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A REVIEW OF NATURAL DYES FROM *LOLIUM PERENNE*, *MEGATHYRSUS MAXIMUS* AND *SACCHARUM OFFICINARUM*: EXTRACTION AND CHARACTERIZATION

Onofuevure, V.,*[1] Adamu, H.M.[1] Ajiya, D.A.[1] Nasiruddeen, B[1] and Ogunmefu, G.S.[2]

[1]Department of Chemistry, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

[2]National Biotechnology Research and Development Agency, Abuja, Nigeria.

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***Corresponding Author: Onofuevure, V.**
Department of Chemistry, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

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ABSTRACT

The study investigated the prospect of eco-friendly dyes extracted from *L. perenne*, *M. maximus* and *S. officinarum*. The aim was to extract and characterise the natural dyes. The extraction process involved Soxhlet techniques of the plants. The dye extracts were purified using chromatographic techniques. Fourier transform infrared spectroscopy techniques were used to characterise the isolates. Extraction was carried out at different temperatures. At a temperature of 80°C, ethanol was more efficient for extracting natural dye from the plants, with absorbance values of (1.4), (1.604) and (1.46) with a 29.88, 30.4 and 29.93 percentage recovery for *L. perenne*, *M. maximus* and *S. officinarum* within the wavelength of 200-800 nm. The dye extracts were subjected to thin-layer chromatography to separate the crude extracts on the basis of polarity and purify the extracts using column chromatography. The purified dye extracts were characterised using FT-IR. The FTIR study suggested the absorption band frequencies 3287.5-2937. 1 cm^{-1} is assigned to N-H stretching, 3362. 1 cm^{-1} to O-H stretching, 2927.7 cm^{-1}, 2881.2 and 937.1 cm^{-1} to symmetric C-H stretching, 1651.2 cm^{-1} and 1047.4 cm^{-1} to C=C stretching, 1461. 1 cm^{-1} vibration to $-CH_2$, 2937. 1 cm^{-1} to C-H stretching, 1640 cm^{-1} to C=O and 1461 cm^{-1} are assigned to C=C stretching in the dye structure. Thus, the findings can inspire further research and development in utilising the plants for natural dye production.

KEYWORDS: Natural dye, Extraction, Characterization, *L. perenne*, *M. maximus*, *S. officinarum.*

INTRODUCTION

The utilisation of plants as natural dyes holds a rich and enduring history across diverse cultures, where specific species have been essential for dyeing textiles, food, and various materials. Beyond their visual appeal, natural dyes exhibit antioxidant, anti-inflammatory, anticancer, and even antiallergic properties. These attributes make them promising compounds for the prevention of chronic diseases and the promotion of well-being (Manzoor *et al.,* 2021; Ghosh *et al.*, 2023). The versatility of these compounds is also reflected in their origins; natural dyes can be extracted from various parts of plants, such as fruits, peels, flowers, stems, roots, and seeds, significantly expanding the range of available sources for the sustainable production of pigments (Yadav *et al.,* 2023; Ghosh *et al.*, 2023). About 500 species of plants have been identified as potential sources of dye, and it is estimated that their various parts synthesise more than 2000 pigments (Gürses *et al.,* 2016). However, about 150 species have been commercially explored (Gürses *et al.,* 2016). This study focuses on extracting and characterising natural dyes from *Lolium perenne*, *Megathyrsus maximus* and *Saccharum officinarum*, probing their potential as dye sources. The Soxhlet extraction technique was employed for the extraction process, and the chromatographic technique was used to purify the crude extracts, and the dye component was characterised using Fourier transform infrared spectroscopy. *Lolium perenne* is an important and widespread perennial grass species (Byrne, 2015; Katova, 2017). It is grown in temperate regions around the world, where it accounts for up to 50% of total grass seed production (Katova, 2019)**.** It is characterised by high productivity and quality of the grass mass (Westermeier *et al.,* 2016; Katova, 2019). It has tufted, dark green, narrow, flat leaves with a width of 2-10 mm (Skerman and Riveros, 1990). *L. perenne* was successfully involved in combating the effects of environmental and soil pollution with metals, indicating that it is a plant with phytoremediation potential (Cruz *et al.,* 2021). *Megathyrsus maximus* (Guinea grass), formerly known as *Panicum maximum,* is a subtropical grass and is considered one of the best fodder species in tropical countries. The plant is more suitable for being used as a pasture, cut-and-carry, and producing hay and silage. The grass contains a percentage of leaf nutrients, nitrogen, phosphorus, potassium, calcium, and magnesium. The highest leaf nutrient levels for nitrogen, phosphorus, potassium, calcium, and magnesium were 1.49%, 0.29%, 3.16%, 0.57%, and 0.45%, respectively (Udumann et *al.,* 2023). *Saccharum officinarum* is a genetically complex plant genus consisting of different species (Pandey *et al.,* 2014). The variation of the plant morphology in quantitative character includes plant height, production stem height, stem diameter, and stem weight. The characters of plant height, production stem height and stem diameter showed low diversity, with KK values between 12.03% and 21.04% (Hamida and Parnidi, 2019). The leaves are long, narrow, and blade-like, with a length of 1-2 metres and a width of 2-5 cm. It is green with a waxy coating and has a midrib and sharp edges. (Moore and Botha, 2014). The study concluded that the plants can be used as a valuable source in the field of colour discovery.

