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Spectroscopy and chemical analysis of natural dye from *Sorghum bicolour*

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Many plants produce very attractive flowers, leaves and fruits. Colour pigments that are derived from nature are believed to be environmentally friendly because of their non-toxic, non-carcinogenic and biodegradable nature. In this project, colour pigment from ethanol extract of *Sorghum bicolour* was investigated using three different spectroscopic techniques (UV-Vis, FT-IR and GC/MS). The effects of dyeing parameters; temperature, pH and the presence of mordant on dyeing quality of the extract were also examined. UV-VIS absorption spectra indicates a λ_{max} peak at 535 nm, value associated to delphinidin dyes under bathochromic shift. Structural characterization by FT-IR revealed presence of C=O, OH and C=C of aromatic. Twelve phytochemicals were identified through GC/MS study.The extract showed colour change from brown to red between pH ranges of 3 to 12. The best dyeing quality was achieved at 30°C with alum pre-treated cloth at neutral pH. Presence of delphinidin dye in *S. bicolour* extract was confirmed from spectroscopy and chemical analysis results; however, further feasibility study has to be carried out on *S. bicolour* before it can be applied as dye in industry.

Key words: *Sorghum bicolour*, spectroscopy, natural dye, delphinidin.

INTRODUCTION

Sorghum bicolour is annual or short-term perennial plant that grows up to 4 m or more in height. Mature glumes of sessile spikelets are either red or reddish brown or straw coloured or yellowish, sometimes flushed with dark red or reddish brown; grain predominantly red or reddish brown (De Wet et al., 1972). Plants, animals and minerals sourced natural dyes are renewable and sustainable bioresource products with minimum negative environmental impact. Biosource dyes have been known since antiquity for their uses, not only in coloration of textiles (Kadolph, 2008) but also as food ingredients (Dweck, 2002) and cosmetics (Frick, 2003).

Awareness of the hazard associated with synthetic dye has led to upsurge in the use of non-allergic, non-toxic and ecofriendly natural dyes. Recently, a number of commercial dyer and small textile houses have started investing on the possibilities of using natural dyes for regular basic dyeing and printing of textile in order overcome negative environmental impacts caused by synthetic dyes (Glover and Pierce, 1993). The choice of dyeing parameters can influence the affinity (substantivity) of dyes for cellulose fibres which depends

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on the type of chromophore (Ross et al., 1989). Solubility, substantivity of the dyes, and their stability in the dyebath are influenced by the pH value of the solution. Dyeing equilibrium is determined by temperature, which at room temperature is shifted strongly toward adsorption on the fibre. At high temperature, usually 80.0°C the equilibrium is attained more rapidly (Klaus, 2003). Natural dyes adhere to natural fibres with the use of a mordant or fixative. Mordant dyes require addition of chemical substances, such as metal salts (mordanted), to give them an affinity for the material being dyed. Highly adhesive, basic metal compounds are formed on the fibre. These compounds are capable of producing insoluble coloured complexes (lakes) with certain azo and anthraquinone derivatives. The shade of the dyeing depends on the type of metallic mordant used (Rath, 1972).

Many plant pigments have been revealed to act as acid-base indicators. The pigments in the plant products such as tea, red cabbage or grapes react with acids or bases resulting in changes at the molecular level which causes their colour to be different at different pH levels. Some examples of natural pH indicators are anthocynins which are pigments that react in a different way to acids and bases. Alizarin isolated from the root of the madder plant, is yellow at pH 5.5 and red at pH 6.8. Another natural dye, Curcumin, or turmeric yellow, is found in curry powder. It turns from yellow at pH 7.4 to red at pH 8.6. Most plants contain pH sensitive anthocyanins that can be used as pH indicators.

