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CHROMATOGRAPHIC SEPARATION AND SPECTRO-ANALYTICAL CHARACTERIZATION OF A NATURAL AFRICAN MINERAL DYE

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ABSTRACT. Chromatographic fractionation and spectroscopic characterization of a natural African mineral dye have been carried out. The chromatographic separation of the dyes made use of column and thin layer chromatographic techniques. Some physicochemical properties of the dye including solubility in polar and non-polar solvents, pH, ash and organic contents were determined. The spectro-analytical techniques used for characterization included energy dispersive X-ray fluorescence (EDXRF), X-ray diffractometry (XRD), Optical microscopy, infrared (IR) and UV-VIS spectroscopy. Four different fractions having colours yellow, grey, orange and purple were obtained from the chromatographic separation. All the fractions were found to contain aromatic nucleus based on IR and UV-VIS spectroscopic data. Other functional groups detected are Ar-NH_2, -$CONH_2$, C=C, C-C and metal-carbon chelate rings. The presence of aromatic amine in the dye provides strong evidence for its use as hair dye. The dye was found to be soluble in both aqueous and non-aqueous solvents. The pH of the dye's aqueous solution was found to be 8.6, and the ash and organic content of the raw dye were 49% and 51% respectively. The XRF revealed that the dye contains twenty elements with concentrations ranging from major to ultra-trace levels. The XRD also showed that the sample contains about forty-six mineral phases which include both inorganic and organic components. The maximum absorption wavelength ($\bullet_{max}$) in UV-VIS of the aqueous solution was found to be 464 nm. The optical microscopic investigation gave indication that the dyes are likely to be of the marine origin.

KEY WORDS: Natural African mineral dye, Chromatographic separation, Composition, Inorganic, Organic, XRF, XRD, Optical microscopic, Marine, Aromatic amine

INTRODUCTION

Dyes are coloured substances which imparts more or less permanent colour to other materials. There are two types of dyes: natural and synthetic dyes; natural dyes include plant dyes, animal dyes and mineral dyes. Mineral dyes come from ocher (yellow, brown, red) limestone or lime (white), manganese (black), cinnabar and lead oxide (red), azurite and lapis lazuli (blue), and malachite (green) [1]. Natural dyes may also be organic or inorganic in nature and are broadly classified into dyes and pigments [2, 3]. Dyes and pigments are essentially different in their degree of solubilization. Dyes are soluble intensely coloured substances that are applied in solution to substrate while pigments are insoluble colouring substances usually applied to substrate in conjunction with some binding agents [3, 4]. Dyes and pigments of natural origin are now used mostly for small-scale textile handicraft [5, 6]. Typical examples of such natural dyes and pigments are extracts of Indigo plant (*Indigofera tinctoria*), logwood (*Haematoxylon camphecianum*), pigments from rhizome (*Zingiber officinale*) and more recently pigment from Teak plant (*Tectona grandis*) [7, 8].

Qualitative analysis of dye using thin layer chromatography studies of some novel analogous of phthalein dyes have been carried out using silica gel G as adsorbent and five different

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solvents systems as developing media. These solvents includes: ethyl acetate-methanol-ammonia (5 mol/L) (40:4:20), n-butanol-acetone-water-ammonia (sp.gr. 0.880) (5:5:1:2) and benzene acetic acid (90:10) [9].

The colour strength of C.I. Basic Red 18 in various solvents has been determined using a single beam instrument set at wavelength 499 nm [10].

Two different dyes vis a vis: methylene blue and malachite green dyes have been separated by column chromatography on alumina. The column was eluted with distilled water and finally with ethyl alcohol, these two solvents washed the green and the blue dyes through the adsorbent respectively [11]. The spectra of quinoxaline dyes were measured in chloroform solution. Quinoxaline dyes absorbed visible light at wavelength from 400 to 680 nm and showed six absorption maxima (λ_1- λ_6) [12].

The TLC technique was found to be quite efficient for identifying three dyes which are closely related with one another structurally. It has been found preferable to classical column chromatography, as it is faster and separations are better [9].