MATERIALS AND METHODS

Chemicals and Reagents

Solvents such as dichloromethane, methanol and silica gel 200-400 mesh of analytical grade were used for the purification of the crude extracts, while ethanol (C_2H_5OH) was used for the extraction of dyes from the plant material.

Collection and identification of plant materials

The matured fresh leaves were harvested from Dorben Polytechnic, Abuja farmyard on 23rd March 2023 and were further identified and authenticated as *Lolium perenne*, *Megathyrsus maximus* and *Saccharum officinarum* from the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. The plants were given voucher numbers *L. perenne* (ATBU/DBSH: 545), *M. maximus* (ATBU/DBSH: 1847) and *S. officinarum* (ATBU/DBSH: 2497). The fresh leaves were washed, chopped into smaller particles and then air-dried for three weeks. The leaves were further reduced to powder form using a grinding machine to facilitate better dye extraction using solvent.

Extraction of Plant Material

In total extraction, 20.0 g of the powdered samples was weighed and poured into a 250 ml flat-bottom flask in the inner part of the Soxhlet extractor, and 200 ml of ethanol was added and heated under reflux with a condenser using a heating mantle at various temperatures of 50°C, 60°C, 70°C, 80°C, 90°C and 100°C to generate absorbance. The process automatically repeated itself, and the solvent in the dye extract was concentrated to obtain the dye extracts. A plot of absorbance against temperature was carried out in each case of the extraction, as shown in Figures 1-3.

Purification of Crude Extracts by Solvent/Solvent Fractionation

The crude extracts obtained from Soxhlet extraction after recovery of extracts by evaporation were subjected to purification by modified sequential solvent/solvent fractionation. Each of the plant crude extracts obtained from the previous sequential Soxhlet extraction was subjected to the sequential solvent/solvent fractionation or solvent washing.

Purification Procedure/Chromatographic Technique

The dyes extracted from the three plant materials were subjected to thin-layer chromatography. Separations of dye extracts were carried out on aluminium foil plates (UV 254) coated with a silica gel layer of 0.25 mm thickness. Thin layer chromatography with the solvent combination dichloromethane and methanol in the ratios (5:2 v/v) for *L. perenne*, (7:3 v/v) for *M. maximus,* and (6:4 v/v) for *S. officinarum* was used for the solvent mixture to separate the crude extracts. The TLC chromatogram of crude *L. perenne* showed eight spots, *M. maximus* showed fourteen distinct spots, whereas *S. officinarum* showed nine distinct spots. The isolated extract was then purified using the slurry method for the column chromatography. The elution process was carried out using mixed solvents, dichloromethane and methanol, in the ratios of suitable solvent for the three crude extracts. The dye extracts were injected into the column for separation, and different isolates from each plant sample fraction were collected and labelled with sample bottles (Ahmad *et al.,* 2021). The different elucidated samples were further tested using thin-layer chromatography to ascertain their purity. The Rf values were determined, and fractions having the same retention factor values were combined and evaporated and labelled isolate A, B and C.

