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*Full Length Research Paper*

# **Spectrophotometric evaluation of rotenone extraction from leaves and seeds of mature *Tephrosia vogelii* plant**

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The percentage yield of rotenone extracted from *Tephrosia vogelii* leaves and seeds was investigated. Ten samples of well ground leaves and seeds were each subjected to extraction in soxhlet extraction system using trichloromethane, ethanol and methanol solvents for 24 h at room temperature. The maximum absorption wavelength of rotenone was determined using Ultra violet-visible spectrophotometer. Different solvent extracts were quantified using high performance liquid chromatography instruments fitted with Ultra Violet detector and their yield expressed as percentage rotenone. Trichloromethane recorded the highest rotenone yield from both leaves (8.3 and seeds 2.7%) compared to the other two solvents. Ethanol was second with 5.9% in leaves and 1.9% in seeds while methanol had 4.8% in leaves and 1.6% in seeds. In general, the leaves extracts were found to have a higher rotenone percentage yield by an average factor of 3 compared to seeds. Rotenone can be commercially extracted from *T. Vogelii* using ethanol that is locally available to complement natural pyrethrum insecticide industry.

**Key words:** Rotenone, *Tephrosia vogelii*, solvents, seeds, leaves.

## **INTRODUCTION**

The legume *Tephrosia Vogelii* (*T. vogelii*) Hook. f. (Fabaceae) is a shrubby plant distributed to many parts in the tropics where it is used as shelter, cover crop, fish poison and as a pesticide. It was identified in Kenya in 1930 in Shimba Hills, Kwale County at the Coast region, 1932 at Mt.Elgon in the western part and in Kiambu County in 1934 in the central part of the country. This plant has over the years been known to have insecticidal and acaricidal activities though it has not been

commercially exploited locally. It is worth noting that Pyrethrum, *Chrysanthemum cinerariaefolium*, was introduced in Kenya in early 1930s by the colonial farmers and a processing plant constructed in Nakuru Town in 1935. Pyrethrum is now grown in the four East Africa countries namely, Kenya, Rwanda, Uganda and Tanzania, by small scale farmers for sale to three processing plants in the region.

In Kenya, pyrethrum is grown in more than 15 counties,

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with the highest yielding crop (3.0%) pyrethrins, found in the highlands of West Pokot County (PBK, 1996).

In East and Southern Africa, *T. Vogelii* is grown in small plantations by small scale farmers for their own use in crop protection, both in the field plantation against moles and in storage against insects (Belmain et al., 2012). Crop production in Kenya is constrained, where the small scale farmers loose almost 30% of their yield to pest in the field and in post harvest storage (Midega et al., 2016). There has been a renewed interest in botanical pesticides because of several distinct advantages that include; i) pesticidal plants are generally safer than conventional synthetic ones due to their degradation rate and metabolites, ii) Plants have more than one molecule as active principle ingredients responsible for biochemical properties hence, insects are highly unlikely to develop resistance. Pyrethrum has six pyrethrins different molecules and has been in use as an insecticide in the last 70 years (Casida and Quistad, 1995).

The insecticidal activity of *T. Vogelii* plant is attributed to, rotenone, tephrosine, deguelin and toxicarol compounds collectively referred to as rotenoids. Rotenone show stronger insecticidal activity than tephrosine and deguelin while toxicarol is toxic to fish (Stevenson and Belman, 2016; Watt and Brandwijk, 1988).

Rotenoids are respiratory enzyme inhibitors, acting between a coenzyme involved in oxidation and reduction in metabolic pathways and a respiratory enzyme responsible for carrying electrons in some electron transport chain (Hassanali et al., 1989). Rotenone is unstable and decomposes rapidly in light and air with a half life ( $t_{1/2}$ ) of 6 h (Kariuki et al., 2014; Mkenda et al., 2015). *T. vogelii* is also found in other parts of the world and was introduced to the United States of America in the sixties for the purpose of commercial production of Rotenone with much success than *Derris* and *Lonchocarpus* spp. which hitherto were the main sources of the pesticide (Yenesew et al., 2004). The plant's extract is not phytotoxic and only moderately toxic to mammals by inhalation than by ingestion. Skin irritation and inflammation of mucous membranes may result from skin contact (Sola et al., 2014; Tavershimal et al., 2015).

*T. Vogelii* extracts have been shown to effectively synergize the insecticidal activities of Pyrethrum by a Co-toxicity Coefficient (CC) factor of 4, when combined at a Pyrethrum:*T. vogelii* ratio of 57:2, against cockroach (*Americana periplaneta*) (Kariuki et al., 2014). This makes *T. vogelii* an important and effective ingredient to support natural derived insecticides that will not only substitute for insect resistive overused synthetic molecules, but will also sustain the environment in addition to increasing income revenues to small scale farmers in Kenya. The aim of this study was to evaluate *T. vogelii* as an additional commercial source of sustainable natural insecticide that is effective and environmentally friendly, capable of enhancing the

activity and hence the demand of pyrethrum uses, to farmers and general public. The successful positive evaluation will not only introduce a new cash crop to farmers, but also promote growing of *T. vogelii* and Pyrethrum in Kenya.

