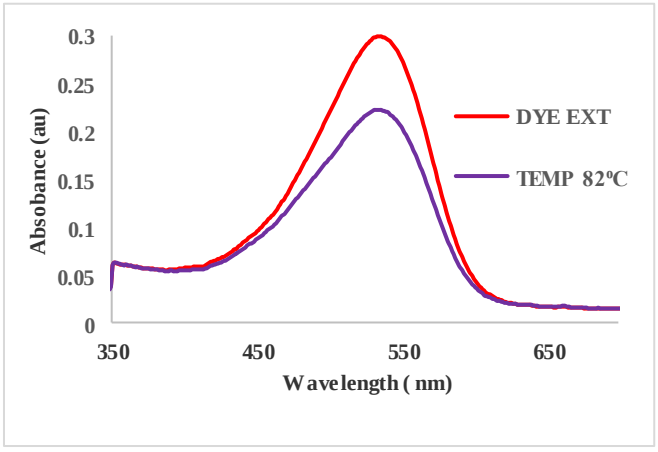

Figure 1: Spectra of betalain dye before subjecting to temperature and after subjecting to temperature at 82°C

4.3 Effect of pH on betalain stability

To examine how pH impacts the colour and betalain concentration, the dye extract was subjected to pH 2, 7 and 10 and the dye was closely monitored for any changes. The dye's

colour changed rapidly from red-violet to dark green at pH 10. The dye was tested at pH 2, 7, and 10 using a UV-VIS spectrophotometer, and the results are shown in Figure 2. According to the results, there was no peak at around 530 nm indicating betacyanin compound degradation in the dye at pH 10. This demonstrated the betalain's instability in alkaline conditions [30, 38]. Thus, the dye is appropriate for use in acidic and neutral environments. Because of the acidic conditions at pH 2, betalain dye undergoes significant changes. The carboxylic acid groups in the betalain molecule are protonated at this low pH, resulting in a positively charged molecule [39]. Because of the low pH, the amine groups in the betalain molecule remain protonated. The absorption spectrum of the betalain molecule can shift towards shorter wavelengths causing a blue shift when the carboxylic acid groups are protonated [33]. This is because the positive charge on the molecule causes more electron delocalization in the chromophore, resulting in a higher energy transition between molecular orbitals [40, 41]. In this study, a wavelength shift to a shorter wavelength was observed, indicating protonation of the carboxylic acid group present in the betalain dye, as a result of which the dye's betalain content was significantly reduced.

The betalain molecule is relatively stable at pH 7, but its colour properties may be affected [42]. At pH, the functional groups of the betalain molecule, such as the carboxylic acid and amine groups, are partially deprotonated and protonated. [43]. This ionization state can cause a shift in the molecule's absorption spectrum, resulting in a change in its colour properties. The degree of conjugation in the chromophore determines the colour of betalain dye [44]. A series of alternating single and double bonds in the chromophore of betalain dye give the molecule its characteristic red, purple, and yellow colours [44]. According to the results, there was a wavelength shift towards a shorter wavelength, indicating protonation of the betalain dye at pH 7. This resulted in a 51% decrease in betalain content. Several factors, such as storage conditions, light exposure, and temperature, can affect the stability of betalain at a pH of 7. [45]. Betalain can degrade under certain conditions, resulting in the loss of its colour properties. It is therefore critical to control the pH, temperature, and other variables that can affect the stability of betalain dye.

The alkaline conditions cause significant changes in betalain dye at pH 10 [46]. The carboxylic acid groups in the betalain molecule are deprotonated at this high pH, resulting in a negatively charged molecule. Because of the high pH, the amine groups in the betalain molecule are partially deprotonated. The deprotonation of the carboxylic acid groups in betalain can cause a significant shift in the molecule's absorption spectrum towards longer wavelengths, resulting in a yellow or orange colour [35]. This is because the molecule's negative charge causes less delocalization of electrons in the chromophore, resulting in a lower energy transition between molecular orbitals. The alkaline conditions at pH 10 can also cause betalain molecule degradation, resulting in the loss of colour properties. The alkaline hydrolysis of betalain can break down the chromophore into smaller fragments, causing the intensity of the absorption spectrum to decrease [32]. Additionally, betalain may not remain stable at elevated pH levels, which could lead to the creation of quinones and other byproducts from degradation. [42]. In this case, betalain degradation was observed because there was no peak observed as shown in Figure 2; this could be due to alkaline hydrolysis of the betalain in the dye extract, resulting in colour loss.

