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Authentication of *Rothmannia whitfieldii* Dye Extract with FTIR Spectroscopy

Onyekachi Onyinyechi Nnorom*, Genevive Chinyere Onuegbu

Department of Polymer and Textile Engineering, Federal University of Technology, Owerri, Nigeria
Email: *kachiadeola@gmail.com

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Abstract

Extraction of dye from dry fruit of *Rothmannia whitfieldii* was carried out using four different extraction methods. Solvent and acid extraction methods gave a colourless supernatant solution after extraction time of 45 minutes at 60˚C. The alkali method gave a deep brown coloured supernatant solution while the aqueous method gave a dark coloured supernatant solution after extraction under the same conditions. From the result of the FTIR spectroscopy characterization of the coloured solutions and the dry powder of *Rothmannia whitfieldii* fruit, it was observed that only the alkali method extracted what can be called a dye with likely presence of tannins. The result also showed that the possible functional groups present in the supernatant solution after aqueous extraction are same with the functional groups present in the dry pulverized *Rothmannia whitfieldii* fruit. Hence, aqueous method did not extract any dye. Similarly, a mixture of the solution after aqueous extraction with drops of alkali solution produced a deep brown coloured solution indicating solubility of the dye component in alkali media.

Keywords

Characterization, Extraction, FTIR Spectroscopy, Natural Dye, *Rothmannia whitfieldii*, Supernatant Solution

1. Introduction

Interest in the study and use of natural dyes especially from plant materials in recent times is on the increase mostly due to environmental hazard and toxic effect of most synthetic dyes on the human skin [1] [2]. In addition, it has also been reported that natural dyes do not stain other fabrics when bleeding [3]. Of all the known sources of natural dye, plant materials are mostly used. The desired colour component of such plant materials must be extracted before it can

be properly applied as a dye on a textile material [4]. Most times, the size of the dry plant material is reduced for better interaction between the plant material and the extracting solvent or solution [5]. It has been observed that different coloured extract can be achieved from a particular plant source depending on the extraction method used. However, a major challenge is how to ascertain the extraction method that will yield a dye and how to confirm which of the coloured extract can be considered a dye.

There are several dye yielding plants in Nigeria, however, for this research work, *Rothmannia whitfieldii* plant was investigated. *Rothmannia whitfieldii* (synonym: *Randiamalleifera*) is a plant of the Rubiaceae family found within tropical Africa from Senegal to Sudan and in the southern part of Africa: Angola and Zimbabwe to be precise [6]. The plant is called *uri* by the Igbos in the eastern part Nigeria where their women use juice from the fresh fruit for body decoration. The juice turns blue-black after a while when rubbed on the body, an old art that is no longer trending. It has reported that most plants have both medicinal and dye potentials [7]. Medicinally, the *Rothmannia whitfieldii* plant has also been used by the Igbos for the cure of measles. But with the advent of well researched pharmaceutical drugs and synthetic body decoration colorants, the plant has been neglected and gradually becoming extinct especially in Nigeria where the tree is being cut down to serve as fire wood. Structurally, the plant is a small tree of about 15 m tall and has fruits of 3 - 7 cm in diameter, with smooth to strongly 10-ribbed, velvety brown pubescent when young but glabrescent when it matures. It has many-seeds crowned by the persistent calyx. The seeds are lens-shaped with a dimension of 7 - 11 mm × 3 - 4 mm [6].

Fresh *Rothmannia whitfieldii* fruit as earlier stated has been used mostly in Nigeria for body decoration and medicinal purpose with little or no interest in its cloth dyeing ability. It is important to note that the dry fruit of *Rothmannia whitfieldii* (shown in **Figure 1**) has not been considered hitherto as a useful source of natural dye. The objective of this research therefore is to ascertain a dye extraction method for the dry fruit using four extraction methods and to characterize any deep coloured supernatant liquid after extraction using Fourier Transform Infra-Red (FTIR) spectroscopy to determine which method can extract a dye.

Figure 1. Dry *Rothmannia whitfieldii* fruits.