## MATERIALS AND METHODS

### Sampling

*T. vogelii* leaves and seeds samples were collected from a farm in Kikuyu area, from 2 year old mature plants. The leaves were harvested, put into polythene bags and brought to the Laboratory in the Department of Chemistry, University of Nairobi for further preparations prior to extraction.

### Sample preparation

The leaves and seeds were spread on laboratory benches and dried at room temperature for two weeks. They were then ground into powder using a high speed blender. 250 g of the ground plant material was weighed in triplicate and stored in paper bags in the lockers away from light.

### Extraction

Each 250 g sample was then transferred into a thimble and assembled the soxhlet extraction system and extracted using either of the solvents viz; 95% trichloromethane, 98% methanol and 95% ethanol. The extraction experiments were repeated with each of the other two solvents separately to give a set of three extracts. The extracts were then evaporated to near dryness on a rotary evaporator at reduced pressure and 40°C to give a dark to light brown oily trichloromethane, methanol or ethanol crude extract. The weight of each extract obtained was determined and then stored in a refrigerator at 0-4°C for analysis for analysis.

### Ultra violet- visible absorption wavelengths of rotenone

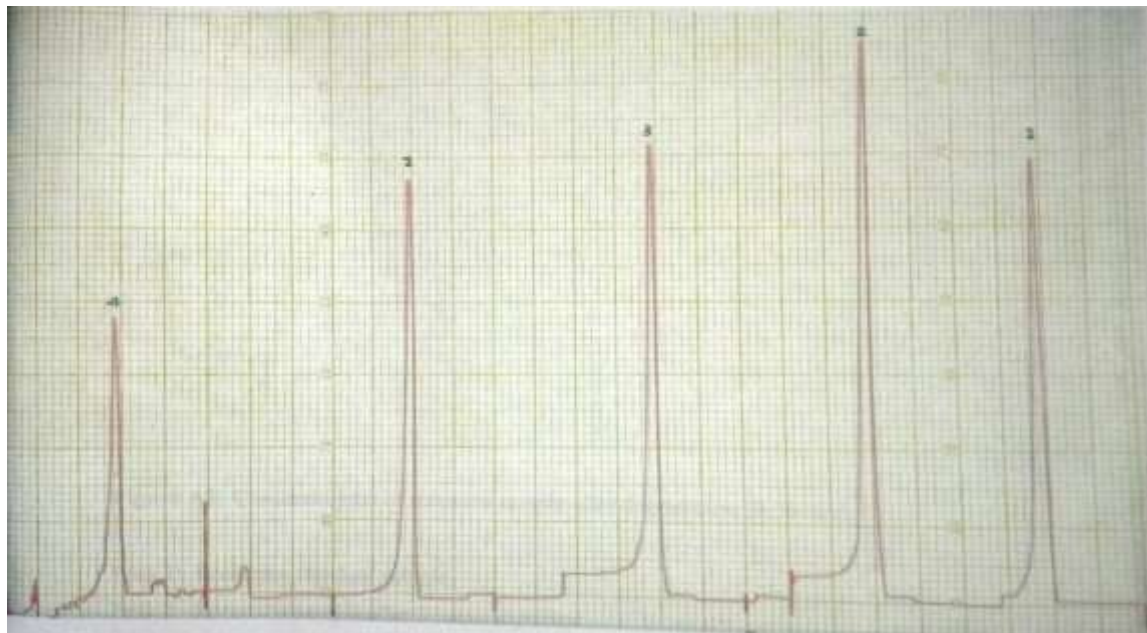
One gram of rotenone standard reference material and trichloromethane extract from *T. vogelii* was each separately dissolved in 100 cm<sup>3</sup> of acetone. This experiment was repeated with each of the other two solvent *T. vogelii* extracts. The absorption wavelength of rotenone standard reference material and the rotenoids extracts were determined while acetone was used as the blank. The maximum absorption wavelength of rotenone was used to monitor the separations in the detector in the HPLC analysis.

### High performance liquid chromatography analysis of rotenoids

The analysis of rotenone was quantitatively analyzed using high performance liquid chromatography fitted with an ultra violet detector. A stationary phase (PR C18, 250 x 4.6 mm) column was used and a mobile phase system comprising of acetonitrile and water at a ratio of 85:15, flowing at a rate of 2 cm<sup>3</sup>/min and a chart speed of 1 cm/min. The liquid chromatography system was switched on and allowed to stabilize for 5 min with the mobile phase before injecting the analyte. The injection volume in each case was 20 µl. The analysis was performed at the maximum absorption wavelength on injecting an appropriate amount of standard solution

**Table 1.** UV/Visible absorption wavelengths of standard Rotenone and *T. Vogelii* extracts.

S/N	Sample	Maximum absorption wavelength ( $\lambda_{nm}$ )
1	Rotenone [95%]	340
2	<i>Tephrosia vogelii</i> Extracts	340,570,695,665,666

**Figure 1.** HPLC *Tephrosia Vogelii* Leaves Extracts Chromatogram. Peak 1, rotenone standard sample; Peak 2, trichloromethane extract; Peak 3, ethanol extract; Peak 4, methanol extract.

of 1 mg/cm<sup>3</sup>. The quantification of the analyte was based on peak areas corrected with the rotenone standard reference material.