pH is one of the most important factors that is closely monitored during the leather dyeing process for the penetration and fixation of the dye onto the leather. As a result, the dye must be stable under a variety of pH conditions. Betalain, in the form of betacyanin, is known to be considerably stable in the pH range of 3 to 7, with greatest stability at pH values between 5 and 6 [30, 38].

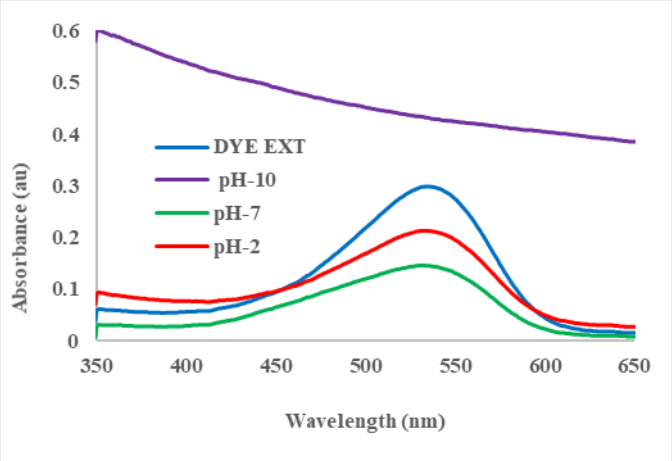

Figure 2: UV-VIS Spectra of betalain dye before subjecting to pH, at pH 2, 7 and 10

4.4 UV-VIS Analysis for betalain content

A UV-VIS analysis was performed to assess the content of betalain in the dye extract. Figure 3 depicts the spectrum obtained after scanning the dye at wavelengths ranging from 200 nm to 1000 nm. The highest absorbance was used to calculate the betalain content. The study's UV-VIS findings showed that a peak was present at 535 nm, indicating the presence of betacyanin in the dye extract, which is responsible for its red-violet colour with maximum absorbance of 0.3 as illustrated in Figure 3. The absorbance was then used to calculate the concentration of betalain using Beer lambert's law as shown in equation (I).Betacyanin and betaxanthin are the two subclasses of betalain [47]. The wavelength of maximum absorbance for betalain has been reported to be in the range of 480 nm and 535 nm due to the colour mixture of yellow-orange betaxanthin and red-violet betacyanin respectively [48]. According to literature [32] betacyanin has two absorption maxima, one in the range of 270-280 nm caused by the cyclo-DOPA residue, and the other in the 535-540 nm range, which is influenced by the solvent used. The absence of a peak in the 480-530 nm range in this case indicated that the sample contained little to no betaxanthins. If the concentration of betaxanthins in the sample is low, UV-Vis spectroscopy may not detect it, resulting in a spectrum with only one peak at 535 nm.

