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***Justicia Carnea* Dyes: Extraction, Characterization, Application and Evaluation of Antimicrobial Activity on Cotton Fabric**

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**ABSTRACT**

This study aims at extracting natural dyes from fresh and dried leaves of *Justicia carnea*. Application of the aqueous extracts of these leaves on fabrics with mordants (cobalt chloride and lime) and without mordants at a dyeing temperature of 80° C was carried out using pre-mordanting, simultaneous- mordanting and post- mordanting processes. The extracts show a good antimicrobial activity to bacteria and fungi. The improvement in fastness properties of the dyed cotton fabrics to rubbing and perspiration showed that mordants as well as mordanting methods have influence on colour efficiency. The FTIR and UV spectroscopy analysis of the dyes revealed some important peaks and functional groups related to colour formation. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, anthraquinones, steroids and phenols in the fresh and dried samples. This research work reported a successful extraction of ecofriendly dyes from *Justicia carnea* that can meet the current yearning for industrial development in the developing countries of the world where biosources materials from nature abounds in large quantities.

**Keywords:** Antimicrobial, dyes, ecofriendly, extraction, fastness, mordants

**1.0 INTRODUCTION**

The production and utilization of synthetic dyes increased at the expense of natural dyes due to low cost of production, high dye uptake and better fastness properties. Most of the synthetic dyes are however made from non renewable petroleum based chemical by-products and coal tar (Berhanu and Ratnapandian, 2017). Non-biodegradable wastes from excessive use of synthetic dyes are major pollutants to the environment (Sohini and Shyamala, 2017) and a serious concern to human existence because they are carcinogenic, led to destruction of ecosystem and causes environmental pollution due to increase in population and industrialization. With these shortcomings associated with synthetic dyes, many people worldwide are anxiously demanding for natural dyes because they can easily overcome major defects of synthetic dyes of harmfulness to human body, pollution and wastewater (Thet *et al.*, 2020).

Natural dyes are coloured materials obtained from natural sources without synthesizing and can be obtained from plants and animals which are biodegradable, anti-allergic, non-toxic, deodorizing and colours soothing to human eyes (Ahmed *et al.*, 2019; Thet *et al.*, 2020) with little or no environmental impact. Despite these advantages, natural dyes are generally

more expensive due to seasonal limitation of source, problems of shades and blending, non uniformity and non reproducibility of shades, poor colour fastness and low affinity due to formation of weak bonds with the fibre (Bhandari *et al.*, 2020; Jose *et al.*, 2020). The quality and quantity of dyes from any natural sources depends on age, climate and processing methods of the raw materials.

Colour fastness is the property of the pigment or dye to retain its original hue especially without fading or bleeding under the influence of various conditions they may likely be exposed to in textile manufacturing and use (Thet *et al.*, 2020). Most natural dyes have low colour fast with fibres, hence mordants which have the ability to enhance the chemical bonding of the dyes and fibres with a view of increasing dye - fibre fixation (Ahmed *et al.*, 2019) are required. Mordants can either be chemical (usually the metallic salts of aluminium, chromium, iron, copper and tin) or natural products like wood ash, lime, stale urine, cow dung etc (Bhandari *et al.*, 2020). Aside applying natural dyes as colourants in textiles, they are also useful in colouration of food and cosmetic materials.

Microorganisms are considered to be present almost everywhere and textile materials could easily provide a

better and conducive environment for multiplication of pathogenic microbes to thrive. Presence of microorganisms on textile materials among other things can cause discoloration, develop stains, break yarns, cause smell and loss of tear and strength (Ijaz, *et al.*, 2020). Since most plants are source of natural herbs, they have the ability to produce many natural antibacterial due to the presence of certain bioactive compounds such as phenols, saponins, tannins, terpenoids etc in them. Antibacterial materials were reported by Mirjalili and Karim, (2013) to have the ability to eliminate or reduce the spread of disease and prevent deterioration of textiles. The use of natural colourants from plants as a means of producing antimicrobial agents has been documented (Alam *et al.*, 2022). Several researchers (Sutrisna *et al.*, 2020; Vaghela and Upasai, 2023; Oforghor *et al.*, 2023) have reported their findings on extraction, characterization and fastness properties of dyes extracted from different bio sources.

