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Phytochemical analysis and extraction of natural dyes from mulberry leaves

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Abstract
Textile chemists and colorists are encouraged to employ eco-friendly methods of dyeing textile substrates due to environmental limitations. Natural compound dyeing, with its practical qualities and environmental advantages, has gained increased attention. The present study investigated the aqueous and alkaline extraction method for obtaining natural colors from mulberry leaves. The extract from mulberry leaves produced a brown to yellow-brown color. The optimal conditions for dye extraction were found to be a M:L ratio of 1:30, a temperature of 80 °C with time duration of 80 minutes. Colour composition evaluation of the natural dye using UV-visible spectra revealed the presence of quercetin and kaempferol at 370 nm. FTIR results showed the presence of flavonoids, phenolic compounds, carbohydrates, halogen group and tannin in mulberry leaves. Overall, it can be concluded that the dye extracted from mulberry leaves could be promoted as an environmentally friendly alternative to synthetic brown dyes for fabrics.

Keywords: Mulberry leaves, natural dye, brown, FTIR, UV-visible spectra, fabrics

Introduction
Humans are drawn to the stunning colours found in nature. Natural dyes have been used for coloring human skin, food, cave walls, fabrics, leather, and everyday objects since the dawn of civilization. There are several sources of plants, animals, insects, and minerals that can be used to extract dyes and pigments. Dyeing has been a practice as long as human civilization has existed. The existence of dyed textiles during archaeological excavations at various locations around the world is proof that dyeing was a common practice in prehistoric civilizations. The first written evidence of dye substance used in China dates back to 2,600 BC (Kramell *et al*., 2014) [13].

Natural dyes include colorants that are made without chemicals, derived from animal, mineral and plant components. With an increased awareness of the environmental and health risks associated with the manufacturing, processing, and use of synthetic dyes, there has been a global resurgence of interest in natural dyes. The non-toxic and biodegradable qualities of natural colors derived from plants have recently given them an economic edge over synthetic dyes (Bhuyan and Saikia, 2003; Samanta and Agarwal, 2009) [5, 20].

The textile sector is experiencing a steady rise in the use of sustainable green technology. In contrast, synthetic dyes and finishing agents pose risks to the environment and living organisms. Consequently, scientists are increasingly involved in the transition to eco-friendly resources in place of synthetic chemicals, without compromising output quality or efficiency. Numerous researchers have explored the use of natural dyes derived from various sources to color different materials (Benkhaya *et al*., 2020) [4]. Brudzyńska *et al*. (2021) [7] revealed that plant-derived colourants contained different chemical groups such as carotenoids, polyphenols, quinones, or alkaloids pave the way for successful track record in the food industry on a broader scale. Plant-derived colourants can be useful for therapeutical properties, such as antioxidant and antibacterial activity, in addition to their ability to dye, which can be a further advantage for their use in the cosmetic, textile, and food industries.

The natural dyed textiles are promising to provide functional properties such as antimicrobial, UV protection, biodegradable, eco-friendly, non-toxic and renewable in nature. Although dye extracts initially appear to be somewhat expensive, but they are economical. This time period can be referred to as the renaissance of natural dyes because so many scientists and researchers are working hard to improve natural dye (Kiron, 2022) [12].

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Sericulture, an agro-based industry has unique features such as reversal flow of income from rich to the poor, more employment generation potential and scope for recycling of byproduct. India, producing about 15% of the world's silk, ranks second-largest silk producer after China. Mulberry (*Morus* spp.) belongs to the Moraceae family included more than 150 species such as the black mulberry (*M. nigra*), red mulberry (*M. rubra*) and white mulberry (M. *alba*). In rural area, sericulture is a profession that generates revenue. Lakshmanan *et al*. (1997) [15] claimed that due to its low investment profile and potential for family employment, it is ideal for small-scale and marginal farming.