#### Determination of percentage yield of rotenone

A stock solution of *T. vogelii* trichloromethane extract was prepared by dissolving 1 g of extract with 100 cm<sup>3</sup> of acetone. 1 cm<sup>3</sup> aliquot of the stock solution was measured and then homogenised with a mobile phase solution. The sample was then injected into the HPLC for analysis. Similar procedure was repeated for methanol and ethanol extracts and each experiment was replicated four times. Concentrations of various extracts and standard deviations were then established. The yield of rotenone in leaves and seeds was then expressed as the percentage concentration of the initial density of the original extract.

## RESULTS AND DISCUSSION

### Maximum absorption wavelength of rotenone in ultra violet-visible spectrophotometer

The establishment of absorption wavelength of Rotenone standard reference material and rotenoids from extract

had several peaks in the scan from the ultra violet to the visible region of the electromagnetic spectrum.

The maximum absorption wavelength of Rotenone standard reference material was established at 340 nm. The *T. vogelii* extracts exhibited five absorption peaks between 340 and 666 nm (Table 1). At 340 nm, the peak was slightly broader compared to the one realized in the case of Rotenone standard reference material. The five peaks were realized from all the three extracting solvents but at varying proportion, thereby signifying extraction efficiencies.

### High performance liquid chromatography (hplc) of *tephrosia vogelii* extracts

The concentration of the *T. vogelii* extracts of the three solvents was analysed using HPLC. The chromatogram in Figure 1 shows the varying peaks of the extracts.

The percentage yield of the solvents' extracts was established on the basis of rotenone in *T.vogelii* leaves and seeds.



**Table 2.** Mean densities and concentration of rotenone in leaves and seeds extracts.

S/N	Solvent	Plant material	Weight of dry material	Concentration of Rotenone, g/kg (sd)	Yield % (sd)
1	Trichloromethane	Leaves	124.1	10.3 ±0.84	8.3±0.71
		Seeds	40.7	4.8 ±0.054	2.7±0.15
2	Methanol	Leaves	85.4	4.1 ±0.08	4.8±0.22
		Seeds	112.5	1.8±0.03	1.6±0.08
3	Ethanol	Leaves	61.0	3.6±0.04	5.9±0.24
		Seeds	78.9	1.5±0.02	1.9±0.1

### Percentage yield of rotenone extracts in leaves and seeds

The three solvents were separately used to extract rotenone from two different parts of a mature plant. Table 2 shows the percentage yields of leaves and seeds extracted with three solvents.

The trichloromethane extracts recorded the highest concentration of rotenone per kilogram of dry material extracted in both leaves and seeds extracts while the least concentration was recorded in methanol extracts. The percentage yield of rotenone in trichloromethane leaves and seeds extracts were 8.3 and 2.7% respectively. All the solvents recorded a higher percentage yield in leaves compared to the seeds.

### Conclusion

Rotenone is therefore highly concentrated in the mature *T. vogelii* plant's leaves than in the seeds by a factor of 3. The extraction efficiency of solvents used (described on the basis of the actual content of rotenone present in one kilogram of dry material seeds or leaves extracted) showed that trichloromethane (8.3%) yield in leaves is about three times the highest percentage yield of 3% w/w pyrethrins, achieved from highest yielding *Chrysanthemum cinararaefolium* flowers in Kenya. Moreover, the amount of *T. vogelii* required to synergise Pyrethrum extract is 28.5 times lower; to achieve an average of 4 times pyrethrins efficacy. In conclusion growing of *T. vogelii* may be commercialized to effectively extract rotenoids from its leaves using trichloromethane or ethanol. Trichloromethane is a relatively harsh solvent, expensive and given that ethanol is locally manufactured, it would well be used for commercial extraction of Rotenoids from the leaves of *T. vogelii*.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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*Full Length Research Paper*

## Preliminary characterization of some natural dyes

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**A preliminary study on the chemical structure of dyes from *Rothmannia hispidia*, *Pterocarpus osun* and *Terminalia superba* was made using chemical tests, UV-visible and infrared spectroscopies. *R. hispidia* dye was found to contain an alkyl amino group (-NHR) and carbon-carbon double bond conjugated with a carbonyl (C = O) group, and also showed maximum absorption at 595 nm in the visible region. *P. osun* dye contains conjugated systems, hydroxyl (-OH) and amino (NHR) groups and showed maximum absorption at 506 nm in the visible region whereas *T. superba* dye is made up of conjugated system, nitro (NO<sub>2</sub>) and hydroxyl (-OH) groups. It showed maximum absorption at 478 nm in the visible region.**