Such work on determining the optimum condition to extract dye using different extraction methods from leaves of *Indigofera tinctoria linn* and from the floral part of *Woodfordia fruticosa* have also been done [4] [8].

2. Materials and Methods

The fresh fruits of *Rothmannia whitfieldii* were sourced from a local market in Owerri, Imo State in the eastern part of Nigeria. Chemicals used for this work were of analytical value, they are sodium hydroxide (NaOH), citric acid ($C_6H_8O_7$) and isopropanol ($(CH_3)_2CHOH$).

Methods

The fresh *Rothmannia whitfieldii* fruits were dried under sun before opening up the fruit. The pulp and seed were removed and pulverized. The powder used was sieved to average particle size of 100 μm using a sieve with aperture size of 100 μm. Four extraction methods used include aqueous, alkali, acid and organic solvent.

1) Extraction

Extraction was done using 100 ml beakers for 45 minutes at 60˚C with a material to liquor ratio of 1:50 (g:ml). 1% citric acid and sodium hydroxide solutions were prepared using the Equation (1) and there pH are 3 for citric acid and 11 for sodium hydroxide.

$$\text{Mass percent} = \frac{\text{weight of solute}(\text{g}) \times 100}{\text{weight of solute} + \text{weight of solvent}(\text{g})} \quad (1)$$

For aqueous extraction, 50 ml of distilled water was poured into a 100 ml beaker and placed in a water bath with the temperature set at 60˚C. As the temperature of the distilled water got to 60˚C, 1 g of the plant powder was added and stirred. The solution was stirred then allowed to stand for 45 minutes while maintaining the temperature at 60˚C. The supernatant liquid was drained off into a sample test bottle using a white synthetic fabric as the sieve. Same procedure was followed using 1% citric acid, 1% NaOH and iso-propanol solutions respectively. The results (shown in **Figures 2-5**) are presented below.

Figure 2. Organic solvent extraction at 60˚C.

Figure 3. Acid extraction at 60˚C.

Figure 4. Alkali extraction at 60˚C.

Figure 5. Aqueous extraction at 60˚C.

2) FTIR Spectroscopy

Transmittance method was used with wavenumber range of 4000 - 650 cm^{-1}, Happ-Genzel apodization, 16 sample scans, 16 background scans and 8 times resolution

Infra-red (IR) spectroscopy of three samples were taken and their absorption spectra with peaks corresponding to certain frequencies (cm^{-1}) (shown in Figures 6-8). Each of the spectra was also interpreted by identifying the possible functional group each peak with assigned number represents (presented in Tables 1-3).

3. Results and Discussion

3.1. Extraction

By visual observation, only aqueous and alkali extraction methods gave a deep

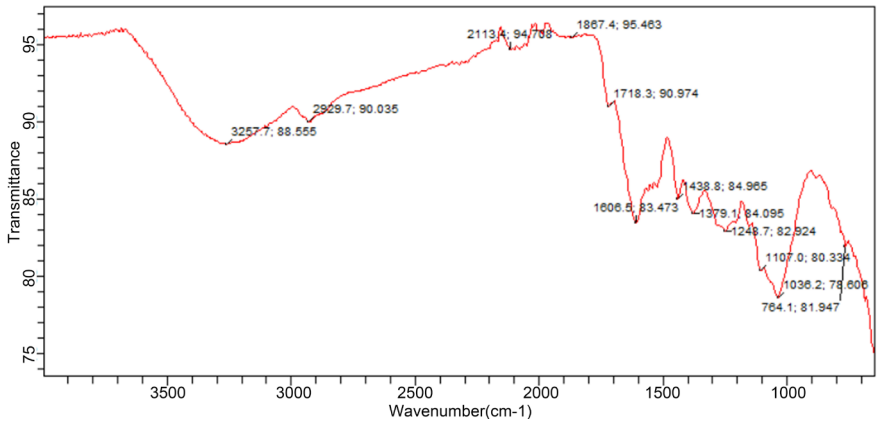

Figure 6. Infra-Red (IR) Spectrum of *Rothmannia whitfieldii* fruit dry powder.

