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Evaluation of the effect of Beetroot Dye on Banana Pseudo Stem and Pineapple Leaves Fibres

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ABSTRACT

Extraction and characterization of dye from beetroot scientifically known as Beta vulgaris was achieved. The dye was extracted using two extraction method namely Soxhlet with methanol as solvent and water where the beetroot powder was soaked in water and heat at 30-40^{0}C for 2 hours. It was observed that due to instability of beetroot dye at high temperature, aqueous dye extraction is preferable over Soxhlet extraction as the one extracted with Soxhlet changed its color from purple to black brown. Moreso, the extracted dye was characterized. Qualitative photochemical analysis, Anti-bacterial activities, Fourier Transformer Infrared, Ultra Violet Spectroscopy are the analysis conducted. The photochemical analysis revealed the presence of Saponin, Flavonoids, Phenols, Betacyanin with the absence of Tanin and Glycosides. The anti-microbial activities of the dye was conducted using to microorganism namely, *Escherichia coli* and *Staphylococcus aureus* with Muller-Hinton Agar. The dye showed antimicrobial activities on *Escherichia coli* while it could not show any antimicrobial activities on *Staphylococcus aureus* microorganism. Also, UV-visible spectrum of the dye was conducted and the results showed the presence of betacyanins which are major compounds found in beetroot dye the major one being betanin. The presence of betanin in the dye was also confirmed by the FTIR as well as gas chromatography. Betanin is a major compound found in beetroot which is soluble in water due to the presence of polar groups such as COOH, OH, and NH_2, thus the beetroot extract is a dye and not a pigment. Furthermore, dyeing of the extracted dye to treated banana pseudo stem and pineapple leaves fibres shows that it is feasible and the color fastness test conducted shows that both aluminum ammonium sulphate and aluminum acetate mordants can be used separately in simultaneous mordanting methods.

Keywords: Beetroot, Extraction, Characterization, Dye, Banana pseudo-stem and pineapple leaves fibres and color fastness.

INTRODUCTION

Beetroot scientifically known as *Beta vulgaris* is a huge, fleshy root that grows in fertile soil with hydrogen potential of 6.0-7.0. The root is reach in natural dye. The dye extracted from beetroot is used in various application such as coloration agent in food and textile industries. Beetroot has a purple color with a pleasant flavor [1]. Beetroot dye has attracted attention of a good number of researchers as it is a natural occurring dye which comes with many advantages such as being environmentally friendly compare to synthetic dye [2]. Due to instability of beetroot color at high temperature aqueous extraction was proven to be the best method to extract beetroot dye. In this method of color extraction, a color rich material is broken into small pieces or in powdered form by grinding. The material is then soaked in water for some time to loosen the cell structure. The broken pieces are boiled in water to get coloring components dissolved in water. The solution is then filtered to separate the coloring solution and non-coloring remnant [3]. This study is aimed in extraction and characterization of a dye from beetroot.

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Plate I: Beetroot (Beta *vulgaris*)

MATERIALS AND METHODS

Sample Preparation and Dye Extraction

Beetroot, a Potential dye yielding plant scientifically known as *Beta vulgaris* of *Amaranthaceae* family was purchased from Jimeta Modern Market, Yola, Adamawa State, Nigeria. The sorted and washed beetroots were peeled and chopped into uniform size (1-5mm) using knife, these uniform slices were sundried for 3 days. The dried beet roots were subjected to grinding manually in a mortal, then the ground material was passed through mesh sieve. The grinded beetroot was then put in an air-tight container for further use [4]. Extraction process was carried out by aqueous extraction method, taken finest powder of beet root, due to highest extraction yield. In Aqueous extraction process distilled water was used in a solid- liquid ratio of 1:10 (g/ml) at 30-40^0 C for 2 hours [5].

Characterization of beetroot Dye

Qualitative Phytochemical Analysis of Beetroot Dye

Phytochemical are the chemical compounds that are produced by plants, generally to help them resist fungi, bacteria, plant virus infections and also consumption by insects and other animals [6].

The extracts of beet root were analyzed for tannin, saponin, flavonoid, glycoside, phenols, and Betacyanin using standards procedures.