The broad range of modern instrumental techniques now available to the analysts for the determination of metal traces in dyes and pigments have largely replaced the classical techniques. These instrumental techniques include X-ray fluorescence (XRF), X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and infrared spectroscopy (IR). It has been pointed out that just as X-ray are useful in medicine and surgery in revealing problems in the human body, they are equally valuable in their ability to give information on the nature of dyes and pigment [13]

X-ray fluorescence (XRF) is an excellent method for the qualitative and quantitative determination of the major trace elements in geological materials and mineral dyes [13]. One advantage of XRF in comparison with wet chemical methods is the accurate analysis of sample which contains minerals such at zircon that are difficult to dissolve. It is also useful for analyzing materials, which contain elements that are unstable in solution. Other advantages are that the method is non-destructive and requires only small amount of sample.

X-ray powder diffraction (XRD) is another method in which a beam of X-ray is directed at a fine powder of randomly oriented grains of crystalline substance. The X-rays are scattered in direction that depend on the crystal structure of the sample and the resulting X-ray diffraction pattern is unique for each crystalline materials [14]. For the purpose of X-ray diffraction, a crystal may be defined as a solid within which there is a three-dimensional atomic array. Solids that do not contain a three dimensional atomic array are called amorphous or glassy. However, this concept of crystal suggest that it is possible to obtain diffraction from particles which do not appear to be crystal, i.e. the external faces may be irregular, provided the external atomic array is satisfactory [15].

Information about the surface features of the sample, its texture, the shape and size and arrangement of constituents of the object that are lying on the surface of the sample or have chemical etching as well as the elemental and molecular compound of the sample can be obtained with the use of scanning electron microscope (SEM).

Infrared spectroscopy (IR) is another analytical tool that can be used in the determination of chemical compound of a dye. It can also be used for the elucidation of the structure of both organic and inorganic components of the dye. It usually reveals the functional groups present in a sample [16].

The combination of the above mentioned analytical tools (i.e. XRF, XRD, SEM and IR) coupled with the information on the solubility test and pH determination can give useful information about an unknown mineral dye and hence be used to characterize the sample.

Extraction and characterization of natural mineral dyes and pigments have not been reported in recent time. In the present investigation, an attempt has been made to fractionate and characterize a natural African mineral dye using a combination of column and thin layer chromatographic techniques, XRF, XRD, optical microscopic, IR and UV-VIS spectroscopic measurements. The purpose of the present investigation is to use combination of the above-mentioned techniques to characterize fully a natural mineral dye being used by local Africans for hair dyeing.

EXPERIMENTAL

Source of the dye. The white rock-like mineral dye (Yombo-fitta) was obtained from Emir's market in Ilorin, Nigeria. The original source of the dye was traced to the southern part of Ghana.

Solubility test. Solubility of the dye was investigated in fourteen different solvents. The solvents include deionised water, hydrogen peroxide, acetone, benzaldehyde, acetaldehyde, ethanol, pet ether, methanol, diethylether, ethylacetate, conc. HNO_3, conc. HCl, acetic acid, and aniline. The test was carried out by adding 10 mg of the well grounded (< 80 μm) dye to 5 mL of each of the above solvents in test-tube at room temperature [17].

Thin layer chromatography (TLC). A very dilute solution of the raw dye was prepared with each of the chloroform, n-hexane, acetone, diethyl ether and ethyl acetate as dissolving solvent. Each of the solution was spotted and examined using TLC precoated silica gel F_{234}, (Merck No 5554) as described by Hamilton and Hamilton [18]. These were developed separately using the following solvent systems, namely ethylacetate/acetone/diethylether (5:2:2). They were developed for 5-10 min. After the development, the chromatograms were dried and spots were observed with different colouration and R_f values.

Column chromatography (CC). The dye was fractionated by column chromatography using silica gel (60 mesh) packed in a glass column of 90 cm length with 3 cm internal diameter. 1.0 g of the raw dye was dissolved in a small quantity of acetone and gently introduced on top of the column. The components were eluted with solvents system ethyl acetate/diethyl ether/acetone (5:2:2).

Eleven fractions were collected and monitored by TLC, precoated silica F_{254} (Merck No 5554) using solvent system, Ethyl acetate/acetone/diethyl ether (5:2:2). Spots were detected visually and under UV-lamp at 360 nm and 254 nm, respectively. Identical fractions were combined after correlating their R_f values. Four major fractions with distinct colour were concentrated to give solid crystals of each of the fraction.