**Key words:** *Rothmannia hispidia*, *Pterocarpus osun*, *Terminalia superba*, dyes, characterization.

### INTRODUCTION

Generally, dyes are used for colouring of foods, drugs, cosmetics, leather, petroleum products, and textiles materials among other things. These materials are dyed for different purposes. For instance, in leather industry, one of the reasons for dyeing the leather is to make it adaptable for fashion styling (Opara et al., 2014). Petroleum products are coloured for identification of fuel adulteration (Ezeokonkwo and Okoro, 2012) and for differentiation of various petroleum products (Rostad, 2010, Meghmani Dyes and Intermediates, 2011). Most substances are generally dyed to enhance appearance and aesthetic value of the finished product. In recent times, many people are becoming more conscious of the need to use natural dyes in food colouring as against synthetic dyes (Dweck, 2009). Some of the approved dyes are being delisted due to legislative action as well

as consumer interest (Garcia and Cruz-Remes, 1993). Again, natural food colourants contain some biological active components such as lycopene, carotenes, canthaxanthin and quercetin, which play vital role in human health (Okafor et al., 2016). Turmeric (a yellow dye) is a good colouring agent (Vankar et al., 2007), which is used as spice and as natural food colorant. Turmeric has also been reported to have a powerful antiseptic effect that revitalizes the skin; while indigo, a dark blue dye has a cooling sensation (Grover and Patni, 2011). Several researches have reported on the anti-diabetic effects of many medicinal plants including *Rothmannia hispidia* and *Pterocarpus osun* dyes, and this has resulted in an increase in the number of people who use these natural compounds to control their disease (Pius et al., 2016; Saki et al., 2014). All these

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information have led to increase in demand for natural dyes in food industry (Okafor et al., 2016). There is also a growing interest in the use of natural dyes in textile coloration, which has been attributed to the strict environmental measures imposed by many countries (Kamel et al., 2005). Synthetic dyes have been associated with toxicity and allergic reactions, whereas natural dyes are non-toxic, non-allergic, non-carcinogenic and more environmentally friendly (Semwal et al., 2012). Natural dyes have better biodegradability and generally show a higher compatibility with the environment. Again, there is a reduction in the use of synthetic dyes as against natural dyes, as histological stain, due to their hazard to human and animal health (Avwioro et al., 2005). According to Morrison (Morrison, 2015), there are four reasons why natural dyes should be chosen over synthetic dyes. First, clothings coloured with natural dyes reduces the challenge of toxic runoff that could be suffered when synthetic dyes are used in textile and dyeing process. Secondly, using dyes from plants that grow in our environment eliminates the problems associated with production of synthetic dyes. Thirdly, natural dyes are non-toxic to work with. Fourthly, in working with natural dyes one gains enlightening experience from direct connection with nature.

Dyes owe their colouring effect to their ability to exhibit a number of characteristics, which include presence of at least one chromophore and one auxochrome, conjugated system, resonance, and ability to absorb light in the visible region (Abrahart, 1977; Gürses et al., 2016). Chromophores are groups with multiple bonds responsible for the colour of a compound. Examples include nitro (-NO<sub>2</sub>), nitroso (-NO), azo (-N=N-), azoxy (-N=N-O) and conjugated system ((-CH=CH-)<sub>n</sub>). Auxochromes on the other hand are colour enhancers. They are groups that make coloured substances act as dyes. Some examples of auxochromes are OH, Cl, COOH, SO<sub>3</sub>H, NH<sub>2</sub>, NHR, NR<sub>2</sub> and OR.

Many plants in Nigeria are sources of natural dyes (Akpuaka, 1992; Nnabugwu and Okoro, 2012), among which are *R. hispidia*, *P. osun* and *Terminalia superba*. Some of the local names by which *R. hispidia* is known in Nigeria include: "okukim", "obong", "asun", "asogbodu", "uriohia" and "owuruokumuo" (Antai et al., 2010). *P. osun* also has many common names depending on the country and location. Some of them are Vene in French, Palissandre in Senegal, Kino in Gambia, Bani or Banuhi in Burkina Faso, Madubiya in Northern Nigeria and Osundudu in Southwest Nigeria (Shobayo et al., 2015). *T. superba* has several common names, such as yellow pine, white afara, limba, black korina (Englis); limba, frakè (French), limba (German), akom (Spanish), mwalambe (Spanish), and afara, afa (Yoruba-Western Nigeria) (Orwa et al., 2009). Crude extracts from *R. hispidia*, *P. osun* and *T. superba* are used locally by some women in Eastern part of Nigeria for body beautification. They are also used locally as a medication

