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Characterization of Natural Dye Extracted from the Leaves of Wild Himalayan Pear (*Pyrus pashia*) by Phytochemical Analysis and FT-IR Spectroscopy

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ABSTRACT

The major goal of extracting dyes from plant (natural) sources is to reduce pollution in the environment. Present days with global concern over the use of eco-friendly and biodegradable materials, considerable research work is being undertaken around the world on the application of natural dyes in textile industry. The present study was carried out to characterize the *Pyrus pashia* leaf dye. Characterization of dye stuff is carried out to understand the nature of natural dye stuff and how it behaves with textile material. It was done by phytochemical analysis and FT-IR spectroscopy. For phytochemical analysis the Pyrus pashia leaf dye was prepared by using aqueous extraction method. Presence of different phytochemicals such as terepenoids, glycoside, quinines, coumarins, anthocyanins, phenols and tannin was detected in the leaf extract of *Pyrus pashia.* The FT-IR analysis of powder dye, undyed silk and dyed silk was carried out. Silk samples were dyed with optimized dyeing recipe. The FT-IR spectroscopy also provided evidences that IR spectrum of dye, undyed silk and dyed silk exhibited variation in their intensity and position of their absorption band.

Keywords FT-IR spectroscopy, Phytochemical analysis, *Pyrus pashia,* Silk dyeing, Characterization.

INTRODUCTION

The importance of plants is known to us well. They provide us with food, fodder, fuel, herbs, medicine, paper. Apart from these they are used in dyeing industry as a source of dye. Natural dyes derived from flora and fauna are thought to be harmless since they are nontoxic, noncarcinogenic and biodegradable in nature (Cristea and Vilarem 2003). Natural dyes are currently popular in cosmetics, leather, food, and medicines, in addition to the textile business. Our country's great biodiversity has supplied us with lots of raw resources, but a sustainable linkage among cultivation, collecting and utilization must be established (Gokhale *et al.* 2004). Natural dyes are used in the tex-

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tile industry for yarns dyeing, fabric dyeing and block printing. (Gopi 2004). In the present study the dye was extracted from the leaves of Pyrus pashia. *Pyrus pashia* also known as wild Himalayan pear, is a small to medium sized deciduous tree. It has finely toothed leaves, attractive white flowers with red anthers and small pear-like fruits. It is native to southern Asia. *Pyrus pashia* is described as an intermediate species between oriental and occidental pear groups. It is one of the most important wild pears. *Pyrus pashia* have played an important role in the evolution of the *Pyrus genus.* As P. pashia shows extensive adaptation to the environment, it is widely used as a pear rootstock in Southwest China (Zong *et al.* 2013). Characterization of the dye can be done with different techniques. Two techniques namely phytochemical analysis and FT-IR spectroscopy were used in the present study. Phytochemicals are the chemical compounds produced by plants, help them flourish and counter attack the competitors, predators and pathogens (Mendoza and Silva 2018). The phytochemical analysis helps in determining the chemical structure, biosynthetic origins which gives information of parent plant and how the growth hormone of the plant is acting (Harborne 1973). Phytochemicals are divided into 2 categories; primary constituents and secondary constituents. The primary groups include proteins, chlorophyll, amino acids and sugar. Terpenoids and alkaloids are the secondary constituents of phytochemicals (Wadood *et al.* 2013). Red pigments derived from natural sources contain a basic structure called as quinines. Quinines are composed of anthraquinones. The fastest shades of blue can be obtained from indigo. The quinone-based dyes, anthraquinones and naphthoquinones are responsible for majority of natural brown dyes (Patel 2011). Most of the yellow pigments derived from natural sources are derivatives of methoxy and hydroxyl substituted isoflavones or flavones (Classification of Natural Dyes 2008). The chemical compounds produced by the bio material (source of natural dye) can be identified using phytochemical analysis. The phytochemical such as glycosides, tannis, phenols etc can be identified using range of protocols based on type of chemical compounds. The ethanol and NaOH extraction of the dye from the flowers of *Spathodia campanulata* was done in soxhlet and it was found that tannins, cardiolides and terepenoids were positive in both the extracts whereas saponins, tannins, cardiac glycosides, cardiolides and terpernoids were positive only in ethanol extract (Parthasarathi and Lokesh 2015). FT-IR spectroscopy technique is used for characterization and identification of molecules including its structure and characteristic absorption spectrum of the sample (Espinosa *et al.*2012). FT-IR spectroscopy generally uses vibrational spectroscopic technique for analysis of chemical in biomedical samples (Chan and Kazarian 2016). Most of the organic as well as inorganic compounds in our atmosphere are at their excited level in infrared (IR) radiation because of the presence of dipole moments (Griffiths and de Haseth 2007). FT-IR relies on the principle of electromagnetic spectrum absorption of infrared light. The resultant absorption spectrum from the bond shows natural vibration frequencies that indicates the presence of various chemical bonds and functional groups present in the sample.