Spectroscopic Techniques

FTIR Analysis of dye extracts

The FT-IR equipment with model number (Agilent Cary 630) from Agilent Technologies, Waltham, MA, USA, was used to identify the specific vibrational chemical bonds characteristic of the individual functional groups present in the dye extracts that were collected from the column. Dye extracts of the isolated compound were prepared, placed in the optical path of the instrument and scanned over the range of the 4000-650 cm^{-1} frequency region at 1 cm^{-1} intervals. The absorption bands were obtained and recorded as frequency (cm^{-1}) against percent transmittance (% (T). The spectrum was interpreted to deduce the functional groups present in the compounds.

RESULTS AND DISCUSSIONS

Results

Extraction Recovery

Table 1: Percentage Recovery of *L. perenne* Dye Using Ethanol

Temp °C	Weight (g)	(%) Recovery
50	16.95	15.25
60	15.850	20.75
70	15.020	24.9
80	14.024	29.88
90	18.128	9.36
100	18.25	7.75

Table 2: Percentage Recovery of *M. maximus* Extract Using Ethanol

Temp °C	Weight (g)	(%) Recovery
50	16.014	19.93
60	15.384	23.08
70	14. 656	26.72
80	14.92	30.4
90	17.220	13.9
100	17.842	10.79

Table 3: Extraction Recovery of *S. officinarum* Extract Using Ethanol (20 g)

Temp °C	Weight (g)	(%) Recovery
50	16.874	15.63
60	16.106	19.47
70	15.380	23.6
80	14.010	29.95
90	17.252	13.74
100	17.220	10.9

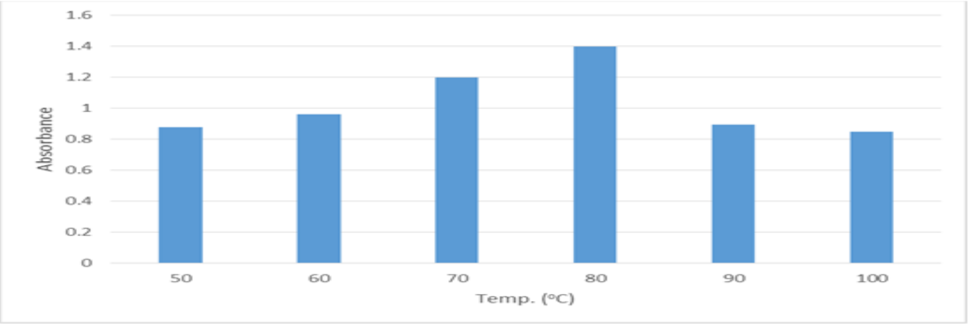

Figure 1: Effects of Different Temperatures on the Absorbance of Dye Extract from *L. perenne* using ethanol as solvent.