Test for tannin

1 ml of the beet root extract was added 1 ml of 5% $fecl_3$ dark blue and greenish black color indicates the presence of tannin [7].

Test for Saponin

1 ml of the beet root extract was added in 1 ml of distilled water shake it for 15 min formation of 1 cm layer of foam indicates the presence of the saponin [8].

Test for Flavonoids

1 ml of the beetroot dye extract was added in 1 ml of 10 % of lead acetate solution. The formation of yellow precipitate indicates the presence of flavonoids [9].

Test for Glycosides

1 ml of the extract was added in 3 ml of chloroform then added 10% ammonium solution. Pink color indicates the presence of glycoside [10].

Test for phenols

1 ml of beet root extract was added in 2 ml of distilled water then added few drops of 10% $fecl_3$ formation of blue green color indicates the presence of phenols [9]

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Test for Betacynins

1 ml of extract was added in 1 ml of NaOH then heat it for 5 min at 100^{0}C, formation of yellow color indicates the presence of betacyanin [11].

Antibacterial activity of Beetroot Dye

Both *Escherichia coli* and *Staphylococcus aureus* were used in this study for antibacterial activity of beet root dye. These cultures were obtained from the laboratory of microbiology Department, Modibbo Adama University, Adamawa State, Yola. Agar well diffusion method was used for the evaluation of antibacterial activity of beetroot extract.

Muller-Hinton Agar was used during the exercises, along with Escherichia coli and Staphylococcus Aureus microorganisms. Three wells of 6 mm were made on each Petri dish. Three beetroot extract solutions were made too via serial dilution, a serial dilution is a step-wise series of dilutions, where the dilution factor stays the same for each step. The stock solution was prepared by diluting 1 gram of beetroot powder in 10 ml of water, 100 % solution. The subsequent solution was prepared by topping 5 ml from the 100 % solution previously prepared to 10 ml, 50 % Solution. The last solution was prepared by topping 5 ml from the 50 % solution previously prepared to 10 ml, 25 % solution [12]. Thus, the beetroot dye antibacterial test was conducted using three solutions namely, 100%, 50 % and 25 % of concentration. The prepared solutions were then transferred into the wells of the incubated agar plates. The plates were allowed to stay for 1 hour to allow the extract to diffuse into the medium, and then incubated for 24 hours. After, the incubation period the diameters of the inhibition zone were measures and recorded in millimeters using a transparent rule [13].

Fourier Transform Infrared (FTIR) Spectrometer of Beetroot Dye

The existence of functional groups or identification of chemical bonding in beetroot dye was evaluated using FTIR analysis. FTIR of beetroot dye was carried out at chemistry laboratory of American University of Nigeria, Yola. A small amount (2-3 drops) of the beetroot liquid dye was placed directly on the plate, Silver Chloride plate was used. The plate was brought close to the moving fan to allow water in the dye to evaporate otherwise, the result will show a board -OH bond due to much water. The measurement was taken in 4000-600 cm^{-1}.

UV -Visible Spectroscopy

Optical properties of beetroot dye were analyzed using UV/V Spectroscopy. The UV/V Spectroscopy was conducted at chemistry laboratory of the American University of Nigeria, Yola. During the analysis quartz cuvette was used and water was used as a reference. The cuvette with water was placed in the pocket that face the beam, the machine was zeroed prior to starting sample analysis. The analysis was conducted in the range of 300-800 mn.

Gas Chromatography

The GC/MS analysis was performed using a Thermo scientific, trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column. For GC/MS detection an electron ionization system with ionization energy of 70 eV was applied, Helium gas has been used as the carrier gas at a constant flow rate of 1 mL/min. The injector and MS transfer line temperature was set up at 280ºC [14].

Extraction and treatment of banana pseudo-stem and pineapple leaves fibres

Both banana pseudo stem and pineapple leaves fibres were extracted using water retting method. After extraction banana was scoured, bleached and mercerized using sodium hydroxide and hydrogen peroxide. Scouring, bleaching and mercerization were carried out with the aim of increasing dye affinity of the fibres.