Spectroscopic analysis

The raw dye and different isolates of the dye were subjected to spectroscopic techniques for the identification of components.

XRF elemental analysis of the dye. The sample was analyzed for trace element using energy dispersive X-ray fluorescence (EDXRF) spectrometry at the Center for Energy Research and Training, Ahmadu Bello University, Zaria, Nigeria. The sample preparation prior to elemental analysis consisted of crushing and grinding in a tungsten carbide Spex Mill followed by other procedures specific for the method of analysis [19]. The EDXRF facility consists of two interchangeable (^{55}Fe and ^{109}Cd). Annular source, a Canberra model SL 12170 silicon solid state detector and the associated pulse processing electronic which are coupled to ADC-Card. The facility runs on PC with Maestro software for spectra acquisition. Sensitivity calibration using thick pure metal foils (Ti, Fe, Co, Ni, Cu, Zn, Zr, Nb, Mo, Sn, Ta, Pb) and stable analytical grade chemical compounds (K_2CO_3, $CaCO_3$, Ce_2O_3, WO_3, ThO_2 and U_3O_8.) The spectra data collected with the Maestro software were first converted to the standard AXIL format and then fitted with a model created from the qualitative information on the spectra using a nonlinear least square strategy of AXIL software package. Quantification of the concentration of detectable elements was made using a modified version of emission-transmission method [20].

XRD analysis. The sample was further analyzed using X-ray diffractometry PW 1800 at the National Steel, Raw material, Exploration Agency, Kaduna, Nigeria. The sample preparation consisted of pulverization and screening to the required mesh size of 63 μm to 100 μm. 0.5 g of the pulverized sample was then weighed and placed in the sample holder of about 10 mm diameter and smoothened to obtain a polished sample face. The sample holder was then introduced into the sample cavity and scanned continuously at 0.020^o angle interval from 2^o (2θ) to 65^o (2θ) at 0.4000 second per step [21].

Optical microscopic analysis. Optical microscopic investigation was carried out for the sample at Geology Department of the University of Ilorin. The petrological slide was prepared following standard procedure [21]. The prepared slide was observed under optical microscope for mineralogical identification and the optical micrographs of sections of the slides were produced using Leitz R3MOT electronic coupled with Leitz dialux 2D camera at the University of Ilorin Teaching Hospital. The films were developed and printed. The interpretation of the results was done following the petrography interpretations of carbonate petrography [22].

Infrared (IR) spectroscopy. The IR spectra of the fractions purified on preparatory thin layer chromatographic (PTLC) plates coated with silica gel F_{254} were run on IR spectrophotometer Perkins Elmer model 457A.

UV-visible spectroscopy. The UV-visible spectra of fractions purified on PTLC plates coated with silica gel F_{254} were also run on UV-VIS spectrophotometer Aquamate V4.60.

RESULTS AND DISCUSSION

Solubility test

The results of the solubility and other physico-chemical tests carried out on the raw dye are shown in Table 1 and 2. It is of interest to note that the sample was soluble in almost all the solvents with the solutions having different colours. Some of the colour appeared to be unstable as they changed after 24 hours. The colour ranges from brown, red, yellow and black. The colour observed may be attributed to the existence of certain complexes involving some elements within the sample and organic species acting as ligands. The sample was found to be soluble in both polar and non-polar solvents. The pH of the resulting aqueous solution shows that the sample solution was slightly alkaline. This may be due to the presence of carbonates or hydrogen carbonate of alkaline and alkaline earth metals in the samples. The wide melting point range (125-145 ^oC) show that the dye is a complex mixture as indicated in the ash and organic contents of the dye (49% and 51% respectively).

Table 1. Solubility of the sample YF, in different solvents with the observed colour change after 24 hours.