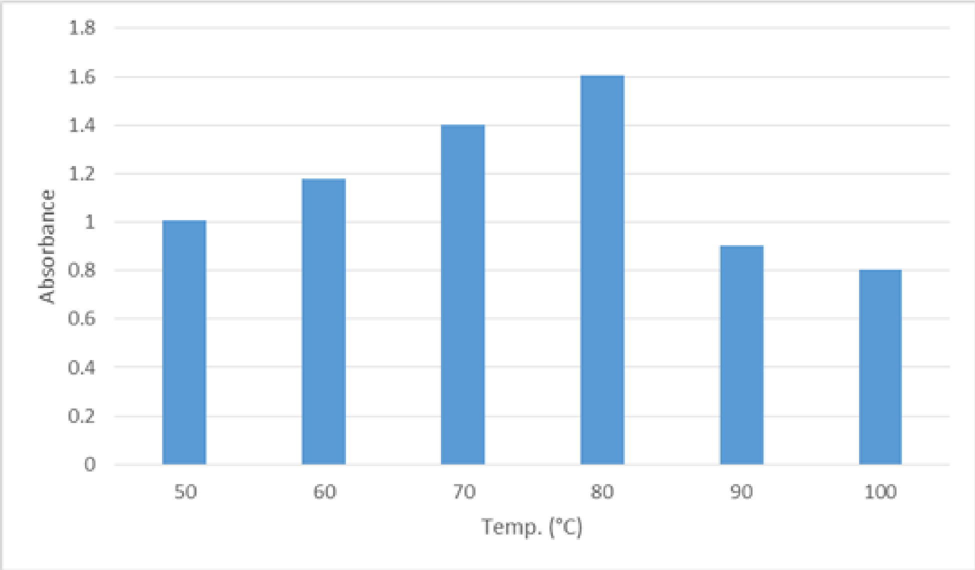

Figure 2: Effect of Different Temperatures on the absorbance of Dye Extracts from *M. maximus using* ethanol as a solvent.

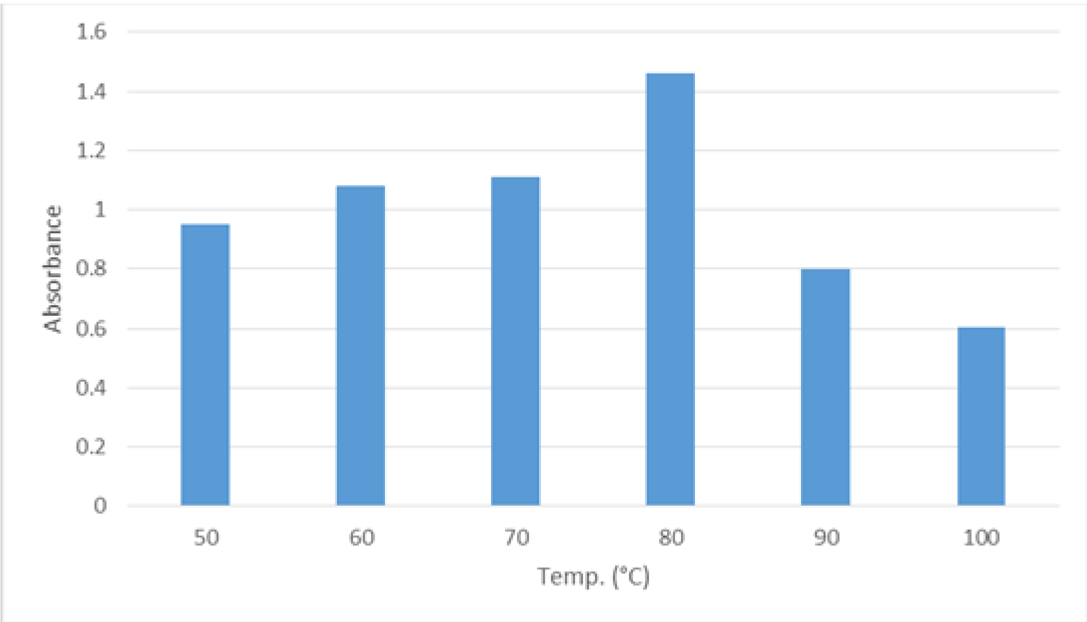

Figure 3: Effects of Different Temperatures on the absorbance of Dye Extract from *S. officinarum* using ethanol as a solvent.