Dyeing of banana pseudo-stem and pineapple leaves fibres

The treated (scoured, bleached and mercerized) pseudo stem banana and pineapple leaves fibres were dyed using beetroot natural dye with the aid of mordants namely, aluminum acetate (Al $(CH_3CO_2)_3$), aluminum ammonium sulphate sulphate ($Al_2(SO_4)_3$) and potassium acetate (CH_3CO_2K) using both pre-mordant and simultaneous mordant. During both type of mordanting 8 % WOF of aluminum acetate [15] was used, and 15% WoF for aluminum ammonium sulphate sulphate [16] and 5% WOF potassium acetate were used.

Pre-Mordanting

For a pre-mordanting technique the treated pseudo-stem and pineapple leaves fibres were soaked in prepared mordant solutions and heated for 30 minutes for a temperature of 70^{0}C after which the fibres were removed and air dried without washing. The air dried pre-mordanted fibres were then placed in a prepared dye bath at PH of 4 adjusted using 3% citric acid, the dye bath was heated by gradually increasing the temperature up to 50-60% for 1 hours, and the dye bath was allowed to cool and was kept for 24 hours after which the fibres were removed and washed under running water, the fibres were air dried and after kept in a nylon for next step of the experiment, color fastness [17].

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Simultaneous Mordanting

During a simultaneous mordanting, solution of each mordant was made using extracted liquid dye as a solvent unlike in pre-mordant where the mordant solution was made using distilled water as a solvent. Afterward, the dried treated fibres were placed in the dye/mordant solution and allowed to simmer at temperature of 50-60 % at PH of 4 for 1 hours. The dye bath was allowed to cool and kept for 24 hours before fibres were removed and washed with running water, the fibres were air dried and after kept in a nylon for next step of the experiment, color fastness [17].

Color Fastness of the dyed fibres

Wash Fastness

Wash fastness was carried out according to American Association of Textile Chemists and Colorists (AATCC) test method 61, using 2 g detergent (viva) and 1 g of sodium perborate diluted in 200 ml of distilled water. The wash fastness rating was measured using standard grey scale for color change [18].

Light Fastness

Color fastness to light was evaluated as per International Organization for Standard (ISO) 105- B ,where the dyed samples were exposed to sunlight for a period of 35 days (800 + hours) and the degree of fading of each sample was observed against the fading of blue wool standard (1-8) [19].

Rubbing Fastness

Color fastness to rubbing was carried out as per AATCC test method 8 by rubbing both wet and dry dyed fibres on a white fabric and the color staining was graded using grey scale for color staining [18].

RESULTS AND DISCUSSION

Dye Extraction from Beetroot

Dye was extracted aqueously from beetroot. However, the dye presented instability at a temperature of 70 ^{0}C as the dye turned brown/black when heated while trying to evaporate water solvent. Also, the beetroot turned brown/black when Soxhlet Extraction was conducted using methanol as a solvent. This is a result of betalains in beetroot. Betalains are a class of pigments that are not stable to heat, light, acidity, and oxidants but betalains, upon heating, change color to yellowish/brown [20]. Also, when the dye was left outside the fridge for 48 hours, fungi started to grow on it. This might be attributed to the high sugar content in beetroot [21]. Therefore, it was learnt that beetroot dye could be extracted below 70^{0}C, which falls in agreement with what was reported by [13] that the beetroot dye is stable at pH and temperature of 4.5 and 50^{0}C, respectively.

Plate II : Beetroot dye Extraction using water and Soxhlet Extraction

Phytochemical Screening of the Dye Obtained from *Beta Vulgaris*

The phytochemical analysis revealed that *Beta vulgaris* contain some secondary metabolites, substances manufactured by plants that make them competitive in their environment. The table below shows the presence (+) and absence of (-) of phytochemical constituents in the tested beetroot sample. In this analysis, we obtain the presence of saponins, flavonoids, phenols, and betacyanin and the absence of tannins and glycoside.