The mulberry plant serves as the sole food source for silkworm. An average of about 25 MT to 30 MT of leaf (without twigs) can be harvested per annum per hectare under row system of cultivation in mulberry garden. However, the leftover mulberry leaves from gardens and the silkworm excrement generated during rearing are often left unutilized or disposed of as waste. In sericulture, waste can be transformed into wealth, as approximately 77% of the total leaves fed to silkworms end up as rearing waste and silkworm excreta. Proper utilization and recycling of these natural resources have become the need of the hour. Various byproducts generated during sericulture activities can be converted into value-added products. Integrating the different byproducts obtained from the sericulture industry, such as wastes from mulberry cultivation, rearing, and reeling, has been a long-felt necessity (Basu, 2015) [3].

It is estimated that effective management and utilization of by-products could lead to value addition of up to 25 percent in various sectors of sericulture. One of the major by-products of sericulture is the waste generated from rearing, including leftover leaves and silkworm excreta. Currently, these by-products are underutilized in sericulture-based industries. Extracting dyes from mulberry leaves acts as natural source for dyeing fabrics. Utilizing mulberry leaves as a byproduct in the textile industries is a potential idea that could reduce the cost of producing natural dyes (Ohama, 2014) [17]. Zhu and Zhang (2014) [23] reported that lutein (30.86%) and β-carotene (26.30%) are the main coloring agents present in mulberry leaf extract.

Therefore, the present research work aimed to promote the use of natural dyes extracted from mulberry leaves due to presence of colouring pigments and their commercialization in related industries through a methodical and scientific approach of identification, extraction, purification, chemical structure elucidation, and characterization.

Materials

Sample collection and preparation

Fresh mulberry leaves were collected from the mulberry garden of Department of Sericulture at Forest College & Research Institute, Mettupalayam, Tamil Nadu. The location of the garden is situated at coordinates 11°32' N latitude and 76°93' E longitude and altitude of 300 meters above sea level. For the study, mulberry leaf samples were obtained from the third and fourth positions from the top and also utilized mulberry plants was used. The collected leaves were thoroughly washed with distilled water to eliminate any dirt or impurities, and then they were chopped into approximately 1 cm-sized pieces.

Standardization of dyes extracted from mulberry leaves

The dye extraction from mulberry leaves was standardized with three different variables *viz*., material to liquor ratio, temperature and time.

Different single-factor conditions were examined, such as the Matter: Liquor ratio (1:10, 1:20, and 1:30), different temperatures (60 °C, 70 °C, and 80 °C) and different time periods (60 min, 70 min, 80 min, and 90 min) for mulberry leaves. After the extraction process, the leaf samples and extracts from the hot water bath were filtered and transferred to a clean, dried, and weighed glass beaker. The extracts were then dried in the hot water bath until the water had evaporated, leaving only the extract in the beaker. The beaker was allowed to cool to room temperature, and the amount of colorant extracted per gram of plant material used was determined.

Using the following equation, the yield of the natural colorant was calculated

$$\text{Dye yield \%} = \frac{\text{Weight of residue (g)}}{\text{Weight of raw material taken (g)}} \times 100$$

Ultra Violet – visible spectrum (UV-vis)

UV-vis spectroscopy is a widely used and versatile technique for detecting molecules in a compound. In this study, the aqueous-extracted sample of mulberry leaves was analyzed using LMSP-UVP1200 UV-visible spectrophotometer. The UV-visible spectrum of the extract samples was measured in the visible region, specifically in the range of 350-800 nm.

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR experiment was carried out using a Shimadzu 8400s spectrometer. In the ideal condition, dried mulberry leaves were finely ground into powder using a mixer grinder and was measured in absorbance mode within a wavelength range of 400-4000 cm^{-1} using a resolution of 4 cm^{-1}. The generated IR spectroscopy was tentatively assigned based on the wavelength.

Statistical analysis

All the measurements were performed in triplicate. Mean values±standard deviations (SD) were calculated. The data were subjected to one way analysis of variance (ANOVA) following Tukey's honestly significant difference post hoc analysis. The significance of variance (p<0.05) between means was calculated using IBM SPSS 16 software.