No.	Solvent	Solubility	Colour of solution	Colour after 24 hour
1	Deionized water	Soluble	Colourless	Golden red
2	Hydrogen peroxide	Soluble	Dark brown	Yellowish red
3	Acetone	Soluble	Light brown	Brown
4	Benzaldehyde	Soluble	Wine red	Golden yellow
5	Acetaldehyde	Soluble	Reddish brown	Brownish black
6	Ethanol	Soluble	Faint brown	Dark brown
7	Petroleum ether	Insoluble	Colourless	Residue
8	Conc. HNO_3	Soluble	Colourless	Golden brown
9	Conc. HCl	Sparingly soluble	Cloudy whitish mixture	White sediment
10	Methanol	Soluble	Light brown	Dark red
11	Diethyl ether	Soluble	Colourless	Residue
12	Ethyl acetate	Soluble	Colourless	Red
13	Acetic acid	Soluble	Faint yellow	Faint yellow
14	Aniline	Soluble	Brown	Dark brown

Table 2. Physiochemical parameters of the raw dye (YF).

Test performed	Results
Appearance of the crystal	White
pH of the aqueous solution	8.6
λ_{max}	464.0 nm
Ash content	49%
Organic content	51%

Table 3. Results of elemental analysis of sample YF by EDXRF techniques.

No.	Element	Concentration in ppm
1	K	0.43 (%)
2	Ca	0.26 (%)
3	Ti	0.07 (%)
4	V	345 ppm
5	Cr	207 ppm
6	Mn	175 ppm
7	Fe	246 ppm
8	Co	66.0 ppm
9	Ni	53.0 ppm
10	Cu	35.0 ppm
11	Zn	26.0 ppm
12	As	21.0 ppm
13	Pb	31.0 ppm
14	Br	13.0 ppm
15	Rb	9.0 ppm
16	Sr	9.0 ppm
17	Y	9.0 ppm
18	Zr	6.0 ppm
19	Nb	30.0 ppm
20	Mo	5.0 ppm

Elemental analysis by EDXRF techniques

The results of EDXRF analysis of the natural dyes are summarized in Table 3. Twenty elements were recorded and their concentrations range from major to ultra-trace levels. The major elements include K and Ca; minor elements are Ti, V, Cr, Zn, Mn and Fe; trace elements are Co, Ni, Cu, As, Pb, Nb and Zr; and the ultra-trace elements include Rb, Sr, Y and Mo. The presence of Br (13.0 ppm) is in accordance with the result of optical microscopy which suggests marine origin for the sample. These results reveal that the dye could have certain degree of toxicological effect due to the presence of some toxic elements such As, Pb, Cr, Co and Zn in the sample. It is of interest to note that all of the first series transition elements except Sc were recorded. The colour of the sample can therefore be linked with the formation of coloured complex compounds by some of these transition elements [23].

Table 4. List of minerals with their chemical formula and identification numbers from ASTM files.

No.	Card ID	Chemical formula
1	42-1379	$Na_{3.7}Ca_{7.4}Al_{18.5}Si_{77.5}O_{19}$
2	39-0225	$C_{48}H_{116}N_4O_{196}Si_{96}$
3	41-0569	$Cs_4Al_4Si_{20}O_{48}$
4	38-0293	$Ca_5Cr_2SiO_{12}$
5	42-0972	$RbYbBr_3$
6.	42-1478	$Ca_4Al_6O_{12}SO_4$
7.	32-0647	$C_2MnO_{4.2}H_2O$
8	44-0465	$Nb_{12}WO_{33}$
9	44-1613	$C_{12}H_4N_8O_{12}$
10	20-1322	$Nb_8W_9O_{47}$
11	42-1564	$C_6Br_4O_2$
12	43-0167	$K_3Nb_3B_2O_{12}$
13	42-1148	$NaCaAlF_6$
14	46-0600	$NaCaAlF_6$
15	44-1937	$CH_8N_3O_3P$
16	34-0106	$BaCd(PO_3)_4$
17	30-0197	Bi_3Y_5
18	38-1082	Li_2ZnGeO_4
19	36-1222	$Tl_6As_5Se_{10}$
20	32-1358	$SnBi_2S_4$
21	26-1493	Na_3VF_6
22	44-1614	$C_{12}H_4N_8O_{12}$
23	29-0559	$CuPb_{13}Sb_7S_{24}$
24	33-1219	$NaCa_2Al_2F_4(PO_4)_2(OH).2H_2O$
25	15-0563	SiP_2O_7
26	21-1183	$Sr_{10}(CrO_4)_3(GeO_4)_3F_2$
27	17-0383	$TiSe_{1.60}$
28	45-1412	$Cu(Pb,Bi)_{12}Bi_4S_{18}$.
29	46-1370	$Pb_{8.2}Bi_{4.3}CuO_{.4}S_{15}$
30	21-0946	$PbSrCl_3$
31	21-0946	Pb_2CrO_5
32	22-1477	Tl_2Te_3
33	17-0754	$(Ca, Na)_4Al_3(Al,Si)_3Si_6O_{24}$.
34	37-1361	$CdZnGe_2O_6$
35	07-0379	$Pb_9Sb_3S_{21}$
36	20-1532	$C_6H_4Cl_3N$
37	27-1144	$EuScO_3$
38	45-0361	$Ba_3InFeGe_4O_{14}$
39	36-0533	$K_3LiNb_6O_{17}$
40	33-1335	$SrHPO_4$