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Table 1: Phytochemical analysis of beetroot (*Beta vulgaris*)

Phytochemicals	***Beta Vulgaris* Dye**
Saponins	+
Flavonoids	+
Phenols	+
Betacyanins	+
Tannins	-
Glycosides	-

+ represents presence & - represents absence

Antimicrobial Activities of Beetroot Dye

Microorganism varies extensively in their degree of susceptibility to the anti-bacterial agent. This research observed various zone of inhibition in beetroot dye after analysis. The zone of inhibition is represented in the table below. The zone of inhibition in the tested dye is *Escherichia coli* (8 mm) for the dye of 100 % concentration prepared in the 1:10 g/ml ratio and *Escherichia coli* (3 mm) for the dye of 50 % concentration. However, the beetroot dye of 25 % concentration could not show any zone of inhibition on *Escherichia coli* while beetroot dye of 100%, 50 %, and 25 % of concentration could not indicate a zone of inhibition on *Staphylococcus aureus*.

Table 2: Antibacterial activity against the zone of inhibition

Sample/ Beetroot dye	Escherichia Coli	Staphylococcus aureus
100%	8mm	-
50%	3mm	-
25%	-	-

UV-Visible Spectrum of *Beta Vulgaris* Dye

Figure 1 shows the results of the UV-Visible absorption spectrum of *Beta vulgaris* dye. Absorption spectra (plots of absorption intensity vs wavelength) characterize specific compounds. In visible spectra, the absorption patterns tend to be broad bands with maxima at longer wavelengths corresponding to more extended conjugation. The position and shape of the absorption band affect the appearance of the observed color. Progressive absorption into the visible region gives orange (420-480 nm), red (480-550 nm), violet (550-600 nm), and blue (600-700 nm), while absorption at 400-480 nm gives green. It has been recorded that the wavelength of the maximum absorption of betalains (betacyanins and betaxanthins) is in the wavelength range of 451 and 586 nm because of the colouration combination of yellow-orange betaxanthins and red violet betacyanins. In this study, the maximum absorption wavelength for Betacyanins is generally red-to-red violet in colour. They absorb in the 543 nm range. Also, the small hump of Betaxanthins, which generally appear yellow, absorbs in the 487 nm range [22].

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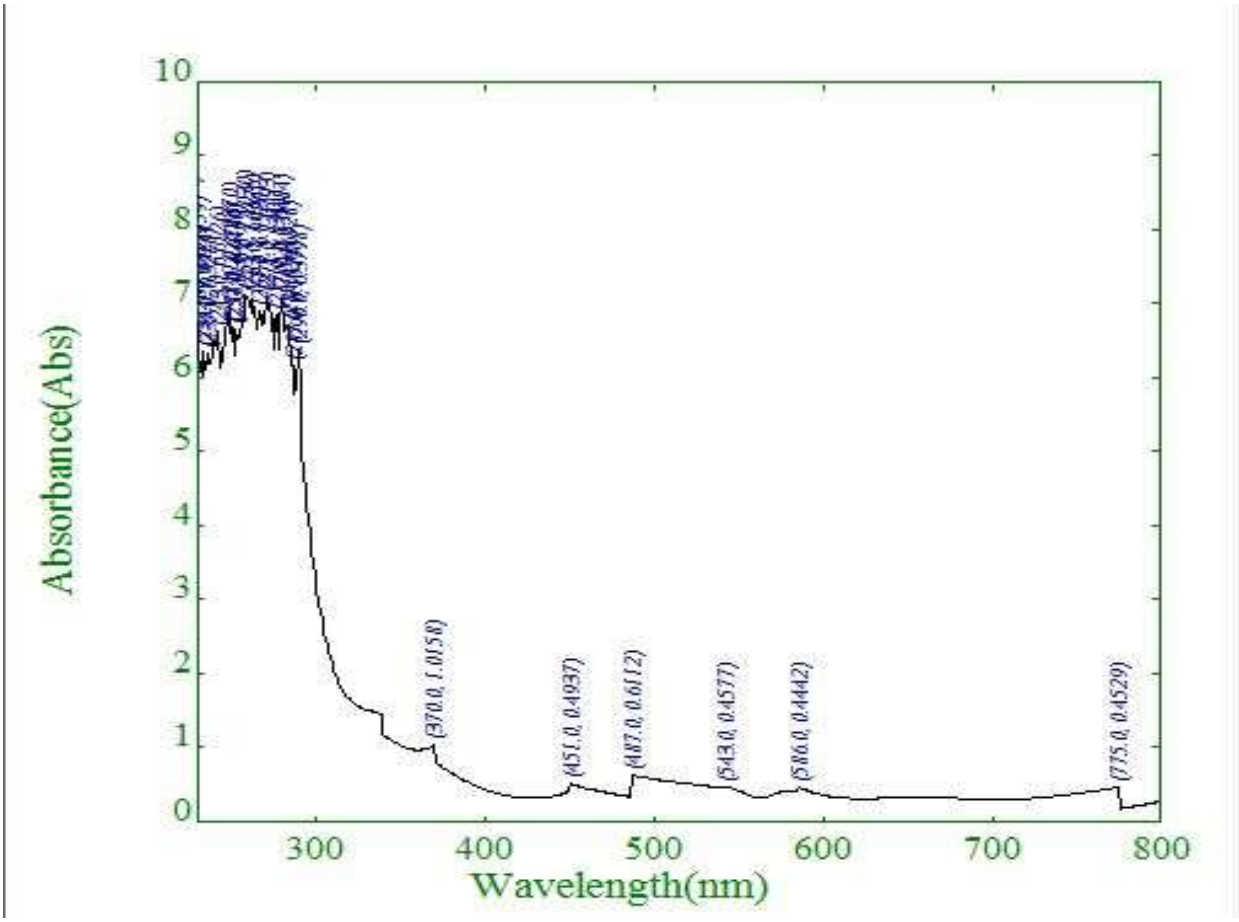