for the treatment of chicken-pox in children (Akpuaka, 1992). Literature has shown that *R. hispidia* has been used for the treatment of many ailments such as diabetes mellitus (Antai et al., 2005) and skin infections (Antai et al., 1995). *Pterocarpus* species is one of such plants which have been used for treatment of type 2 diabetes (Mukherjee et al., 2006). The stem bark powder of *Pterocarpus* spp. has also been used in treating diarrhea and the wood powder has been externally applied in the treatment of inflammations, headache, mental aberrations, and ulcers (Krishnaveni et al., 2000). *T. superba* has broad spectrum against the growth of betalactamin multi-resistant bacteria (Anago, 2009). The bark of the plant is used in treatment of some broncho-pulmonary ailments (Akoegninou et al., 2006), diarrhea and gonorrhoea (Neuwinger, 2000). Other useful properties exhibited by *T. superba* include antifungal (Ahon et al., 2011) and hypoglycemic activities (Wansi et al., 2007). In addition to the aforementioned, the dyes from *R. hispidia*, *P. osun* and *T. superba* plants have been shown to be potential dyes for colouring gasoline, diesel, kerosene, and candle wax (Nnabugwu and Okoro, 2012). These dyestuffs and many others (Akpuaka, 1992; Nnabugwu and Okoro, 2012; Akpuaka et al., 2001) have been extracted and isolated from many plants in Nigeria, but not much work has been done to investigate their chemical structures. Considering the several applications of these dyes and the growing interest in the use of natural dyes, there is urgent need to elucidate the structures of these dyes. Moreover, the knowledge of the dye structures is useful for forensic investigations and the study of art history. The present preliminary work investigated the basic chromophores and auxochromes present in dyestuffs from *R. hispidia*, *P. osun* and *T. superba* using chemical tests, UV-visible and infrared spectroscopies. This is a vital step in the right direction towards understanding the makeup of some of these natural dyes.

## MATERIALS AND METHODS

Melting points were determined on a Fisher John's melting point apparatus and are uncorrected. Ultraviolet and visible spectra were obtained on a Unico-Uv2102 PC spectrophotometer using 1 cm quartz cells. The solvent used was ethanol or chloroform as the case may be. The absorption maxima were recorded in nanometers (nm). Infra red (FTIR) spectra were obtained as KBr discs on a magna-IR system 750 spectrophotometer. Chemical analysis was done at the Post-Graduate Laboratory, Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka.

### Extraction and isolation of dyestuffs

The plant materials were collected at Nsukka in Enugu State, Nigeria, and were identified by Mr A. O. Ozioko of Department of Botany, University of Nigeria, Nsukka. The dyestuffs were extracted and isolated as reported in the literature (Nnabugwu and Okoro, 2012).

**Dyestuff from *R. hispidia***

About 275 g of *R. hispidia* seeds were crushed using a grinder and then soaked in 55 ml distilled water for 72 h in an airtight container. The soaked seeds were later agitated and there after filtered with a Buckner funnel. The light yellow filtrate was stirred vigorously with a magnetic stirrer for several hours until a dark blue solution was obtained. The solution was kept at room temperature for 2 weeks during which the dark blue dye precipitated out and collected at the bottom of the containing vessel as sediment. The supernatant liquid was poured out while the dark blue *R. hispidia* dye sediment was air-dried at room temperature.

**Dyestuff from *P. osun***

About 150 g of ground stem wood of *P. osun* was steeped in 600 ml of ethanol (96%) for 72 h. After agitation, the steeped wood was filtered. The dark-red filtrate was concentrated via simple distillation to about one-third its original volume. 600 ml of water was added to the concentrate so as to precipitate the dye. After filtration, the precipitate was collected and purified by recrystallization from 96% ethanol and distilled water mixture (3:1) along with activated charcoal. The dark-red dye was dried at room temperature.

**Dyestuff from *T. superba***

About 200 g of the *T. superba* stem wood was ground and soaked in 1.3 L of 96% ethanol for 72 h. The wood was agitated and filtered to obtain an orange-red filtrate. The filtrate was concentrated by simple distillation followed by evaporation through reduced pressure. An orange-red dye obtained was purified by recrystallization from 96% ethanol and 0.5 g charcoal.

**Qualitative analysis**

The dyes being organic compounds were qualitatively analyzed by standard methods used for analysis of organic compounds as described in Vogel (Furniss et al., 1989). Specifically, the following tests were carried out on the dyestuffs, including melting point determination.

**Ignition test**

About 0.01 g of each of the dyestuffs was placed in an ignition tube and heated until ignition occurred.

**Solubility test**

The solubility tests of the dyestuffs in 1 ml portion of ether, water, 5% NaOH, 5% NaHCO<sub>3</sub> and 5% HCl were carried out using 0.1 g of the dyestuff in each case.

**Sodium fusion test (Lassaigne's test)**

About 50 mg of each of the dyestuffs was placed in an ignition tube and four pieces of metallic sodium (2 mm cube) were added to each of the tubes. The tubes were heated gently at first (in a Burnsen burner) and then more vigorously until fumes have ceased to evolve. While the tubes were still red hot, each was dipped in a clean mortar containing about 10 ml of distilled water. The tubes were then crushed inside the mortar and the solution was filtered. The filtrates were used to test for the presence of sulphur, nitrogen and halogens.

(i) Test for Sulphur: Few crystals of sodium nitroprusside were added to 5 ml of each of the filtrates. Purple or deep blue violet colour indicates the presence of sulphur.

(ii) Test for Nitrogen: A little quantity of ferric chloride was added to 5 ml of each filtrate and heated. The hot solution was cooled under tap and few drops of ferric chloride were added to it followed by acidification with dilute sulphuric acid. Blue or greenish blue colour indicates the presence of nitrogen.

(iii) Test for halogens: Concentrated HNO<sub>3</sub> was added to 5 ml of each filtrate, heated and cooled under tap. Few drops of silver nitrate solution were then added. Precipitate indicates the presence of a halogen.