XRD analysis

The complementary results were also obtained from the XRD analysis which shows that the sample contains forty five different minerals phases. The identification numbers used were extracted from the American Society of Testing of Material (ASTM) files and the Joint Committee of Powder Diffraction Standard [23, 24] obtained with PC-APD Software. The dye contains the following minerals with their names, formulae and identification numbers: Nickeldiagua 2. 18-9, 11 ($C_{18}H_{22}N_6NiO_6.H_2O$) (46-1711), cesium fluoride iodide ($Cs_2F_2I_2$) (46-1090), barium hydrogen phosphate ($BaH(PO_3)_3$) (37-0285), thallium scandium fluoride ($TISCF_6$) (45-0902) and potassium hydrogen sulfate ($K_5H_3(SO_4)_4$) (17-0597). The presence of thallium scandium fluoride ($TlScF_6$) also confirmed the presence of scandium (Sc), which was not detectable in the XRF results. The other probable but very minor components with their chemical formulae and identification number were shown in Table 4. It is of interest to note that apart from the inorganic minerals, there were also organic components in the sample. Organic components that were found in the sample include $C_{12}H_4N_8O_{12}$, $C_6Br_4O_2$ and $C_6H_4Cl_3N$.

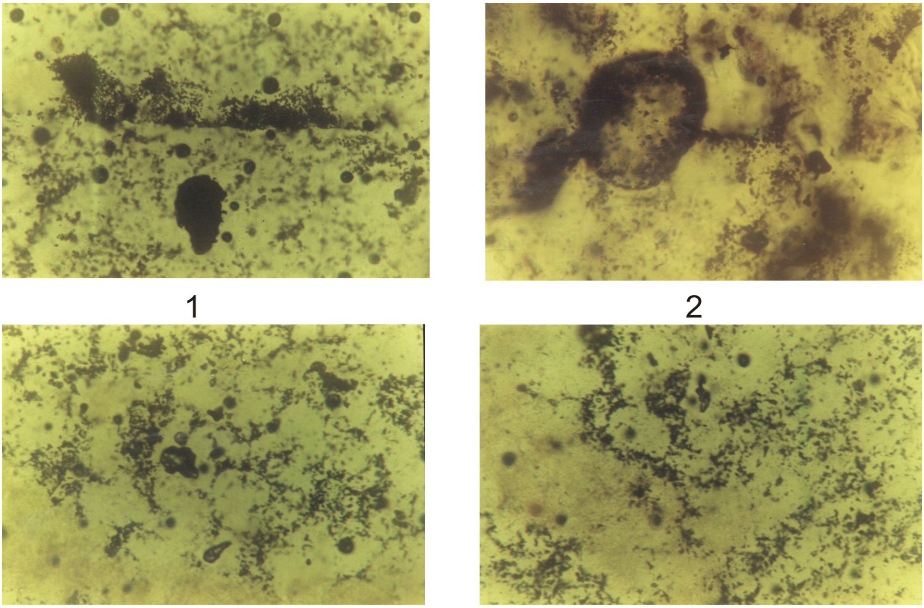
Optical microscopic analysis

Figure 1 and Table 5 describe the micrograph of sections of the slides prepared from the dye. It was observed that the mineral dye consists of mud as the groundmass. The opaque mineral observed, in the sample is likely to be iron materials. Other components observed were fossil traces, and algae, the rock sample also show features that has been subjected to a level of diagenesis (especially micritization and dissolution, and has not really undergone recrystallization [21]. There are evidences of rotten bioclasts (fossil material) decaying to form something like peloid.