Figure 1: UV-Visible Spectrum of Beetroot dye

Fourier Transform Infrared (FTIR) spectroscopy

Figure 1 is the FTIR spectral analysis of beetroot dye carried out to identify the type of functional groups in the beetroot dye. The FTIR spectra of the tested sample were registered in the spectral range of 4000–1000 cm^{-1}. The spectrums of aqueous extract of beetroot dye have different absorption bands characteristics of functional groups of betanin. The peak at 1032 cm^{-1} corresponds to C-O stretching vibration. From the spectra, the solution shows strong broadband at 3336.87 cm^{-1} that belongs to the alcohol -OH bond stretch. The peak absorbed at 2850 cm-1 was assigned to the extension stretching vibration of the C–H bond in alkane, while the absorption band at 1212 cm^{-1} was ascribed to the C–O bond of the carboxylic acid stretching vibration. The peak absorbed at 2043 cm^{-1} is due to C≡ of C- stretch, while 1615 cm^{-1} was ascribed to the C=N bond stretching vibration. The peak at 1459 cm^{-1} corresponds to aromatic C-C stretching. The peak at 1032 cm^{-1} was ascribed to the C-O-C linked symmetric stretching vibration, while the peak absorbed at 778.26 cm^{-1} is due to the presence of C-H bend in aromatic [23]. The spectral region also observed the main vibrational characteristics related to the betanin molecule's carbonyl compounds. Overall, FTIR spectra were obtained justifying the presence of betacyanin pigment, which was proved by a hydroxyl group and double-bound aromatic ring [24]. Moreover, the bands at 2361 cm^{-1}, 2523 cm^{-1} and 2043 cm^{-1} are responsible for the presence of alkyne groups that occur in plants as triglycerides, a composition of three fatty acids esterified to a glycerol molecule, available in plant fat [25].The results of FT-IR show that dye extracted from Beetroot contained C-O stretching vibrations at the peak of 1212 cm^{-1} and at the peak of 3336.87 cm^{-1} representing the O-H stretching vibration. Moreover, the result showed the peak at 1459 cm^{-1} representing the aromatic C-C stretching. For betanin dye, most active functional groups such as C=O and O-H are usually from carboxylic acid in betanin. The presence of the carboxyl group in betanin promotes a strong hydrogen bonding, which gives a significant shift of the O-H bond. The presence of polar groups such as COOH, OH, and NH_2 gives them an affinity for water and specific solubility, which is why the components of betalains cannot be called pigments but dyes.