Acid-base indicators are substances which appear as characteristic colours in different pH. They have different colours in their ionized form and non-ionized form and are usually weak organic acid or base. At equilibrium the two forms of indicator are present in solution. The most important structure changes for indicator dyes are; Reversible transition between acid and base form of a molecule (pH indicator); Reversible transition between reduced and oxidized forms of a molecule (redox indicator), and reversible transition between the free molecule and its complex with a cation (metal indicator) (Ingrid, 2001).

$$HIn_A \rightleftharpoons In_B + H^+$$

To be suitable as an indicator molecule, the absorption spectra of In_A and In_B must be different. The equilibrium shifts to the right or left depending on the pH of the solution. In an acidic medium, the equilibrium shifts to the left and the colour of the indicator is mainly the colour of the non-ionized form. In alkaline medium, the equilibrium shifts to the right and the colour of the ionized form predominates.

Carcinogenic property of synthetic dyes and their persistence in air make the attention of scientists sifted to natural dyes. There is not enough information about colour properties of *S. bicolour* extract. However, the purple coluors produced by plants are related to the flavonoids group called anthocianins (Espinosa-Morales et al., 2012). Therefore, more studies are needed in order to include dye properties of *S. bicolour* extract in colour data base. For this purpose, spectroscopic techniques are used to characterize the pigment while chemical analyses are used to determine the chemical properties of colorant in the extract.

EXPERIMENTAL

Extraction

Dried leaves of *S. bicolour* obtained from the open market in Oyo (Oyo State, Nigeria) were used in this study. The powdered sample 450.0 g was extracted with 1.5 L of distilled ethanol. The extraction was done for 7 days and the extract was filtered using Whatmann No.2 filter paper. The filtrate was evaporated in vacuo to give a residue (60.50 g), which was kept at 4°C until further use.

UV-Vis spectroscopy analysis

An aliquot of the extract was introduced in a quartz cell (1 cm pathway) and analyzed in UV/Vis spectrophotometer. A scan from 190 to 900 nm was performed in order to generate the characteristic absorption spectra of the sample.

FT-IR spectroscopy analysis

2 mg of powered extract was mixed thoroughly with 200 mg of potassium bromide (KBr) and homogenized in an agate mortar. The mixture was then placed in the sample compartment of Infrared Fourier Transform Spectroscopy (FT-IR) for analysis.

GC/MS analysis

Analysis of phyto-component of the extract was carried out by using Gas Chromatography-Mass Spectroscopy (GCMS-QP 2010) PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector which was used with RTX5MS column packed with 100% grade dimethylpolysiloxane. The analysis was carried out under the following conditions: injection volume 0.5 µL, inlet temperature 250°C, split ratio (1:20). Helium was used as a carrier gas with a flow rate of 0.7 mL/min. Oven temperature was set at 50°C and held for 1 min at this temperature. Thereafter, it was raised to 175°C with a flow rate of 25°C/min. In the last step, the temperature reached 230°C with the speed of 4°C/min and held for 5 min at 230°C.

Peak enrichment technique of reference compounds was used in identifying volatile component. The peaks identified by GC-MS were further confirmed by comparing their spectra with those of NIST Library mass spectra.

Dyeing process

Stock solution was prepared by dissolving 30 g of the extract in 100 mL of distilled water. To 5 ml of aliquot 100 ml of distilled water was added and this was replicated into 32 flasks. Fourteen pieces of cleaned white cloths of equal length were boiled in alum solution. Effect of temperature was investigated by varying the temperature of dyeing solution; 0, 30, 60 and 80°C. A piece of cloth was then added to each solution and dyed for 15 min. For the effect of pH, a piece of cloth was immersed each into dyes containing dilute