Results and Discussion

Ultra Violet -visible spectrum (UV-vis analysis)

The UV-vis spectrum of the natural dye obtained from mulberry leaves is shown in Figure 1. The results (Table 1) revealed that mulberry leaves dye extract showed maximum absorbance peak value of wavelength at 370 nm. Minimum absorbance peak was found at 674 nm spectrum.

Ginkgo biloba leaves extract showed maximum absorbance at 370 nm indicating the presence of kaempferol, quercetin, and isorhamnetin (Bilia, 2019) [6]. Cerovic *et al*. (2002) [8] showed that *Spinacia oleracea* leaf extract possess quercetin and kaempferol at an absorbance of 370 nm and 367 nm respectively. Notably, the spectrum exhibited absorbance peaks at 674 nm, which can be attributed to chlorophyll, aligning with the findings of Ohama (2014) [17] who reported that mulberry leaf extract obtained using ultrasonic energy exhibited a maximum peak absorbance at 638 nm. Lefebvre *et al*. (2020) [16] revealed that chlorophyll b is the compound found in peak absorbance of 646 nm from the extract of *Hedera canariensis* leaves.

Table 1: UV-vis spectrum of mulberry leaves dye extract

Wavelength (nm)	Absorbance
674	0.3018
370	3.8633
369	3.3152

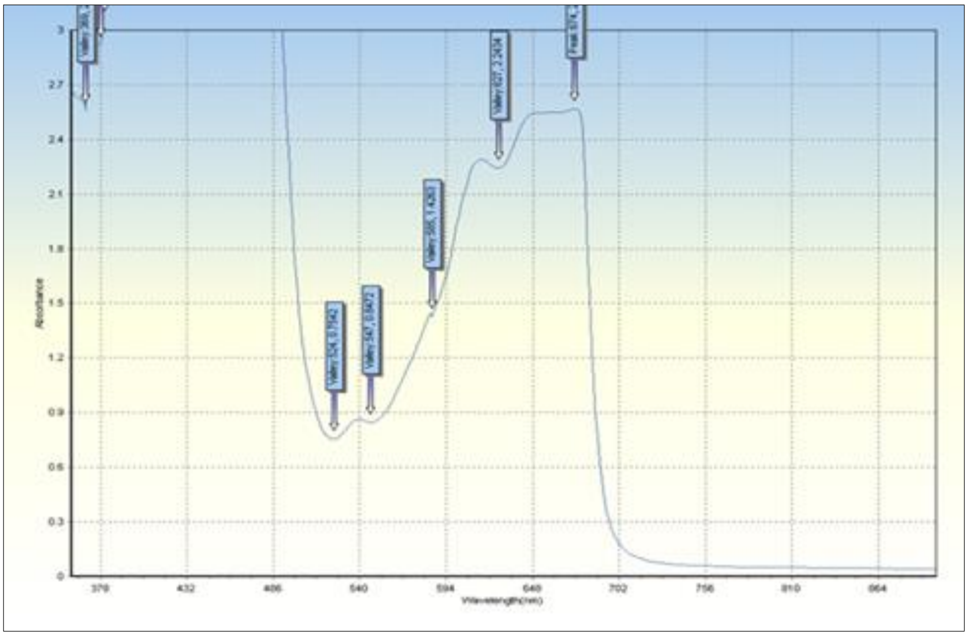

Fig 1: UV-vis spectrum of aqueous extract of mulberry (V1) leaves

FTIR Spectroscopy

The characteristics peak of extracted dye from mulberry leaves showed a band at 3842.20 cm^{-1} which is due to stretching vibrations of -OH group with intermolecular hydrogen bonding. Similar report was studied by Kumbhar *et al*. (2018) [14] in dye extracted from *Ficus amplissima* leaves showed a band at 3414 cm^{-1} due to the stretching vibrations of -OH group with intermolecular hydrogen bonding. Bródka *et al*. (2022) [22] revealed that the FT-IR spectra of mulberry leaves indicated the presence of flavonoid molecules in the range of 900-1700 cm^{-1}. The stronger band at 686.66 cm^{-1} and 648.08 cm^{-1} showed for C-Br stretch, 617.22 cm^{-1} band for C-I stretching, 555.50 cm^{-1} for C-Br stretching and 478.35 cm^{-1} - 432.05cm^{-1} band stretches for alkyl halides. Rather *et al*. (2020b) [19] analyzed the dye extracted from mugwort leaves using FT-IR spectroscopy. The presence of acid and carboxylate groups, specifically neochlorogenic acid, was confirmed by the presence of a peak at 523 cm-^{1} (−COOH deformation peak). Additionally, the peaks observed around 670−650 cm^{-1} were attributed to the C−O out-of-plane bending vibrations of tannins. The FT-IR spectra of extracted dye solution from mulberry leaves confirmed the presences of alcohol, halogen group, flavonoids, phenols and tannins in mulberry leaves.