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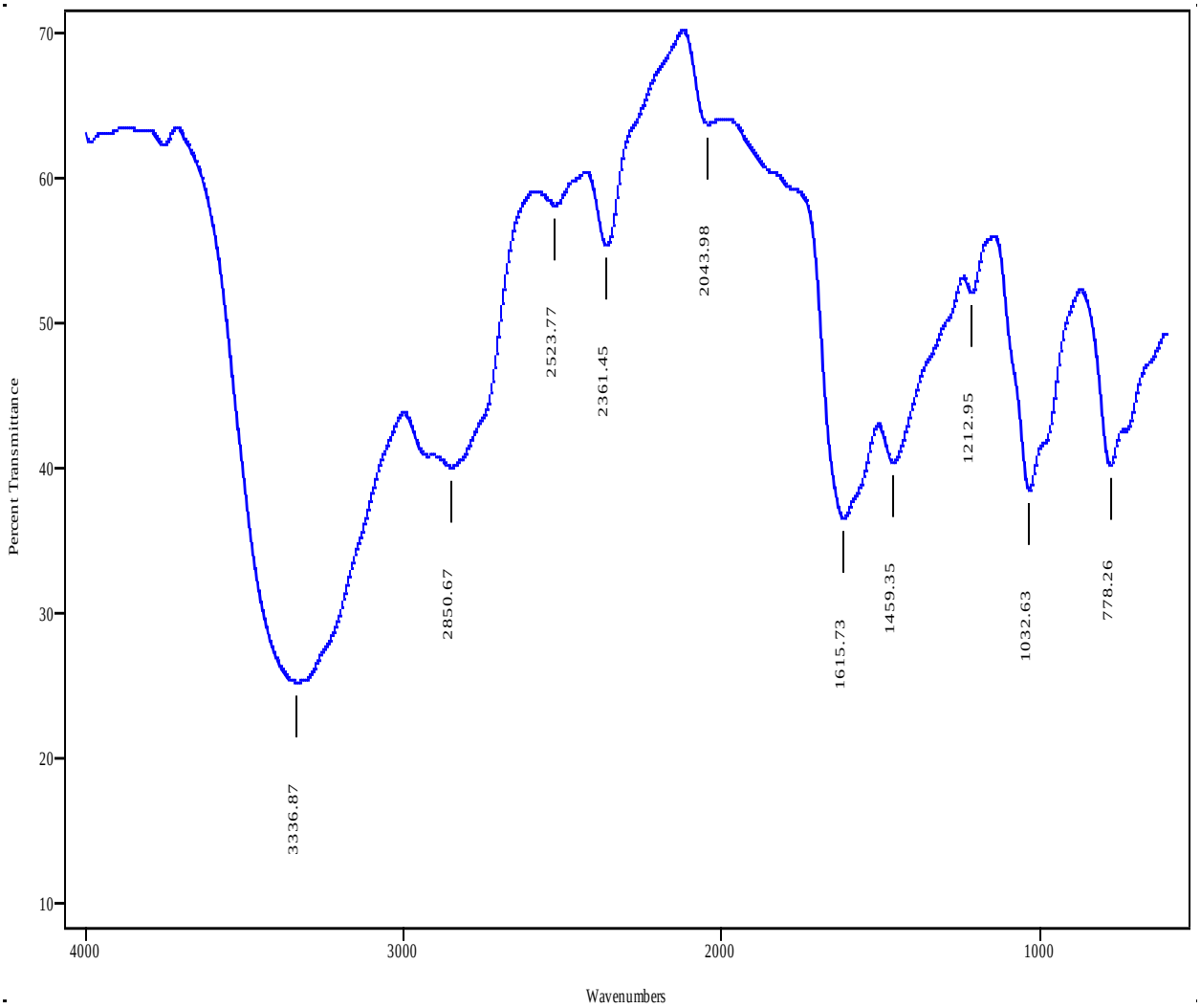

Figure 2: FTIR of Beetroot Dye

Gas Chromatograph

GC/MS was carried out to detect betanin which the major components in beetroot dye and is responsible of the red-violet color of beetroot dye. The GC/MS spectra obtained presented a peak at retention time of 3.310 minutes which represent betanin [26].