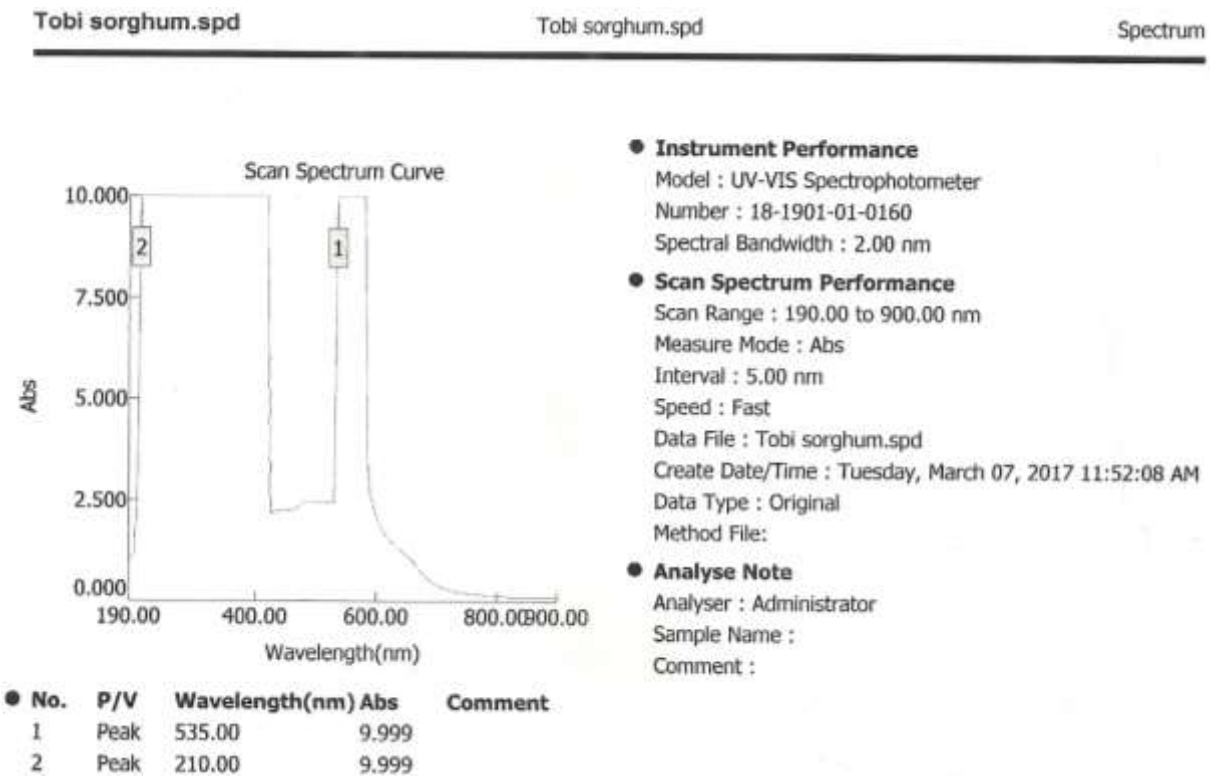

Figure 1. UV-VIS spectra from ethanol dye extract obtained from leaves of *Sorghum bicolour.*

hydrochloric acid, distilled water and dilute sodium hydroxide solution. For the effect of mordant, ordinary cloths and those soaked in alum solution were used for dyeing. All experiments were carried out in duplicate. After dyed cloths had been dried, they were soaked in distilled water for 15 min. The bleaching of the colour was then monitored colorimetrically (Peters, 1975).

pH indicator

Solutions of various pH 1 - 14 were prepared and 3 drops of the plant extract were added to each solution to observe the colour change. Furthermore, 0.1 M solutions of HCl, H_2SO_4, KHP and CH_3COOH were prepared in 4 different conical flasks. To each conical flask, 3 drops of extracts were added and titrated against standardized 0.2 M NaOH solution.

RESULTS AND DISCUSSION

The characteristic red colour manifested by the dye extract could be the consequence of the presence of delphinidin (anthocyanins) derivatives. Figure 1 shows the absorption spectra generated during the analysis of leaves extract of *S. bicolour.*

A maximum absorbance peak at uv range (λmax) was detected at 535 nm. This value is closed to the value of delphinidin-3-cis-coumaroylrut-5-glucoside (532 nm) reported by Eva et al. (2006). Other delphinidin derivatives reported in her findings range from 522-530 nm.

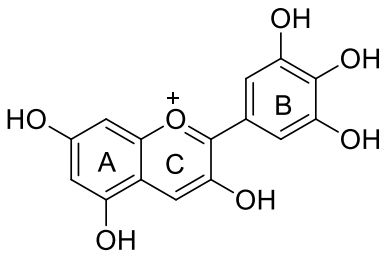

Figure 2. Structure of Delphinidin.