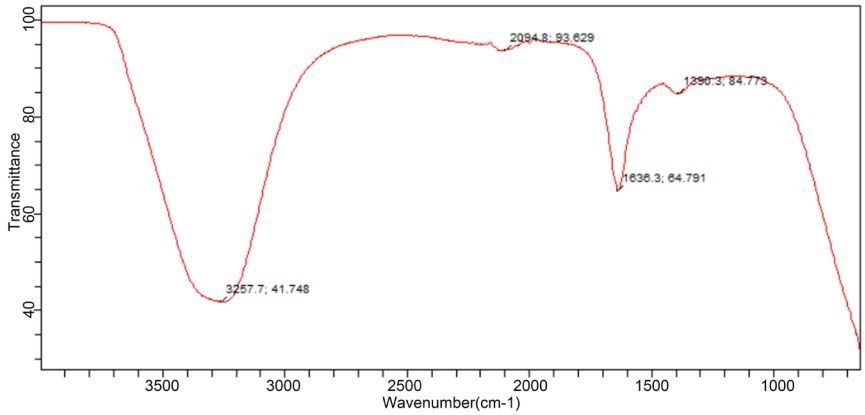

Figure 7. Infra-Red Spectrum of *Rothmannia whitfieldii*, extract with 1% alkali solution at 60˚C.

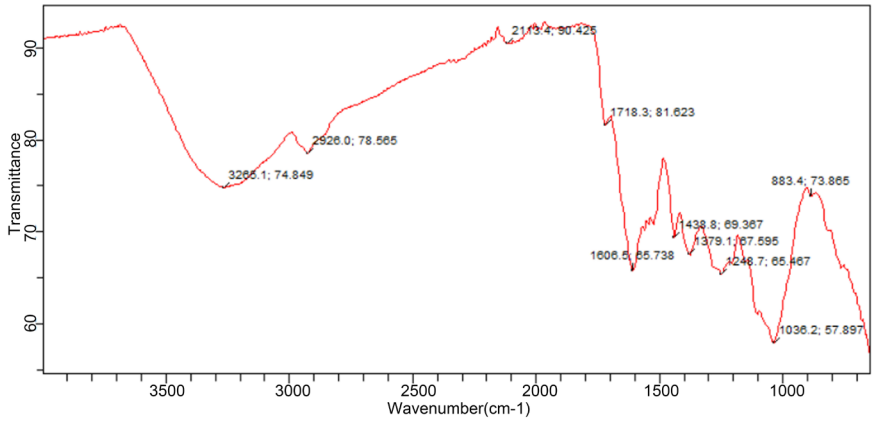

Figure 8. Infra-Red Spectrum of *Rothmannia whitfieldii*, extract with distill water at 60˚C.

coloured liquid after the extraction time (**Figures 2-5**). While alkali extraction gave a deep brown colour liquid, aqueous extraction gave a black colour liquid. Acid and isopropanol extraction methods gave a clear coloured liquid. In keeping with the objectives of this research, only the deep coloured liquids after extraction were characterized with the FTIR spectroscopy. This is because intense

colour and solubility are some of the properties a compound that can function as a dye must possess [9].

It was observed that 5 ml of the supernatant liquid from the aqueous extraction when mixed with drops of 1% NaOH solution changed colour from black to deep brown. Whereas there was no change in the colour of 5 ml of deep brown colour liquid from the alkali extraction when mixed with drops of water. This perhaps indicates that the natural dye molecule in dry *Rothmannia whitfieldii* fruit does not dissolve in water but dissolves in alkali solution. A solute will not dissolve in a solvent if their solubility parameters and/or affinity are different [10]. This is the likely reason for non-extraction of dye from the plant material with isopropanol, and acid solution. On the other hand it can be deduced that the dye molecules of the plant were dissolved by sodium hydroxide solution resulting to the deep brown colour observed.