**Test for functional groups**

Specific tests for functional groups were done on the dyestuffs to determine the functional groups present in them.

**Test for aldehydes and ketones**

(i) About 0.5 g of each dyestuff was added differently to 2, 4-dinitrophenyl hydrazine in ethanolic phosphoric acid. Dark red precipitate indicates the presence of aldehyde or ketone.

(ii) Tollen's reagent test: 2 ml of Tollen's reagent was added to each of the dyestuffs and then heated to 35°C. Silver mirror indicates the presence of aldehyde while absence of silver mirror indicates the presence of ketone.

**Test for amines**

(i) HCl sodium nitrite test: Concentrated HCl was added to each of the dyestuffs and cooled. Then sodium nitrite was added to the solution. A brown precipitate indicates the presence of aromatic amines.

(ii) Acetyl derivative: 6 ml of water and 0.2 ml of acetic anhydride were added to 0.2 g of each of the dyestuffs. Formation of a derivative (gray colour) confirms the presence of amines.

**Test for phenols**

(i) **Methanol in iron (III) chloride test:** Methanolic anhydrous iron (III) chloride solution was added to a solution of the dyestuff. A green solution indicates the presence of monohydric phenol.

(ii) **Neutral iron (III) chloride test:** Neutral iron (III) chloride solution was added to a few crystals of the dyestuff. Brick red solution indicates the presence of monohydric phenols.

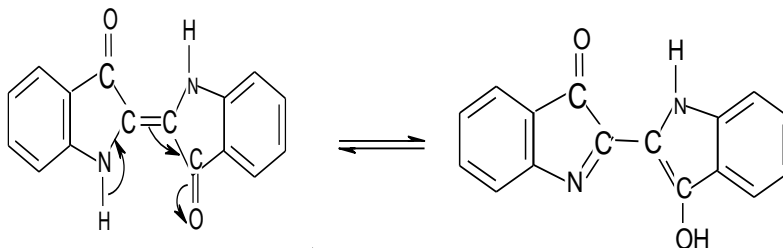
(iii) **Benzoate derivatives:** 10 ml of dilute sodium hydroxide and 0.6 ml of benzoyl chloride were added to 0.6 g of the dyestuff. Formation of the solid derivative indicates the presence of phenols.

**Test for Nitro group**

Each dyestuff was mixed with zinc dust and concentrated HCl and then heated. The cooled solution was tested for aromatic amine. Also, the presence of brown precipitate indicates the presence of nitro group.

**Test for alkaloids**

About 0.2 g of each of the dyestuffs was boiled with 2 ml of 2% HCl



Scheme 1. Structures

on a steam bath. The mixture was cooled, filtered and 1 ml of a portion of each of the filtrate was treated with 2 drops of the following reagents:

- (i) Dragendorff's Reagent: Dirty-green precipitate indicates the presence of alkaloids.
- (ii) Mayer's Reagent: A blue precipitate indicates the presence of alkaloids.
- (iii) Wagner's Reagent: A reddish-brown precipitate indicates the presence of alkaloids.
- (iv) Picric acid (1%): A green precipitate indicates the presence of alkaloids.

#### Application of the dyestuffs on textile fibres

***R. hispidia* dyestuff:** The dye bath, which is the vat, is made in two parts, the stock liquid and the vat (Finar, 2003). The stock solution is made up of water (10 ml), caustic soda (0.5 g) dissolved in water, dyestuff (0.2 g) and sodium hydrosulphite. To prepare the vat, 40 ml of warm water (38°C) was poured into a 250-ml beaker and about 0.2 g of sodium hydrosulphite sprinkled over the surface. The stock solution was then carefully poured into the vat solution and the mixture stirred gently. Four pieces of pre-washed fabrics (cotton, silk, polyester and nylon) were wetted in water and carefully put into the dye bath. They were left in the dye bath for about 20 min after which they were pulled out, allowed to drip out and air-dried. They were allowed to dry under direct sun light.

***P. osun* dyestuff:** 1.0 g of the dye was dissolved in 550 ml ethanol heated to 70°C. Pieces of pre-washed white cotton, silk, polyester and nylon were immersed in the dye bath and the temperature maintained at 70°C for 30 min. The fabrics were then removed, washed with cold water and dried at room temperature.

***T. superba* dyestuff:** About 0.4 g of the dyestuff was dissolved in 30 ml of ethanol heated to 70°C. The dye bath was used to dye white cotton, silk, polyester and nylon as in *P. osun*.

#### Colour fastness of the dyestuffs

The dyed fabrics were subjected to fastness tests to determine the fastness of the dyestuffs to washing, sunlight, acid and alkali.

- (i) Fastness to washing: All the dyed fabrics were soaked in laundry soap for 1 h, washed and rinsed with cold water. The fabrics were then allowed to dry at room temperature.
- (ii) Fastness to sunlight: The dyed fabrics were exposed to sunlight for 10 h every day for 2 days.
- (iii) Fastness to acid (10% HCl): Pieces of dyed fabrics were spotted with dilute HCl (10%) and allowed to dry under room temperature.