Delphinidin is formed by two aromatic rings (Figure 2) the benzopiril group (A and C ring) and the phenolic group (B ring). Delphinidin which may have H, -OH, $-OCH_3$ or sugar moiety substituent groups. Their characteristic colour depends strongly on the number of substituents at the B ring (Myjavcová, et al., 2010).

FT-IR spectra in Figure 3 show a strong signal at 3401 cm^{-1} corresponding to the characteristic O-H vibration which is corroborated by C-O vibration at 1239 cm^{-1}. The signals at 1606, 1509 and 1446 cm^{-1} are C=C aromatic group vibrational frequency. A weak absorption band at 1712 cm^{-1} is assigned to C=O in ring.

Gas chromatography-mass spectrometry analysis of the leaf extract resulted in the identification of twelve (12) compounds. The major components are ethyl oleate

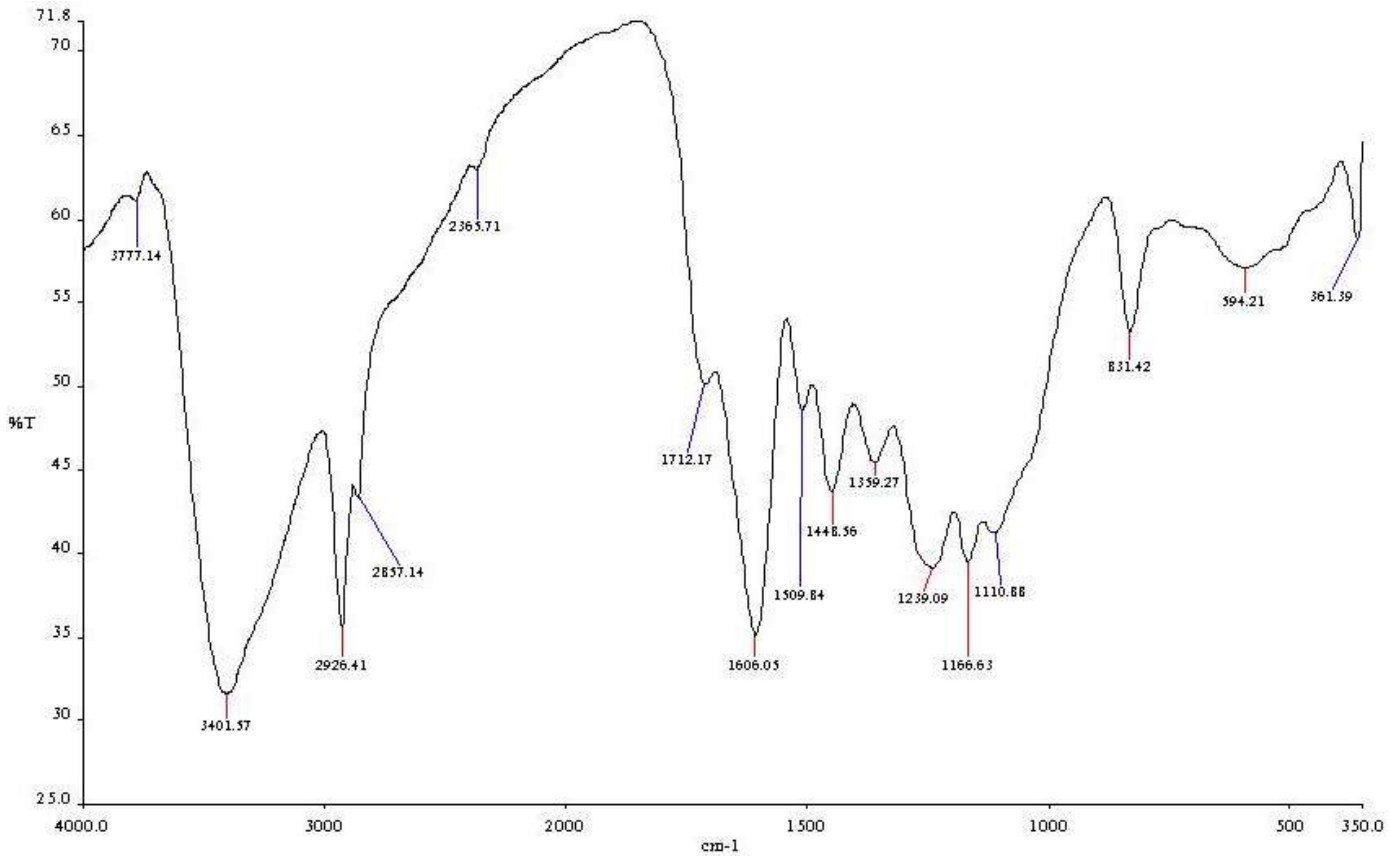

Figure 3. FT-IR spectra of *Sorghum bicolour* extract.

Table 1. Phytocomponents identified from *Sorghum bicolour* leaves extract.

S/No.	RT	Name of the compound	Molecular formula	MW (g/mol)	Peak area (%)
1	9.471	Ethyl paraben	$C_9H_{10}O_3$	166.176	9.10
2	9.911	1-Hexadecanol	$C_{16}H_{34}O$	242.44	1.47
3	11.160	5-Octadecene, E	$C_{18}H_{36}$	252.486	1.93
4	12.318	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	2.89
5	12.576	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	19.02
6	13.986	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282.468	7.86
7	14.265	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.4986	19.51
8	14.316	Ethyl Oleate	$C_{20}H_{38}O_2$	310.52	23.13
9	14.562	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.5304	9.41
10	16.535	Eicosanoic, ethyl ester	$C_{22}H_{44}O_2$	340.5836	1.68
11	16.787	n-propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.541	2.48
12	17.997	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.62	1.52

(23.13%), Linoleic acid ethyl ester (19.51%), Hexadecanoic acid ethyl ester (19.02%), octadecanoic acid ethyl ester (9.41%), ethyl paraben (9.15%), and octadeca-9-enoic acid (7.86%). Table 1 lists the constituents in order of elution from HP-5 capillary column.