MATERIALS AND METHODS

Extraction of dye and dyeing of silk fabric

Extraction of dye was carried out at neutral pH. Two grams dye powder mass was extracted in 100 ml distilled water. Extraction was done at 80°C for 1 hour. Extract was filtered and pH of the dye was found neutral. The extract was used to dye presoaked silk fabric sample at 80°C for the duration of 1 hour in HTHP (High Temperature High Pressure) beaker dyeing machine. After dyeing the sample was removed from the dyeing machine, allowed to cool, rinsed under running tap water and dried in shade.

Phytochemical analysis of *Pyrus pashia* leaves dye

Identification of chemical compounds, produced by the biomaterials can be carried out using phytochemical analysis. Ten grams of dye powder was soaked in 300 ml distilled water for 12 hours followed by boiling at 100°C temperature for 1 hour in open dye bath. The extract was allowed to cool and filtered. Table 1 illustrates the details of the standard protocols for identification of different active chemical compounds which is given by Central Sheep and Wool Research Institute (2019).

Table 1. Phytochemical analysis of dye extracted from *Pyrus pashia* leaves.

Sl.No.	Chemical compound	Protocol	Identification/ confirmation
1	Saponins	2 ml of dye extract was shaken vigorously in a test tube	Stable foam will form
2	Phenols and tannins	In a test tube 2 ml of dye extract was added followed by the addition of 2 ml of ferric chloride (2%)	Color will change to bluegreen
3	Terepenoids	In a test tube 2 ml dye extract was added followed by the addition of 2 ml of chloroform and 2 ml of H_2SO_4	Reddish brown color will form at the interface
4	Flavonoids	2 ml dye extract + 2 ml of zinc dust/ magnesium and HCl drops were added drop wise in a test tube	Color of the solution will turn into red
5	Glycosides	In a test tube 2 ml dye extract + 0.1 ml of glacial acetic acid were added, followed by the addition of few drops of ferric chloride (5%) Few ml of concentrated sulfuric acid was added to this mixture	A brown ring will appear at the interface having greenish blue color solution
6	Quinines	1ml of dye extract was mixed with 1ml of concentrated sulfuric acid. Further confirmed with sodium hydroxide.	Red color formed Blue/ green color appeared after the addition of alkali
7	Coumarins	1ml of dye extract was mixed with sodium hydroxide (10%)	Yellow color will appear
8	Flavanones and Anthocyanins	1ml of dye extract was mixed with sodium hydroxide (10%) followed by the addition of sulfuric acid	Appearance of yellow to orange color and blue colour will confirm the presence of flavanones and anthocyanins respectively

FT-IR

FT-IR spectra of dye powder, raw silk and dyed silk was recorded for the detection of functional groups present in *Pyrus pashia* leaf dye. FT-IR spectrum represents the overall chemical composition and allows characterization of chemical groups in the form of fingerprints. It is an analytical technique used to identify organic material and in some cases inorganic material too. This technique uses infrared (IR) light to observe properties of a solid, gas or liquid.