- (iv) Fastness to alkali (10% NH<sub>4</sub>OH): Pieces of the dyed fabrics were immersed in dilute ammonium hydroxide solution (10%) for 1 min. The fabrics were removed, and dried under room temperature.

## RESULTS AND DISCUSSION

### Qualitative analysis

All the dyestuffs burn with smoky flame leaving no residue. This shows that they are all aromatic compounds. The inability of the *R. hispidia* dye to dissolve in any of the normal solubility test solvents, but very soluble in alkaline sodium hydrosulphite is consistent with the solubility of vat dyes. The solubility tests also show that *P. osun* and *T. superba* are probably phenols. However, it should be noted that the presence of more than one functional group might have such a profound effect on the solubility of the dyestuff that it is often impossible to make deductions about the functional groups present from solubility data (Jansen and Cardon, 2005). The chemical analysis further revealed that the *R. hispidia* dye is an alkaloid and thus contains basic nitrogen. It also contains a ketone carbonyl group (RCOR'). The presence of a tertiary amine was observed in this dye, contrary to the expected secondary amine in an indigo dye. This observation is attributed to possible tautomerism in the dye whereby one of the -NH groups rearranges to -N=C (Scheme 1).

The sodium fusion test shows that the *P. osun* dye contains nitrogen. However, the alkaloid test was negative indicating that the nitrogen present in the dye is not basic. The dye also contains phenolic and amino groups. The *T. superba* dye also contains nitrogen, which is not basic. Also, there is the presence of nitro and phenolic groups in the dye (Table 1).

### Spectral analysis

The second column in Table 2 shows the UV-Visible spectra of the dyes. In *R. hispidia* dye, the peak within the region of 595 nm is due to  $\pi \longrightarrow \pi^*$  transition resulting from multiple conjugation. This absorption band occurs in the yellow region and so the compound is blue (that is, complementary colour). The band at 324 nm

**Table 1.** Results of the qualitative analysis of the dyestuffs.

<b>Preliminary tests</b>	<b><i>Rothmannia hispida</i></b>	<b><i>Pterocarpus osun</i></b>	<b><i>Terminalia superba</i></b>
m.p (°C)	>300	130-132	100-102
Ignition test	Aromatic compound	Aromatic compound	Aromatic compound
Solubility test	Insoluble in all normal solubility test solvents, but soluble in alkaline sodium hydrosulphite	Soluble in both NaOH(aq) and NaHCO <sub>3(aq)</sub>	Soluble in both NaOH(aq) and NaHCO <sub>3(aq)</sub>
<b>Sodium fusion tests</b>			
(i) Nitrogen test	+	+	+
(ii) Sulphur test	-	-	-
(iii) Halogen test	-	-	-
<b>Functional Group test</b>			
<b>Amines</b>			
(i) Conc HCl/NaNO <sub>2</sub>	+	+	-
(ii) Acetyl derivative	+	+	-
<b>Phenols</b>			
(i) Methanol in iron III chloride	-	+	+
(ii) Neural iron III chloride	-	+	+
(iii) Benzoate derivative	-	+	+
<b>Nitro group</b>			
Zinc dust + conc HCl test	-	-	+
<b>Aldehydes and ketones</b>			
(i) 2, 4-dinitrophenyl hydrazine test	+	-	-
(ii) Tollen's Reagent	+	-	-
<b>Test for alkaloids</b>			
(i) Dragendoff's reagent	+	-	-
(ii) Mayer's reagent	+	-	-
(iii) Wagner's reagent	+	-	-
(iv) Picric acid solution (1%)	+	-	-

- (Absent), + (Present).

could have resulted from  $n \rightarrow \pi^*$  transition of double bonded hetero-atom (Finar, 2003),  $C = O$ ,

whereas the peak at 294 nm denote the absorption band of  $\pi \rightarrow \pi^*$  transition resulting

from conjugation which is the characteristic of benzene derivative (Finar, 2003). *P. osun* dye

**Table 2.** Spectral data of the dyestuffs.

Dyestuff	UV – visible (nm)	Infrared (cm <sup>-1</sup> )
<i>Rothmannia hispidia</i>	294( $\pi \longrightarrow \pi^*$ ), 324( $n \longrightarrow \pi^*$ ), 595 ( $\pi \longrightarrow \pi^*$ )	-
<i>Pterocarpus osun</i>	226 ( $\pi \longrightarrow \pi^*$ ), 474 ( $\pi \longrightarrow \pi^*$ ), 506( $\pi \longrightarrow \pi^*$ )	3400 (O-H of phenol) (N-H str. NH <sub>2</sub> of primary amine, 2980 (O-H str. in aromatics), 1610, 1440 (C=C str. of aromatics), 1640-1560 (N-H deforming in aromatic amine), 1350-1280 (C-H str. in primary amine), 260-1000 (C-O str. in phenols), 830 (1,4-disubstituted in aromatics)
<i>Terminalia superba</i>	344 ( $n \longrightarrow \pi^*$ ), 478 ( $\pi \longrightarrow \pi^*$ )	3380 (O-H in phenols), 2930 (C-H str. in aromatics), 1580 and 1400 (C=C str. in aromatics), 1440 and 1310 (-NO <sub>2</sub> str. in aromatic nitro compounds), 1310 (C-O str. in phenols), 820 (C-N str. in aromatics or 1,4-disubstitution in aromatics).