According to the research findings, every dye has its own optimal temperature range for dyeing. High temperatures have adverse effects on dyeing because precipitate started forming around 60°C and above (Liu et al., 2003). Tables 2 and 3 show the intensity of bleached dye under various dye parameters at 420 nm. The best

Table 2. Intensity of bleached dye in various temperature at 420 nm.

Temperature (°C)	Absorbance	% Transmittance
0	0.05	90
0*	0.035	93
30	0.04	90
30*	0.02	95
60	0.085	82
60*	0.025	97
80	0.062	88
80*	0.06	87

* Indicates dyeing with cloth soaked in alum.

Table 3. Intensity of bleached dye in various media at 420 nm (60°C).

Medium	Absorbance	% Transmittance
0.1 M NaOH	0.24	58
0.1 M NaOH*	0.065	86
0.1 M HCl	0.05	90
0.1 M HCl*	0.07	86
Distilled H_2O	0.07	86
Distilled H_2O*	0.04	92

* Indicates dyeing with cloth soaked in alum.

Table 4. Dyeing parameter.

Optimal conditions for dyeing / Extract	Temperature	pH medium	With or without alum
	30°C	Neutral (distilled H_2O)	With alum

Table 5. 0.2 M NaOH vs 0.1 M HCl.

Indicator	Colour in acidic medium	Colour in basic medium	Volume of acid used (ml)	Titre value (ml)
Extract	Golden yellow	Red	25	44
Methyl red	Pink	yellow	25	43.5
phenolphthalein	Colourless	pink	25	40.9

dyeing quality was achieved at neutral pH when distilled water was used for dyeing instead of acid or basic solution. There was great improvement in dyeing quality, especially the ability to retain colour, when mordant was added. This supports the claim that mordant function is to bind colours on cloths. Table 4 shows optimal conditions for dyeing process.

The sets of colours obtained by using *S. bicolour* extract as pH indicator were compared with that of phenolphthalein, methyl red and methyl orange. Acid base titrations were carried out with strong and weak acids. Table 5 revealed that the values of extract and methyl red were very close compared to that of phenolphthalein. From Tables 6, 7 and 8 the three indicators have nearly the same values except in Table 8 where the value of methyl red is lower than that of phenolphthalein and extract.