RESULTS AND DISCUSSION

The aqueous extract of *Pyrus pashia* leaf dye was assessed for the presence of phytochemicals. The extract was assessed for identifying the presence of phytochemicals namely, saponins, phenols, tannins, terpenoids, flavonoids, glycosides, quinones, coumarins, flavanones and anthocyanins. All the phytochemicals were analyzed qualitatively (Table 2).

Table 2 reveals the presence of terpenoids, glycosides, quinones, coumarins, anthocyanins, phenols and tannin. Lia *et al.* (2015) isolated terpenoids from the leaves of *Pyrus pashia*. Seddiqui *et al*. (2015) also reported tannins, phenols, terpenoids, sugar, saponins, flavonoids and alkaloids in the chloroform, ethyl acetate and n-butanol soluble fractions of *Pyrus pashia* leaf dye. It was reported by Harborne (1973) that quinines based pigments range in color from pale yellow to almost black. Jez *et al.*(2000) reported that anthocyanins pigments give orange, red, purple and blue hues. According to Forkmann (1991), anthocyanins help in giving bronze and brown hues.

Pyrus pashia has played an important role in the evolution of the *Pyrus genus*. As *P. pashia* shows

Table 2. Qualitative phytoconstituents screening of *Pyrus pashia* leaf dye.

Sl. No.	Phytochemicals	Results
1	Saponins	-
2	Phenols	+
3	Tannins	+
4	Terpenoids	+
5	Flavonoids	-
6	Glycosides	+
7	Quinones	+
8	Coumarins	+
9	Flavanones	-
10	Anthocyanins	+

Key (+) present,(-) absent.

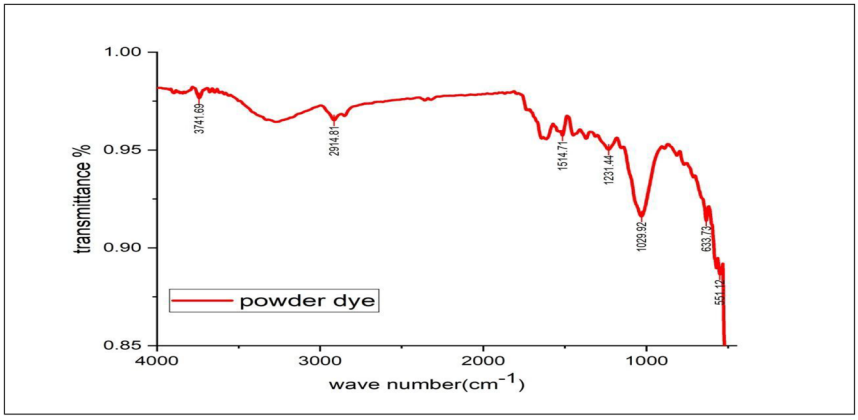

Fig. 1. FT-IR spectrophotometric chromatogram showing functional group peaks of *Pyrus pashia* leaf powder dye.

extensive adaptation to the environment, it is widely used as a pear rootstock in Southwest China (Zong *et al.* 2013). Pharmacological and phytochemical analysis of fruit confirm the presence of alkaloids, steroids, flavonoids and tannins, β-sitosterol, lupeol, βsitosterol—β-D-glucoside. It was also revealed that it showed antimicrobial activity against *K. pneumonia, S. flexneri* and *E. coli* (Saklani and Chandra 2012). The leaves of *P. pashia* also possess anti-mutagenic, anti-carcinogenic and anti-oxidant properties (Challice and Williams 1968). The leaves are rich in phenolic compounds which is responsible for its antioxidant property (Tsering *et al.* 2012). The *P. pashia* was assessed for detection of presence of phytochemicals and positive results for saponins, alkaloids, terpenoids, tannins, anthraquinones and flavonoids were reported (Janbaz *et al*. 2015). Presence of tannins in the dye helps in improving the wash and light fastness property of the dyed textile (Kumar and Bharti 1998, Sudhakar *et al*. 2006). According to Babel *et al.* (2013), compounds like phenol, terpenoids, flavonoids, alkaloids, glycosides, steroids and tannins gave antibacterial property to textile substrate.