It was observed that the sample apparently dissolved in Canada balm mounting medium to give a light to dark brown or reddish brown colour. It contains fine-grained material dominantly (about 80%) composed of sub-rounded to irregular shaped pigments. The pigment is dark green coloured in plane polarized light. The pigment appears dispersed with some aggregations feathers or filamentory like. There were also occurrence of few (about 10%) large grains of quartz and feldspar and some (less than 10%) light-brown to yellow finely elongated crystals of biotite.

Table 5. Observed features from optical microscopy of the dye.

Section	Important petrographic features	Remarks
1	Opaque mineral observed	Likely to be iron materials
2	Fossil and algae traces	Evidence of marine environment that has been subjected to a level of diagenesis (especially micritization and dissolution)
3	Evidences of rotten bioclast	Decaying materials forming something like peloid (fossil materials)



1

2

3

Figure 1. Micrograph of SEM of YF.

Infrared (IR) spectroscopic measurement

The results of IR analysis of the dye are presented in Table 6. The absorptions at 1375, 1381, 1460, 2925 and 2931 cm^{-1} might probably be due to the solvent (Nujol) [25]. These wave numbers were marked with superscript (1) in the table. IR band of 1618 cm^{-1} can be attributed to C=O stretching frequencies of some metal chelates in the sample. Absorption peaks at 1610 and 1650 cm^{-1} have been assigned to the carbonyl C=O stretchning modes, suspected for the metal chelates. Other spectra data of metal chelate complexes reported include 1525 cm^{-1} for C=C chelate ring, 1490-1520 cm^{-1} for C-O stretching, 1328-1375 cm^{-1} for C=C stretching and 1185-1240 cm^{-1} medium weak C-O stretching [25]. Therefore, the 1375 cm^{-1} from the raw dye sample can be assigned to C=C chelate ring of some metal chelate complexes within the sample. The ν_{asym} C=O has been reported to be dependent on the inductive effect of the constituents, such as electron withdrawing which increase ν_{asym} C-O and electron releasing, which decrease its relative value to that of the ligands [26, 27]. Hence the little variation in the spectra observed might be due to some substituents that may be present in the sample, e.g. NO_2 or C_6H_5 groups. In one of the studies reported in the literature [28], IR broad peaks in region of 3300-3550 cm^{-1} could be assigned to OH stretching frequency of coordinated water in chelate spectra of Ba(II), Mn(II), Co(II), Ni(II), Zn(II) and Na. Hence IR bands, 3193(b), 3296(b) and 3375(w. sh) cm^{-1} from raw dye were likely due to coordinated water of some of the complex compounds in this sample.

Table 6. IR spectra data (cm^{-1}) of the dye.

Peak	Assignment
703 (s)	Bending frequency in aromatic ring
818 (s)	PF_2S_2, C-C-O sym. stretch
937 (w)	Mo-O stretching
1059 (m)	Chelate ring
1119 (s)	C–O chelate ring stretch.
1253 (s)	Coordinated amine group
1308 (m)	Nujol
1375 (vs)^1	Nujol, C=C chelate ring
1460 (s)^1	Nujol
1515 (w)	C-O stretching NO_2 asym stretch, aromatic
1618 (m)(sh)	C=O stretching
2688 (w), 2725(w)	Aromatic C-H
2925 (b)^1	Nujol
3193 (w) (b), 3296 (w) (b)	O-H stretching of coordinated water in chelate complex
3375 (w)(sh)	N-H

Intensities in parenthesis: b-board, vs-very strong, s-strong, m-medium, w-weak, sh-sharp, superscript ^{1} – frequency due to solvent used..

Column and thin layer chromatography results

The results of the fractionated pools collected on the base of equal R_f values from column chromatography and purified on TLC are as shown in Table 7. Four colours were separated out from the original raw dye having R_f values of 0.93 (yellow), 0.84 (ash), 0.73 (orange) and 0.52 (purple). This show that the raw dye contains multi-component characteristics of a mixture and this was also evident in the broad absorption pattern.

Table 7. Thin layer chromatography results of major components from column chromatography.