Conclusion

Presence of delphinidin in *S. bicolour* extract was evidence from spectroscopy and chemical analysis results. Natural dyes are limited in application because

Table 6. 0.2 M NaOH vs 0.1 M KHP.

Indicator	Colour in acidic medium	Colour in basic medium	Volume of acid used (ml)	Titre value (ml)
Extract	Light brown	Red	10	8.3
phenolphthalein	Colourless	Pink	10	8.5

Table 7. 0.2 M NaOH vs 0.05 M H_2SO_4.

Indicator	Colour in acidic medium	Colour in basic medium	Volume of acid used (ml)	Titre value (ml)
Extract	Light brown	Red	10	5.0
Methyl red	Pink	Yellow	10	4.9
phenolphthalein	Colourless	Pink	10	5.1

Table 8. 0.2 M NaOH vs 0.1 M CH_3COOH.

Indicator	Colour in acidic medium	Colour in basic medium	Volume of acid used (ml)	Titre value (ml)
Extract	Brown	Red	10	20.6
Methyl red	Pink	Yellow	10	17.3
phenolphthalein	Colourless	Pink	10	20.6
Methyl orange	Orange	Yellow	10	3.5

they are easily degraded by light and heat. Nevertheless, natural dyes are non-hazardous and more desirable than synthetic dyes. Biosource dyes are non-allergic and they are more suitable for children and babies. Moreover, they are biodegradable and pose less pollution problems. However, further stability study has to be carried out on *S. bicolour* pigment before it can be used by industry as dye.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

De Wet JMJ, Harlan JR, Kurmarohita B (1972). Origin and evolution of guinea sorghums. East Africa. Agric. For. J. 37:114-119.

Dweck AC (2002). Natural ingredients for colouring and styling. Int. J. Cosmetic Sci. 24:287-302.

Espinosa-Morales Y, Reyes J, Hermosín B, Azamar-Barrios JA (2012). Characterization of a Natural Dye by Spectroscopic and Chromatographic Techniques Mater. Res. Soc. Symp. Proc. 1374.

Eva SC, Florian S, Reinhold C (2006). Anthocyanins, Colour and Antioxidant Properties of Eggplant (*Solanum melongena* L.) and Violet Pepper (*Capsicum annuum* L.) Peel Extracts. Z. Naturforsch. 61:527-535

Frick D (2003). The coloration of food. J. Rev. Prog. Colour 33:15-32.

Glover B, Pierce JH (1993). Are natural colourants good for your health? J. Soc. Dyers Colourists 109(1):5-7.

Ingrid J (2001). Natural pH Indicators: What are they and how do they work?:Acids and Bases equilibrium. Nelson Science 10.

Kadolph SJ (2008). Natural dyes: a traditional craft experiencing new attention. Delta Kappa Gamma Bull. 75(1):14-17.

Klaus H (2003). Industrial Dyes: Chemistry, Properties, Applications. Wiley- VCH Verlag Weinheim.

Liu RLH, Chiu HM, Yeh RYI (2003). Colloid interaction and coagulation of dye wastewater with extra application of magnetites. Int. J. Environ. Stud. 59:143-158.

Myjavcová R, Marhol P, Kren V, Simanek V, Ulrichová JI, Palíková I, Papouskova B, Lemr, K, Bedñar P (2010). Analysis of Anthocyanin Pigments in Lonicera (Caerulea) Extracts. J. Chrom. 12(17):7932-7941.

Peters RH (1975). Textile Chemistry Vol. III - The Physical Chemistry of Dyeing. Elsevier, Amsterdam, The Netherlands.

Rath H (1972). *Lehrbuch derTextilchemie* Springer Verlag, Berlin 3rd ed. P 578.

Ross E, Köthe J, Naumann R, Fischer W, Jäschke U, Mayer WD, Wieland G, Newman EJ, Wilson CM (1989). .Indicator Reagents. Ullmann's Encyclopedia Ind. Chem. A14:127-148.