FT-IR spectra was investigated to manifest the existence and formation of newly formed bonds over the treated silk due to the application of dye from the leaves of *Pyrus pashia.* The Fig.1. shows the FT-IR spectrophotometric chromatogram depicting functional group peaks of *Pyrus pashia* leaf dye powder. Broad peak was perceived between 3741.69 to 2914.81 cm^{-1} along with small group peaks at 3741.69 cm^{-1} and 2914.81 cm^{-1} and strong intense peak was formed at 1029.92 along with lower intense peaks between 1514.71 to 551.12 cm^{-1}. The band between 2981 to 2833 cm^{-1} may include symmetrical and asymmetrical stretched bands of alkanes, alkenes and alkynes (C-C, C=C, C≡C) of lipids and fatty acids. The bands formed between 1771 to 1104 cm^{-1} may be due to the presence of carbonyl groups (C-O) of aldehydes (-CHO), ketones (-C=O), carboxylic acids (-COOH) and esters (-C=O), nitro compounds (-NO), aliphatic amines (C-N), phosphoryl groups (P=O) and nucleic acids. The band formed at 1029 cm^{-1} and 633 cm^{-1} may be due to very strong intensity of =C-O-C symmetrical and asymmetrical stretching and C≡C stretching respectively.

According to Mishra and Jahan (2018), FT-IR spectrum of *Pseudomonas fluorescens* -24 showed a presence of aromatic O-H stretch at 3318.9 cm^{-1}.

The Fig. 2 shows the FT-IR spectrophotometric chromatogram showing functional group peaks of undyed silk. Peaks manifested at 3742.56 cm^{-1} may be due to O-H bond stretching. Peak manifested at 3273 cm^{-1} may be due to the presence of strong and free hydroxyls (OH) of water, alcohols, phenols, organic acids, due to OH bond stretching via Ar-OH intra-molecular hydrogen bond, stretched amide and amine groups of water and proteins respectively. The peak (cm^{-1}) between 2981-2833 may include presence of symmetrical and asymmetrical stretched bands of alkanes, alkenes and alkynes (C-C, C=C, C≡C)

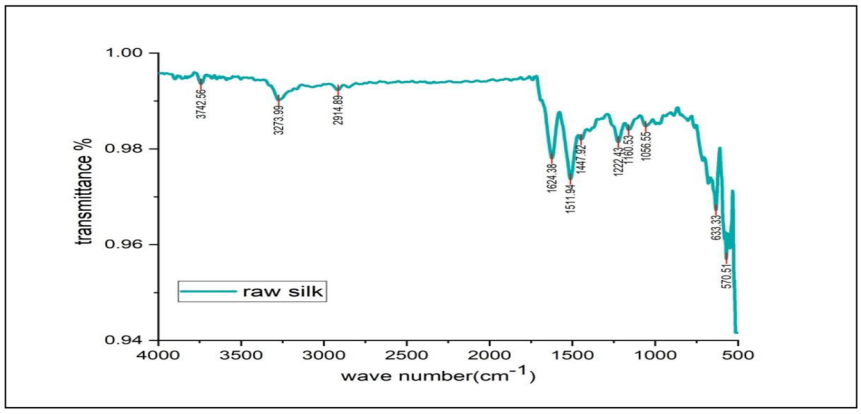

Fig. 2. FT-IR spectrophotometric chromatogram showing functional group peaks of undyed silk fabric sample.

of lipids and fatty acids. The peaks between 1771-1104 may be formed due to the presence of carbonyl groups (C-O) of aldehydes (-CHO), ketones (-C=O), carboxylic acids (-COOH) and esters (-C=O), nitro compounds (-NO), aliphatic amines (C-N), phosphoryl groups (P=O) and nucleic acids. A medium to small intensity peak was formed at 1447.9 cm^{-1} may be due to C=C stretching of aromatic compounds while medium to strong peaks formed in the range 1020-1075 cm^{-1} implies =C-O-C symmetrical and asymmetrical stretching.