3.2. FTIR Spectroscopy Analysis

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. The fact that materials are a unique combination of atoms, no two compounds produces exactly the same infrared spectrum. Therefore, infrared spectroscopy can be used to positively identify (qualitative analysis) different kinds of materials. Also, the size of the peaks in the spectrum is a direct indication of the amount of material present [11].

Each of the peaks identified with a wavenumber in an IR spectrum represent a certain functional group in the compound being investigated. It should be noted that infrared spectroscopy is used majorly to confirm the presence of functional groups in a compound and not for determination of the structure of a compound. For the purpose of interpretation infrared spectrum can be split into four distinct regions [12].

1) 4000 - 2500 cm^{-1}: absorption of single bonds to hydrogen, e.g. C-H, O-H, N-H

2) 2500 - 2000 cm^{-1}: absorption of triple bonds, e.g. C≡C and C≡N

3) 2000 - 1500 cm^{-1}: absorption of double bonds, e.g. C=C, C=O

4) 1500 - 400 cm^{-1}: absorption owing to other bond deformations, e.g. rotating, scissoring and some bending.

There are some exceptions, e.g. N-H bending is observed at 1550 - 1620 cm^{-1}. The region between 1500 - 400 cm^{-1} is the fingerprint region because it is unique to each compound. This region helps to identify particular molecules because no other compound will have the same pattern of absorptions.

The content of **Figure 4** and **Figure 5** extracts based on the identified peaks in the IR spectrum shown in **Figure 7** and **Figure 8** are presented in **Table 1** and **Table 2**, while the content of the *Rothmannia whitfieldii* fruit dry powder based on **Figure 6** is presented in **Table 3** below. The interpretation given to each peak is based on extensive work done by series of researchers in the field of IR spectroscopy [13] [14] [15] [16].

Table 1. Functional groups in *Rothmannia whitfieldii* extract using alkali solution; based on **Figure 7** spectrum.

Wavenumber (cm^{-1})	Bond	Functional group
3257.7	O-H stretch, H-bonded	alcohols, phenols
2094.8	-C(triple bond)C-stretch or N=C=S stretching	Alkynes or Isothiocyanate
1636.3	C=C stretching or N-H bending or C=C stretching	Conjugated alkene or Amines or Cyclic alkene
1390.3	C-H bending or O-H bending or N-O symmetric stretch	Alkane or Phenol, alcohol or Nitro

Table 2. Functional groups in *Rothmannia whitfieldii* extract using distill water; based on **Figure 8** spectrum.

Wavenumber (cm^{-1})	Bond	Functional group
3265.1	O-H stretch, H-bonded	alcohols, phenols
2926	C-H stretch	Alkanes
2113.4	-C(triple bond)C-stretch	Alkynes
1718.3	C=O stretching	Cyclo or aliphatic ketone
1606.5	N-H bending or C=C stretching	Amines or Cyclic alkene
1438.8	C-H bend or C=C stretch (in-ring)	Alkanes or Aromatics
1379.1	C-H bending	Alkanes
1248.7 - 1036.2	C-N stretching	Aromatic amines
883.4	=C-H bend or C-H bend	Alkenes or Aromatics

Table 3. Functional groups of *Rothmannia whitfieldii* fruit dry powder; based on **Figure 6** spectrum.

Wavenumber (cm^{-1})	Bond	Functional group
3257.7	O-H stretch, H-bonded	alcohols, phenols
2927.7	C-H stretch	Alkanes
2113.4	-C(triple bond)C-stretch	Alkynes
1867.3	C-H bending or C=O stretch	Aromatic compound
1718.3	C=O stretching	Cyclo or aliphatic ketone
1606.5	N-Hbending or C=C stretching	Amines or Cyclic alkene
1438.8	C-H bend or C=C stretch (in-ring)	Alkanes or Aromatics
1379.1	C-H bending	Alkanes
1248.7 - 1036.2	C-N stretching	Aromatic amines
764.1	C-H bend	Aromatics