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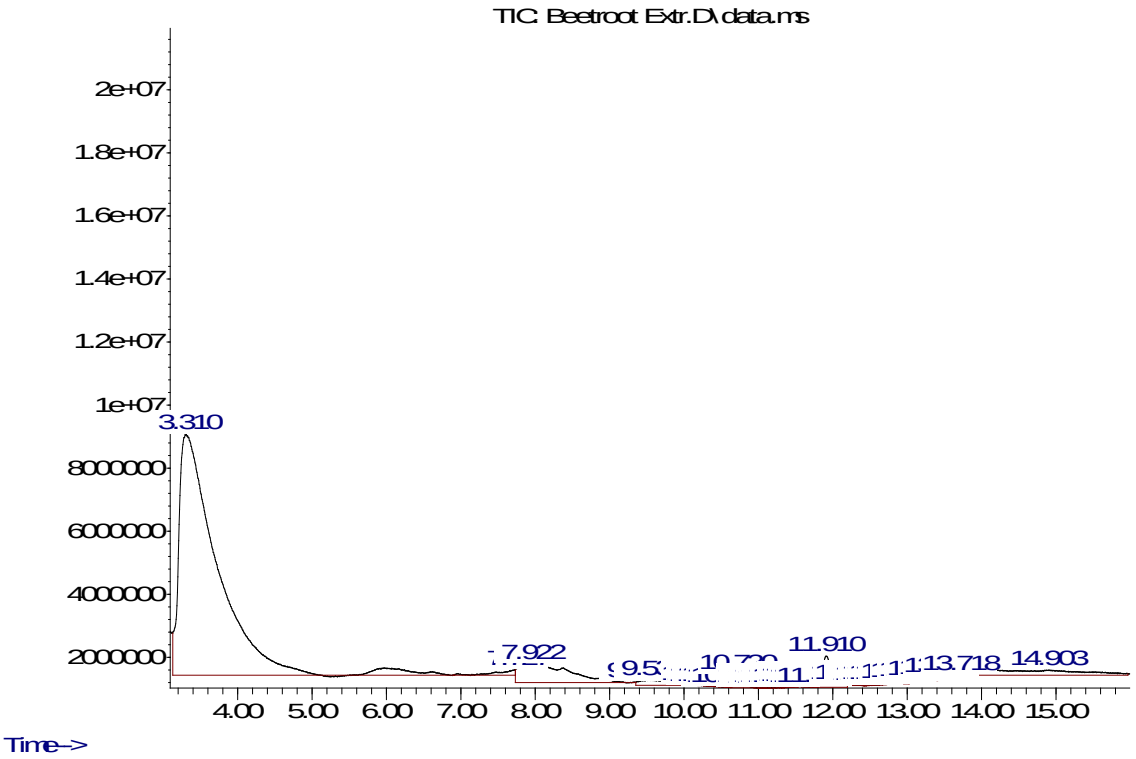

Figure 3: GC/MS spectra of beetroot dye

Extraction and treatment of banana pseudo-stem and pineapple leaves fibres

The treatment of fibers was conducted to get rid of fat, wax and other impurities as well as undesired colors bonded to fibers that could not be removed through water retting. The best scouring concentration of NaOH was found to be 17.5 %, the best bleaching concentration of H_2O_2 was found to be 2 % while mercerization was carried out at NaOH is 15% for banana pseudo-stem fibres. On the other hand, for pineapple leaves fibres, 1.25 % NaOH was used for scouring, 10 % H_2O_2 was used for bleaching while 10% NaOH was used for mercerization.

Dyeing of banana pseudo-stem and pineapple leaves fibres and Color Fastness of the dyed fibres

Wash Fastness

Pre-Mordanting		
Type of Mordants	Pseudo Stem Fibres	Pineapple Leaves Fibres
Aluminum ammonium sulphate	2-3	2-3
Aluminum Acetate	3	3-4
Potassium Acetate	1-2	2-3
Simultaneous Mordanting		
Type of Mordants	Pseudo Stem Fibres	Pineapple Leaves Fibres
Aluminum ammonium sulphate	3-4	3-4
Aluminum Acetate	4-5	4-5
Potassium Acetate	2-3	3