Code	Fractions	Colour	R_f values
YFW_1	1 – 4	Yellow	0.93
YFW_2	5 – 6	Ash	0.84
YFW_3	7 – 9	Orange	0.73
YFW_4	10 – 11	Purple	0.52

IR analysis of Chromatographic fractions of YF

The IR spectra data (Tables 8 and 9) show that all the four main fractions from the sample contain aromatic rings. Bands at 727-882 cm^{-1} and 1515-1520 cm^{-1} can be assigned to aromatic nitro group [29]. Bands 3334-3348 cm^{-1} and 3587-3956 cm^{-1} are assigned to the aromatic amines and phenolic groups, respectively. The finger print region at 960-999 and 1053-1096 cm^{-1} may be assigned to C-N stretching of aromatic NO_2. Other bands at 1325-1456 cm^{-1} present in the sample fractionates can be assigned to aromatic C-H stretching.

UV-Vis spectroscopic measurement

The results show that the raw dye absorbed at visible region while all the fractions absorbed within the UV region (Table 10) which indicate the presence of both saturated aliphatic and unsaturated hydrocarbon that are not coloured in the raw dyes [16]. It was observed that there was shifting of absorption λ_{max} (464.0 nm) of aqueous solution of the dye to lower wavelength (hypsochromic shift) in the solutions of all fractionates of the samples in ethanol. The λ_{max} shift range between 371-396.5 nm for all the fractionates. These observations can be explained in terms of polarity of the solvent used. Ethanol is less polar than water thereby resulting in solution with slightly lower absorption λ_{max} compared to that of water. However, the absorption intensities of fractionates varied from strong to weak. Higher intensity was recorded for fraction YFW_1 (hyperchromic effect). Others are with lower intensities (YFW_2, YFW_3 and YFW_4) (hypochromic effect). This solvent effect may be responsible for different colours of the solution in different media observed under solubility test [17]. The results of the absorption spectra of the raw dye and the isolates are shown in Table 10.

Table 8. IR results of chromatographic fractions YFW_1 (yellow colour) and YFW_2 (ash colour).

YFW_1	Assignment	YFW_2	Assignment
727	Aromatic ring	698	Aromatic ring
999	C-N str. of aromatic NO_2	882	Aromatic ring
1096	C-N str. of aromatic NO_2	999	C-OH bending
1388	C-H bending of CH_3	1145	$C-NO_2$ of aromatic ring
1456	Aromatic C=C str.	1456	Aromatic C=C str.
1661	Aromatic overtone/combination	1690	Aromatic overtone/combination.
2381	C≡N str.	2376	C≡N str.
2974	C-H str. of CH_3	2896	C-H str of CH_3
3339	N-H str.	2974	C-H stretches
3679, 3747	O-H str.	3344	N-H str.
		3616, 3743	O-H str.

Table 9. IR results of chromatographic fractions and YFW_3 (orange colour) and YFW_4 (purple colour).

YFW3	Assignment	YFW4	Assignment
882	Aromatic ring	732, 882	Aromatic ring
970	C-N str. of aromatic NO_2	1106	C-N str. of aromatic NO_2
1145	$C-NO_2$ of aromatic ring	1456	Aromatic C-H str.
1451	Aromatic C=C str.	1651	Aromatic overtone/combination.
1670, 1894	Aromatic overtone/combination	2974	C-H str.
2371	C≡N str.	3334	N-H str.
2891, 2974	C-H str	3757, 3956	O-H str.
3309, 3348	N-H str.		
3752, 3888	O-H str.		

Table 10. UV-Vis spectra data of the raw dye and purified fractions.

Pigment	Maximum absorption wavelength λ_{max} (nm)
Raw dye (YF)	465
Purified fraction YFW_1	372
Purified fraction YFW_2	386
Purified fractions YFW_3	383
Purified fractions YFW_4	397

CONCLUSION

It is evident from the results that the dye is a mixture of many coloured components. The components include aromatic amine, which might probably be the reason why it is used as hair dye. The result has also shown that the sample is a mineral dye containing mixture of different kinds of minerals. The mineral comprises organic and inorganic components and can be said to have been obtained from marine environment. Research work is currently going on the dye to ascertain the structure of each of the components, using the combination of IR, UV-Vis and GC-MS and NMR.

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