Spectroscopic Data of Dye Extracts

Table 5: The Relevant Functional Groups Found in *L. perenne*

Wave number (cm^{-1})	Mode of Vibration	Functional group
3272.6	O-H stretching	O-H alcohol
1640.6	C=O stretching	C=O amides

1467	C-H bending	C-H alkanes compound
1121.9	C-O stretching	C-O alcohol
1047.4	S=O Stretching	S=O of sulfoxide

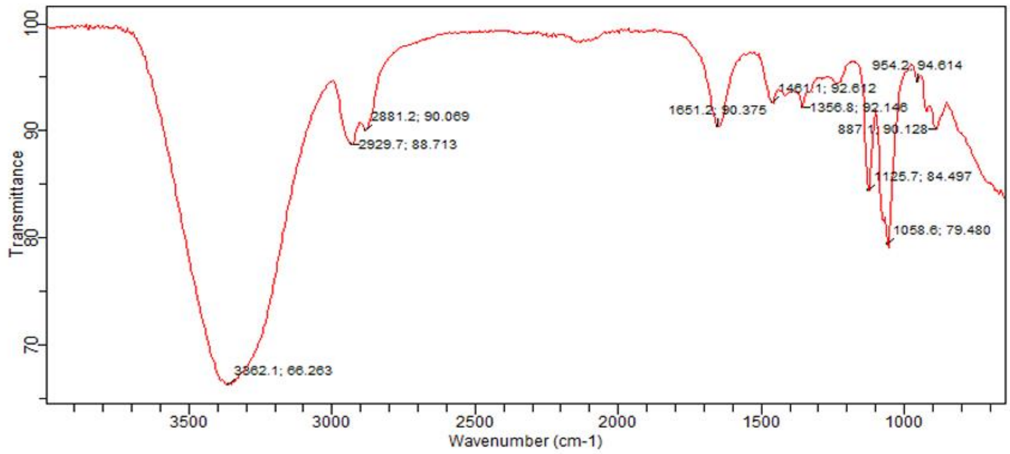

Figure 4: FTIR Spectrum of Dye Extract from *L. perenne*

Table 6: The Relevant Functional Groups Found in *M. maximus*

Wave number (cm^{-1})	Mode of Vibration	Functional group
3362.1	O-H stretching	O-H alcohol
2929.7	C-H stretching (asymmetric)	C-H alkane
2881.2	C-H stretching (symmetric)	C-H alkane
1651.2	C=C stretching	C=C alkane and aromatic
1461.1	C-H bending	$-CH_2-$ alkane
1356.8	NO_2 stretching	NO_2 Nitro compound

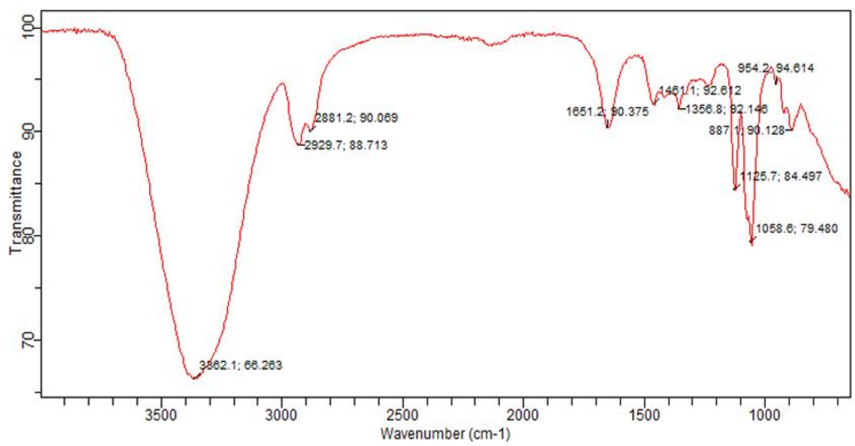

Figure 5: FTIR Spectrum of Dye Extract from *M. maximus*

Table 7: The Relevant Functional Groups Found in *S. officinarum*

Wave number (cm^{-1})	Mode of Vibration	Functional group
3287.5	N-H stretching	N-H amine
2937.1	C-H stretching	C-H alkanes
1640	C=O stretching	C=O amides
1461	C=C stretching	C=C of aromatic