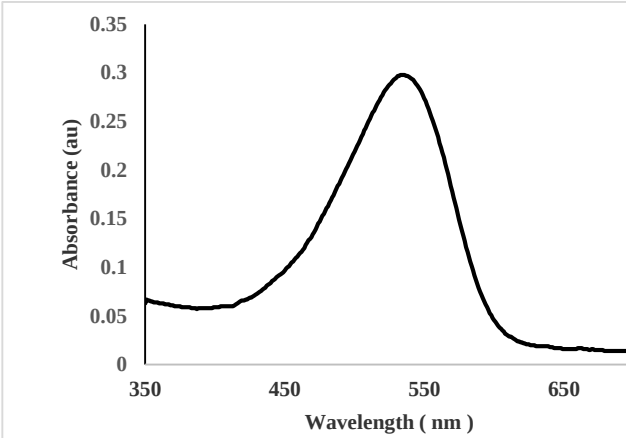

Figure 3: UV-VIS spectra of betalain dye with the highest absorbance at 535 nm

4.5 FTIR Analysis of betalain dye

The dye extract underwent Fourier Transform Infrared Spectroscopy to identify its functional groups. The scan was conducted in the 4000-400 cm^{-1} range, as illustrated in Figure 4. The resulting spectra showed a wide and intense band at 3291.89 cm^{-1}, suggesting the existence of hydroxyl groups in the extract based on the OH Stretching vibration. [49]. The C-H stretching and C-H bending of the hydrocarbon chain were assigned to the two peaks observed at 2884.99 and 2829.06 cm^{-1}, respectively [50]. The wide peak observed at 1634.38 cm^{-1} was attributed to the stretching of both symmetric and asymmetric C=O bonds [51]. The peak could be attributed to the stretching of the C=N bond of the aldimine bond, which binds the betalamic acid in the dopa cycle [52, 53]. The peak observed at 1565.92 cm^{-1} in the extract was assigned to the N-H bend of the secondary amine. Additionally, the peak at 1445.39 cm^{-1} was attributed to the C-C stretching of the aromatic compound present in the extract [54]. The presence of C-O stretching in the ether and aliphatic amine, as well as the C=N stretching in the aromatic amine, were responsible for the peak observed at 1319.07 cm^{-1} [54]. The confirmation of betalain's presence was possible due to the existence of the hydroxyl group and the double bond aromatic ring in the spectra. Since the dye possesses OH and NH_2, it has an affinity for water and some solubility, which distinguishes it from a pigment and classifies it as a dye.[51]. The findings of this study were consistent with those found in the literature. [51].

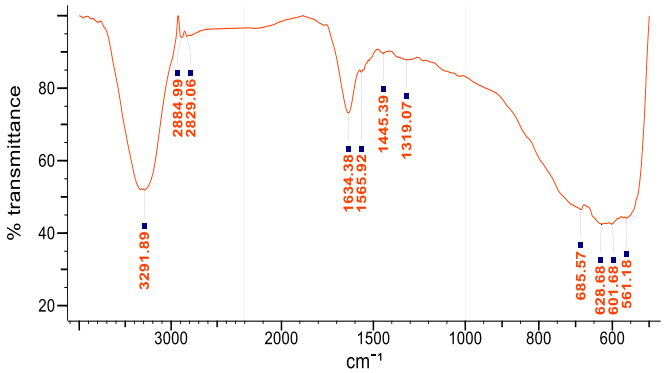

Figure 4: FTIR spectra of betalain dye extract scanned in the range of 4000cm^{-1} – 400$cm^{-1}$for the functional group of betalain dye

5. Conclusion and Future Scope

Natural dye was successfully extracted from beetroot peels using the simple aqueous method and characterized using UV-VIS and FTIR Spectrophotometer. According to the findings of this study, dye extract from beetroot peels can be utilized for leather dyeing because the dye absorbed light in the visible region (535 nm) and contained a chromophore (betalamic acid) in the betacyanin compound, which is responsible for the dye's colour. The dye had a conjugated system, as confirmed by the FTIR analysis, making it a suitable dye for application. The dye concentration was 275 mg/L, which was higher than the minimum standard requirement. Based on the findings presented in Figure 1,2 and 3, it was observed that the dye exhibited stability in the pH range of 2-7 but unstable beyond a pH 7 and above 82 ºC. As a result, the dye should be stored and applied in an acidic or neutral environment at temperatures below 82 ºC. In conclusion, natural dye from beetroot peels (Beta Vulgaris) has proven to be a promising alternative for the leather industry. The study demonstrated that beetroot peels contain betacyanin which is capable of producing vibrant colour on the leather. As a result, using beetroot peel extract in leather dyeing can reduce the environmental impact of synthetic dyes while also providing economic benefits to leather manufacturers. Further research should be conducted to exploit more dye-producing sources, particularly locally available sources, as well as the cost-effectiveness of natural dyes in order to promote their commercial use in the leather industry.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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