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Light Fastness

Pre-Mordanting		
Type of Mordants	Pseudo Stem Fibres	Pineapple Leaves Fibres
Aluminum ammonium sulphate	3	3
Aluminum Acetate	3	4
Potassium Acetate	2	2
Simultaneous Mordanting		
Type of Mordants	Pseudo Stem Fibres	Pineapple Leaves Fibres
Aluminum ammonium sulphate	4	5
Aluminum Acetate	5	5
Potassium Acetate	3	4

Rubbing Fastness

Pre-Mordanting				
Type of Mordants	Pseudo Stem Fibres		Pineapple Leaves Fibres	
	Wet Rubbing	Dry Rubbing	Wet Rubbing	Dry Rubbing
Aluminum ammonium sulphate	3	5	3-4	5
Aluminum Acetate	3	5	3-4	5
Potassium Acetate	1-2	4	2	4
Simultaneous Mordanting				
Type of Mordants	Pseudo Stem Fibres		Pineapple Leaves Fibres	
	Wet Rubbing	Dry Rubbing	Wet Rubbing	Dry Rubbing
Aluminum ammonium sulphate	3-4	5	3-4	5
Aluminum Acetate	3	5	3-4	5
Potassium Acetate	2-3	4-5	2	4

In dyeing both banana pseudo stem and pineapple leaves fibres some selected mordants viz, aluminum acetate, ammonium aluminum sulphate and potassium acetate were used. All those mordants were applied on the fibres using pre-mordanting and simultaneous mordanting methods separately. The three tables above show data for color fastness to washing, rubbing and light for the selected mordants using different mordanting methods. The color change and color staining were graded using both grey scale and blue wool scale. Aluminum ammonium sulphate presented improved fastness to wash, 2-3 in pre mordanting to 3-4, when simultaneous mordanting was carried out for both banana pseudo stem and pineapple leaves fibres. For light fastness aluminum ammonium sulphate showed also a significant improvement when simultaneous mordanting was used for both fibres. However, there was a slight improvement on rubbing fastness in banana pseudo stem fibres from 3 in pre mordanting to 3-4 in simultaneous mordanting while there was not improvement in rubbing fastness on pineapple leaves fibres. More so, both fibres presented a strong rubbing fastness (5) when a dry rubbing was carried out for both mordanting methods. Aluminum Acetate mordant presented improved wash fastness (3 to 4-5 for banana fibres and 3-4 to 4-5 for pineapple fibres) when simultaneous mordanting was carried out. Also, Aluminimu Acetate presented improved light fastness (3 to 5 for banana fibres and 4 to 5 for pineapple leaves fibres) when simultaneous mordanting was carried out. Moreover, aluminum acetate presented no improved fastness to wet rubbing for both fibres using both pre and simultaneous mordanting. Also, aluminum acetate showed strong dry rubbing fastness (5) for both mordanting methods for both banana and pineapple leaves fibres. Potassium acetate mordant presented improved fastness to wash for both fibres when simultaneous mordating method was used. From 1-2 to 2-3 for banana fibres and from 2-3 to 3 for pineapple leaves fibres. For fastness to light test also, potassium acetate registered an improvement when simultaneous mordanting methods was used for both fibres. Furthermore, for rubbing fastness there was an improvement for both wet and dry rubbing when simultaneous mordanting was used for banana pseudo stem fibres while there was no improvement for pineapple leaves fibres for both dry and wet rubbing.

In terms of color fastness rating obtained with the mordants used, simultaneous mordanting method exhibited the best results for both banana pseudo-stem and pineapple leaves fibres. A high-quality colored textile possesses an acceptable amount of color fastness rating at least 3 [18]. Results from this study therefore, demonstrates that dye from beetroot can be used to dye treated banana pseudo-stem and pineapple leaves fibres simultaneously using aluminum acetate and aluminum ammonium sulphate separately as mordants.

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CONCLUSION

Beetroot have betanin as a major component which is responsible of the purple color of its dye and is soluble in water due to its polar properties. Beetroot dye showed the antimicrobial activities on some microorganism which show its potentiality as a good natural dye to use in food and textile industries. Also, the phytochemical analysis profile of the beetroot dye shows a positive health impact to human unlike synthetic dye. Moreover, it was studied that the extracted dye can be used to dye natural fibres such as banana pseudo-stem and pineapple leaves fibres.

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