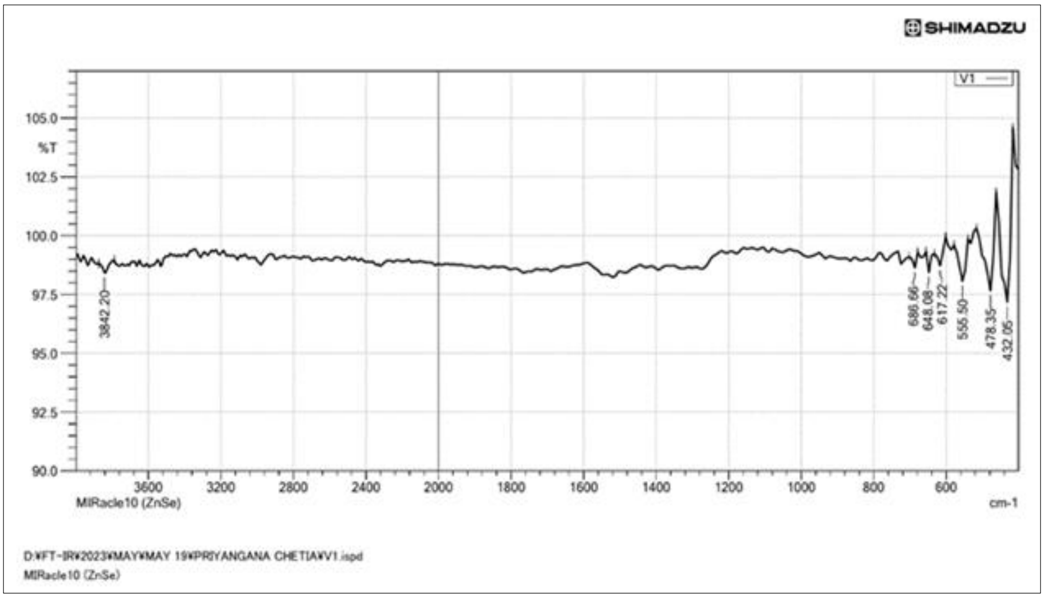

Fig 2: FT-IR spectrum of mulberry leaf extract

Conventional extraction of dye
The experimental parameters, including temperature, extraction time, and matter to liquor ratio (MLR), were optimized for the extraction of pigment from mulberry leaves (Table 2, 3 & 4).

Table 2: Dye yield at various time intervals, concentrations, and a constant temperature (60 °C)

MLR	60 min	70 min	80 min	90 min
1:10	5.26±1.41^{c}	8.5±0.5^{b}	13.26±0.65^{a}	9.2±0.8^{b}
1:20	9.5±1.80^{b}	9.5±1.5^{b}	13.07±0.23^{a}	9.39±0.89^{b}
1:30	9.28±0.75^{c}	11.48±1.03^{b}	13.38±0.59^{a}	8.37±1.16^{c}

Values were expressed as Mean±SD in triplicate

Values with different alphabets in each column are significantly different at α = 0.05 by Tukey's post hoc test.

Table 3: Dye yield at various time intervals, concentrations, and a constant temperature (70 °C)

MLR	60 min	70 min	80 min	90 min
1:10	5.63±1^{c}	10.59±1.28^{b}	15.48±0.68^{a}	6.59±1.13^{c}
1:20	11.62±0.78^{b}	13.66±0.99^{a}	14.62±0.93^{a}	9.52±1.14^{c}
1:30	11.44±0.60^{c}	14.96±0.81^{b}	18.39±0.64^{a}	15.63±0.67^{b}

Values were expressed as Mean±SD in triplicate

Values with different alphabets in each column are significantly different at α = 0.05 by Tukey's post hoc test.