It has been revealed from the FT-IR spectrum of degummed silk that at wave number above 3000 cm^{-1} strong bands were found (OH and NH stretching vibrations) and various amide bands were also found in the range of 1700–600 cm^{-1}, which are typical of polypeptides and proteins (Davarpanah *et al.* 2009).

Table 3 shows the wave length at which peaks were formed in dye powder, dyed and undyed silk fabric samples. The data depicted that four peaks of dye powder i.e., 3741.69, 2914.81, 1231.44 and 1029.92 cm^{-1} had disappeared in FT-IR spectra of dyed silk. It can be clearly seen from data that three peaks of undyed silk i.e. 3742.56, 2914.89 and 1160.53 cm^{-1} had disappeared in FTIR spectra of dyed silk. A slight shifting of peaks has been observed in the FT-IR spectra of dyed silk as compared to FT-IR spectra of undyed silk. The main difference was found in the peak located at 570.51cm^{-1} (Fig. 2), which was shifted to 548.88 cm^{-1} (Fig. 3). IR spectra of dye, undyed silk and dyed silk exhibited variation in their intensity and position of their absorption bands.

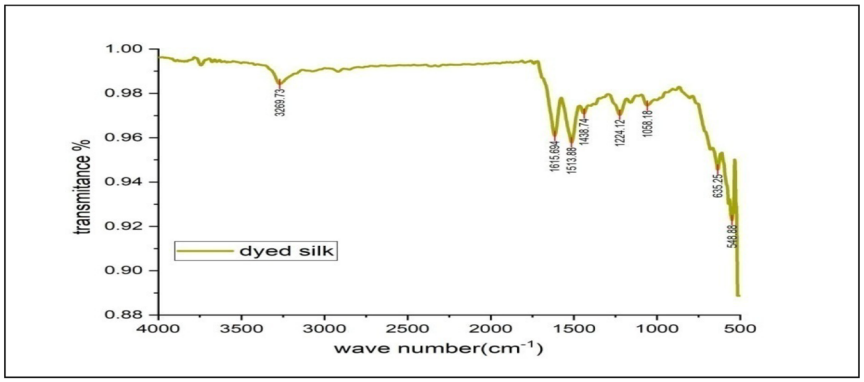

Fig. 3. FT-IR spectrophotometric chromatogram showing functional group peaks of dyed silk fabric sample.

Table 3. Characteristic functional groups of *Pyrus pashia* leaf dye powder, undyed and dyed silk fabric samples.

Wave number (cm^{-1})			Vibrational Assignment/
Dye powder	Undyed Silk	Dyed Silk	characteristic functional group
3741.69	3742.56	-	O-H stretching
-	3273.99	3269.73	Alcohol OH stretch (strong intensity)
2914.81	2914.89	-	C-H stretching with strong intensity
-	1624.38	1615.694	C=C alkenes (weak intensity)
1514.71	1511.94	15.13.88	N-H bending (amides with medium to strong intensity)
1231.44	-	-	C-O stretch of Ar-O-H or C-F stretch of alkyle halides
-	1447.9	1438.74	C=C stretching of aromatic compounds
-	1160.53	-	C-O stretch of alcohols
1029.92	1020-1075	-	=C-O-C symmetrical and asymmetrical stretching
633.73	633.33	636.25	C-X (strong intensity)
551.12	570.51	548.88	

CONCLUSION

The leaf extract of *Pyrus pashia* plant is rich in phytochemical compounds such as terepenoids, glycoside, quinines, coumarins, anthocyanins, phenols and tannin. The FT-IR technique did enable dye components of the dyeing materials in silk fabric to be distinguished. The FT-IR analysis of the dye powder, dyed fabric and undyed fabric showed the presence of different functional groups such as amides, alkenes, carbonyl group, alcohols and aromatic amines.

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