**Table 3.** Results of application of dyestuffs on textile fibres.

Dyestuff applied	Colour of dyed fabrics			
	Cotton	Polyester	Silk	Nylon
<i>Rothmannia hispidia</i>	-	-	-	-
<i>Pterocarpus osun</i>	Dark red	Dark red	Dark red	Dark red
<i>Terminalia superba</i>	Deep orange	Orange	Light orange	Deep orange

showed absorption band at 474 to 506 nm which is due to  $\pi \longrightarrow \pi^*$  transition resulting from multiple and extended conjugation. The band occurs in the blue-green region; hence, the dye is dark-red. The absorption band at 226 nm denotes  $\pi \longrightarrow \pi^*$  transition resulting from conjugation, which is characteristic of benzene derivative (Finar, 2003). The absorption band shown by *T. superba* dye at 478 nm is attributed to  $\pi \longrightarrow \pi^*$  transition of multiple conjugation system and it occurs in the green-blue region of the spectrum; therefore, the dye is orange in colour. The observed band at 344 nm is assigned to  $n \longrightarrow \pi^*$  transition of N=O. It is noteworthy at this juncture that no dye gives a pure shade since no dye reflects only one band of wavelength (Finar, 2003). The infrared spectra of the dyes are shown in column 3 of Table 2. However, the IR spectrum of *R. hispidia* dye was not run due to unavailability of suitable solvent to dissolve it. The absorption band shown by *P. osun* at 3400 cm<sup>-1</sup> has been attributed to the presence of O-H (hydrogen bonded) in phenols (Furniss et al., 1989) and N-H (stretching) in secondary aromatic amines, which is hydrogen bonded. The band at 1350 to 1280 cm<sup>-1</sup> shows the presence of C-N (stretching) in secondary amines (Furniss et al., 1989; Silverstein et al., 2005). While the absorption bands of C - O (stretching) in phenols are observed at 1350 and 1260 to 1000 cm<sup>-1</sup>. These bands result from interaction between O-H bending and C-O stretching (Furniss et al., 1989; Silverstein et al., 2005). Other bands have been assigned as indicated in Table 2. The *T. superba* dye showed an absorption band at 3380

cm<sup>-1</sup> which is attributed to O-H stretching in phenols that is hydrogen bonded (Furniss et al., 1989). The band at 2930 cm<sup>-1</sup> is due to C-H stretching in aromatic nuclei whereas the bands at 1580 and 1440 cm<sup>-1</sup> are C=C stretching in aromatics. The bands at 1440 and 1310 cm<sup>-1</sup> are also attributed to -NO<sub>2</sub> vibrations in aromatic nitro compounds whereas the band at 1310 cm<sup>-1</sup> could also be due to C-O in phenols (Furniss et al., 1989; Silverstein et al., 2005). The observed band at 820 cm<sup>-1</sup> could be due to C-N stretching in aromatic nitro compounds. It could also be due to 1,4-disubstitution in aromatics. However, it should be noted that the interaction of the NO<sub>2</sub> out-of-plane and ring C-H out-of-plane bending frequencies destroys the reliability of the substitution pattern observed for nitro aromatics in the longer wavelength region of the spectrum (Silverstein et al., 2005).

A summary of the dyeing properties of the dyes is shown in Tables 3 and 4. The *R. hispidia* dye was not able to colour any of the fabrics. This is due to the inability of air to oxidize the reduced dye; hence, the original blue dye was not regenerated in the fabrics as expected. The dyed fabrics showed that nylon and cotton have the greatest affinity for *P. osun* and *T. superba* dyes. This is an indication that both dyes are direct dyes for nylon and cotton.

## Conclusion

Although the structure of the dyes could not be elucidated

**Table 4.** Results of fastness tests.

Dyestuff applied	Fastness tests			
	Washing	Sunlight	Acid (10% HCl)	Alkali (10% NH <sub>4</sub> OH)
<i>Rothmannia hispida</i>	-	-	-	-
<i>Pterocarpus osun</i>	+	+	+	+
<i>Terminalia superba</i>	+	++	++	+

-(not fast), +(moderately fast), ++(fast).

due to lack of NMR data, the results from the chemical and spectral analyses gave vital information about the structural constituents of the dyes. The chemical analysis and the characteristic absorption in the ultraviolet and visible regions coupled with the solubility in alkaline sodium hydrosulphite give credence that the *R. hispida* dye is an indigo dye (Finar, 2003) and contains O=C-C=C-C=O and -NHR as chromophore and auxochrome respectively. The results from chemical and spectral analyses also show that the *P. osun* dye is made up of a chromophore which is a conjugated system and auxochromes which are hydroxyl (-OH) and secondary amine (-NHR) groups. The results also show that there is presence of nitro (-NO<sub>2</sub>) group, conjugated system and hydroxyl group in the *T. superba* dye. However, there is need to elucidate the exact structure of the dyes through other spectra analysis such as NMR and Mass spectroscopy.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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