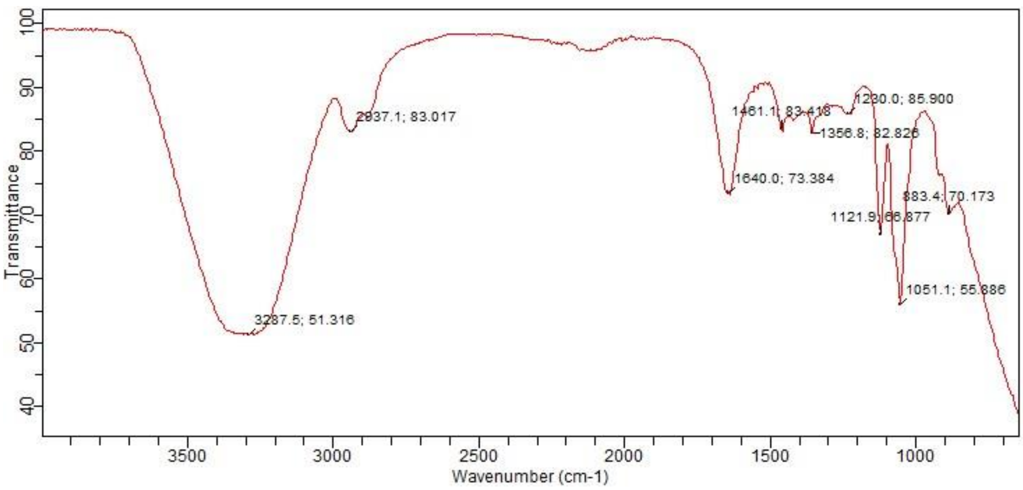

Figure 6: FTIR Spectrum of Dye Extract from *S. officinarum*

DISCUSSIONS

Results of Solvent Extraction at Different Temperatures

Figures 1-3 and Tables 1-3 present the solvent extraction of dyes at different temperatures obtained from *L. perenne, M. maximum and S. officinarum* from the sequential Soxhlet extraction. The results showed the relationship between absorbance and temperature changes. The study revealed that the ethanol solvent used, at a temperature 80°C, which was in agreement with Razak *et al.* (2011), was observed to have a maximum absorbance value of (1.4), (1.604) and (1.46) with a 29.88, 30.4, and 29.95 percentage recovery for *L. perenne, M. maximus* and *S. officinarum* within the wavelengths of 200-800 nm. Ethanol as an organic solvent has been extensively used to extract natural organic dyes from various plant species (Peschel *et al.,* 2006). The results show that high yield was obtained at a temperature of 80°C, which suggested that some natural organic dyes are stable at temperatures less than 80°C. The increase in dye yield is as a result of an increase in temperature, which may be caused by an increase in pigment molecule diffusivity and pigment solubility; these properties are related to the increase in the internal energy of molecules, which increases the extract concentration (Cacace and Mazza, 2003). Temperatures more than 80°C probably caused a decrease in the extract concentration due to chemical structure degradation of pigments. Thus, a temperature of 80°C proves to be the optimum temperature for extraction with high yield.

Spectroscopic Data of Dye Extracts

***L. perenne* Dye Extract using FTIR**

From Table 5 and Figure 4, the absorption band at 3272.6 cm^{-1} vibration attributed to O-H stretching indicates the presence of an alcohol group, and the bands at frequencies 1640 and 1047.4 cm^{-1} due to C-H bending suggest the presence of an alkane or alkyl group. The band appearing at the 1121.9 cm^{-1} region can reasonably be ascribed to C-O stretch, which confirmed the presence of tertiary alcohol, which is also in agreement with Adeyanju *et al.* (2021). FT-IR results were comparable with those reported by Adeyanju *et al.* (2021), who reported bands at 2920.4 cm^{-1}, 1675.4 cm^{-1}, 1578.5 cm^{-1}, 1507.7 cm^{-1}, 1427.6 cm^{-1} and 1269.2 cm^{-1} for natural and modified turmeric dye extracts.