The IR spectra shown in **Figure 6** and **Figure 8** above have close similarity even at their fingerprint region. Similarly, in **Table 2** and **Table 3** we observe that their chemical content which is represented by the peaks and functional groups as interpreted are largely related. A slight difference is observed at peaks 1867.3 cm^{-1}, 1107 cm^{-1} and 764.1 cm^{-1} identified in **Figure 6** but not identified (that is, no wavenumber was assigned to it by the spectroscopy) in **Figure 8**, and peak 883.4 cm^{-1} identified in **Figure 8** but not identified in **Figure 6**. The non-identification has been traced to the poor intensity of such peaks [17]. **Figure 8** spectrum is from a solution (**Figure 5**) the presence of water also contributed to this observable difference. However, the bonds and the functional groups suspected with peak 883.4 cm^{-1} in **Figure 8**, are same with those suspected with peaks 1867.3 cm^{-1} and 764.1 cm^{-1} in **Figure 6**, and peak 1107 cm^{-1} (in **Figure 6**) falls within 1248.7 cm^{-1} - 1036.2 cm^{-1} seen in **Table 2** and **Table 3**. There similarity is also identified in their colour; *Rothmannia withfieldii* fruit dry powder has a black colour corresponding to the colour of the aqueous extraction in **Figure 5** which gave the IR spectrum represented in **Figure 8**.

Figure 7 is the IR spectrum of the deep brown liquid after extraction with alkali solution. The interpretation of this spectrum is presented in **Table 1**. Extensive work has been done on the dyeing capacity of this extract which has been identified to be a direct dye that dyes best at temperatures above 55˚C [17]. Peak 1390.3 cm^{-1} in **Figure 7** an O-H group indicates the presence of phenol groups which implies that the extract contains tannins. The brown colour of the extract could be a confirmation to this. It has been noted that tannins are the most important ingredients required for brown colour shades when dyeing with natural dyes [18]. The presence of phenols at this peak can also imply the presence of carthamine or catechins (flavonols) which is also found in most plant based natural dyes. Jansen [6] though reported that there is no available chemical study of the colourants or dye-precursors present in the flowers and fruits of *Rothmannia whitfieldii,* he however presumed that the fruit pulp contains alkaloids with dyeing properties like several other *Rothmannia* species with similar uses. According to Nnorom, *et al.* [17] the peak at 1636.3 cm^{-1} is confirmed to be a carbon to carbon conjugated double bond. This is the covalently unsaturated group known as a chromophore, responsible for absorption of in the UV or visible region while the O-H groups at peak 1390.3 cm^{-1} a covalently saturated group could be the auxochrome [19].

4. Conclusions

We can therefore conclude from the FTIR spectroscopy result that all functional groups found in **Figure 6** are same with those in **Figure 8**. Hence, both compounds are the same. This implies that the black coloured supernatant liquid from aqueous method of extraction is not a dye. This assertion was further confirmed by the change in colour of the aqueous extract from black to deep brown when drops of 1% NaOH solution were mixed with it. The dark coloured liquid from aqueous extraction method is therefore attributed to the scattering of fine (minute)

particles of the black coloured powder of the dry *Rothmannia whitfieldii* fruit (pulp and seed) when put in hot water.

Similarly, from the FTIR spectroscopy, we have been able to ascertain that the dye component of dry *Rothmannia whitfieldii* fruit was only extracted by the alkali solution and not water. Visual observation of the supernatant liquid after extraction with alcohol and acid solution also confirmed that the dye component of dry *Rothmannia whitfieldii* fruit is not soluble in both and not extracted in both.

We have therefore succeeded to prove that dry *Rothmannia whitfieldii* fruit which hitherto is not considered useful is a good source of natural dye. It has also been established that to extract dye from dry *Rothmannia whitfieldii* fruit powder, alkali extraction method should be used. Unlike Jansen [6] who only made a presumption, we have confirmed that *Rothmannia whitfieldii* fruit contains flavonols due to the IR peak at 1390.3 cm^{-1}.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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