Table 4: Dye yield at various time intervals, concentrations, and a constant temperature (80 °C)

MLR	60 min	70 min	80 min	90 min
1:10	8.85±0.61^{c}	12.24±0.57^{b}	16.44±0.54^{a}	8.33±0.72^{c}
1:20	11.11±0.80^{c}	15.33±0.50^{b}	17.19±0.78^{a}	8.66±0.67^{d}
1:30	13.3±0.67^{c}	17.40±0.49^{b}	22.29±0.44^{a}	17.11±0.29^{b}

Values were expressed as Mean±SD in triplicate

Values with different alphabets in each column are significantly different at α = 0.05 by Tukey's post hoc test.

The results revealed that maximum extraction was observed with 1:30 matter to liquor ratio at a temperature of 80 °C for 80 minutes.

Table 2 illustrated how the dye yield percentage was influenced by the extracted colorant's concentration at fixed temperature 60 °C. The outcome clearly demonstrated the increased colorant concentration enhanced dye output by a factor up to 1:30. Among all the MLR's, 1:30 concentration significantly varied with different time periods, with highest dye yield (22.29%).

Saravanan *et al.* (2013) [21] reported a good dye uptake with a M:L ratio of 1:30 concentration using *Acalypha indica* Linn leaves. Ali *et al.* (2009) [2] revealed that colour strength increased with increase in (L: R) *i.e.*, (1:30) in dye extraction of henna leaves. Khan *et al.* (2013) [11] claimed that dye extract from red calico leaf at M:L ratio of 1:30 using gamma radiations. Adeel *et al.* (2021) [1] analyzed the same colourant concentration for dye extraction from tea leaves using microwave radiation methods.

The extraction period and the extract's dye yield percentage at fixed temperature at 70 °C, was shown in Table 3. The dye yield percent increased with increase in extraction time. The results showed among all the MLR's 80 minutes which had the highest dye yield compared to other time duration. Rather *et al.* (2020a) [18] obtained results that align with the present findings, as they optimized the dye extraction process from *Cinnamomum camphora* waste/fallen leaves at 80 minutes duration. Islam and Mohammad (2018) [10] optimized teak leaves at 90 minutes duration by reflectance spectroscopic. A longer period of time leads in a lower yield content so Haggag *et al.* (2022) [9] revealed that mulberry dye yield increased with increase of dyeing temperature at 100 minutes.

Among the three different temperatures under which dye extraction was carried out, yield with 80 °C was found to be maximum. The Table 4 makes it is evident that, while using conventional heating, the extraction temperature and dye yield percentage are directly related to each other. Ohama (2014) [17] found the same extraction temperature from mulberry leaves using ultrasonic extraction. The present study is in line with Kumbhar *et al.* (2018) [14], who reported that a dyeing temperature of 80 °C was suitable for *Ficus amplissima* Smith leaves dye extraction. Saravanan *et al.* (2013) [21] claimed that colour strength is increased with increased dyeing temperature at 80 °C for dye extraction of *Acalypha indica* leaves. Haggag *et al.* (2022) [9] extracted dye from mulberry leaves using conventional heating at 100 °C.

Conclusion
The results of the present study revealed that mulberry leaves extract was found to be potential natural dye source for textile dyeing. The dye solution showed absorption maxima at 370 nm and minimum spectra at 673 nm indicated the presence of flavonols (kaempferol and quercetin) in mulberry leaves. Alcohol, phenols, flavonoids, tannins and halogen group in mulberry leaves were also confirmed by the FT-IR spectra. The optimal conditions of M:L ratio 1:30, temperature 80 °C and time duration of 80 minutes for dye extraction. Hence, mulberry leaves could be a promising and alternate source for natural dyeing in textile industry in the replacement of synthetic dyes.

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