***M. maximus* Dye Extract using FTIR**

FT-IR analysis of the sample in Table 6 and Figure 5 revealed the presence of many organic functional groups present in *M. maximus,* indicating their respective compounds. Comparison of the absorption frequencies of various organic functional groups mentioned by Maiyo *et al.* (2019) revealed bands at frequency 3362.1 cm^{-1} vibration due to the O-H stretch, which indicates the presence of alcohols, which is in agreement with Maiyo *et al.* (2019), and the absorption of 2927.7 cm^{-1} and 2881.2 cm^{-1} vibration is due to the C-H symmetric stretch, which suggests the presence of alkanes. The band displayed at frequency 1651.2 cm^{-1} vibration is due to C=C stretch, which suggests the presence of alkene and aromatic, which is in agreement with Maiyo *et al.* (2019). The band displayed at frequency 1461.1 cm^{-1} vibration is due to $–CH_2$ bending, indicating the presence of alkane, which is in agreement with Adeyanju *et al.*, 2021. The band at 1356.8 cm^{-1} is assigned to NO_2 stretch, which confirmed the presence of nitro compounds. It was found that FT-IR results were comparable with those reported by Maiyo *et al.* (2019), and Adeyanju *et al.* (2021) reported bands at 3131.94 cm^{-1}, 2935.48 cm^{-1}, 1597.61 cm^{-1}, 1400.77 cm^{-1}, 1325.99 cm^{-1}, 1213.87 cm^{-1}, 1065.85 cm^{-1}, 608.31 cm^{-1}, 2918, 2849, 1745, and 1462 cm^{-1} for natural and beeswax. Sugarcane peel wax also exhibited similar absorption patterns.

S. officinarum Dye Extract using FTIR

Table 7 and Figure 6 describe the various functional groups present in the *S. officinarum* extract. From Table 7 and Figure 6, the absorption bands at 3287 cm^{-1} are assigned to N-H stretch which confirmed the presence of amine group, and the band at frequency 2937.1 cm^{-1} vibration region assigned to C-H stretching indicates the presence of alkane groups which agreeing with (Abdel-Hameed *et al.,* 2013). The IR spectrum of the dye extract from *S. officinarum* showed absorption at 1640 cm^{-1} which suggest the presence of C=O or carbonyl groups, possibly from ketones or aldehydes. The absorption bands at 1461 cm^{-1} are assigned to C=C stretching which confirmed the presence of aromatic groups. This observation agreed with the findings made by different researchers (Amir *et al.,* 2018; Jabar *et al.,* 2020; Mongkholrattanasit *et al.,* 2021) in natural dyes for textile dyeing.

CONCLUSION

The present study investigated natural dyes from *L. perenne, M. maximus, and S. officinarum* as a potential dye source. Of the various temperatures used, ethanol was a more efficient solvent for extraction at a temperature of 80°C with a high yield for the three plants. The dye extracts were subjected to thin-layer chromatography to separate the crude extracts on the basis of polarity and purify the extracts using column chromatography. The purified dye extracts were characterised using FT-IR techniques; the absorption band frequencies 3287.5-2937.1 cm^{-1} are assigned to N-H stretching, 3362.1 cm^{-1} to O-H stretching, 2927.7 cm^{-1}, 2881.2 and 937.1 cm^{-1} to symmetric C-H stretching, 1651.2 cm^{-1} and 1047.4 cm^{-1} to C=C stretching, 1461.1 cm^{-1} vibration to $–CH_2$, 2937.1 cm^{-1} to C-H stretching, 1640 cm^{-1} to C=O and 1461 cm^{-1} to C=C stretching in the dye structure. The study concluded that the presence of compounds justifies the use of the plants as promising results with potential applications as natural dyes.

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