Nature has blessed Nigeria with many green plants from where dyes could be extracted. Among these is the *Justicia carnea* plant. The genus *Justicia*, belongs to the large family of *Acanthaceae* consisting of about 600 species distributed in both hemisphere in the tropics and temperate regions and widely used traditionally as blood tonic for years past in Nigeria (Okocha *et al.*, 2023). *Justicia carnea* is a flowering plant widely distributed in various parts of Africa especially Nigeria. The shrubs of *Justicia carnea* are grown around homesteads as fence (Onochie *et al.*, 2020). The plant is called “Ogwu obara” and “Ewe eje” meaning blood leaf in Igbo and Yoruba speaking parts of Nigeria respectively. Some tribes in Nigeria have given it names like “Hospital too far”, “Blood of Jesus”, “Blood leaf” and Jehovah Witness leaf” (Okocha *et al.*, 2023).

Traditionally, several species of *Justicia* have been



reported to be used in the management of many health conditions. Investigation by the authors from local users of the leaves revealed that most anaemic patients, pregnant and women that have just completed their menstrual cycle use the leaf extract obtained either by soaking or boiling in water to replenish their blood. Ajuru *et al.*, (2022) and Orjiakor *et al.*, (2019) reported that its application as blood booster has not been certified and without chemical tests.

The aim of the research work was to evaluate the performance of dyes extracted from *Justicia carnea* leaf in cotton fabric dyeing in order to explore the possibilities of obtaining better colours using different mordants and mordanting processes and also to determine the antimicrobial activity of the dyed cotton fabrics against two common pathogenic bacteria (*Bacillus spp* and *Streptococcus*) and a fungi (*Candida albican*) with a view to get a cheap, safe, fascinating and easily available colourants for the expansion of dyeing industries.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample collection

*Justicia carnea* was collected from Molasuru area in Ore, Odigbo Local Government area, Ondo State, Nigeria. The plant leaves were identified in Biology Laboratory of Osun State Polytechnic, Iree, Nigeria by a herbarium staff as *Justicia carnea* with voucher number 0143

### 2.2 Samples pretreatment

The fresh leaves were washed and divided into two parts. One part was air dried at room temperature for 7 days and pulverized using a locally made laboratory grinding machine. The powdered sample was stored in airtight containers until used while dye was extracted from the other part immediately.



**Before drying****After drying****Plate 1: Fresh and dried *Justicia carnea* leaf samples****2.0 MATERIALS AND METHODS**

The cotton fabric was purchased in Alamisi market Ikorun, Ifelodun Local Government Area of Osun State, Nigeria while the chemicals (lime and cobalt chloride) used were of analytical grade.

**3.1 Methods****3.1.1 Extraction of dye from the samples**

Aqueous extraction of dye from fresh and dried leaves of *Justicia carnea* was done by weighing 8 g each of the samples in different 500 ml beaker. 400 ml of distilled water was added and the beakers were heated in water bath for 1 h at temperature of 80 °C. After extraction, the solutions were allowed to cool and thereafter filtered separately using muslin cloth and stored for dyeing process.

**3.1.2 Phytochemical analysis**

The phytochemical screening of the extracted dyes was determined using standard methods of Wanyama *et al.*, (2011) and Divya and Ravi, (2013).

**3.1.3 UV-Vis spectroscopy analysis**

1 ml of the total aqueous dye extract was diluted in 100 ml of distilled water. An aliquot was introduced in a 1 cm path way quartz cell and analyzed in Philip Harris spectrophotometer. A scan from 300 to 600 nm was performed in order to generate the characteristics absorption spectra of the sample.

**Fourier transform infrared spectral analysis**

5 mg of the dye extracts were mixed thoroughly with 195 mg of KBr until homogenized in an agate mortar. The mixture was placed into the sample compartment of the Fourier Transform Infrared Spectrophotometer (PerkinElmer system 2000 FTIR spectrophotometer) where the transmission spectra of all powder samples in the wave number range 400-4000 cm<sup>-1</sup> were collected at a resolution of 4 cm<sup>-1</sup> with 100 scans for each spectrum.

**3.0 RESULTS AND DISCUSSION**

The phytochemical screening (Table 1) revealed the presence of flavonoids, steroids, phenols, anthraquinones, tannins and alkaloids. Phenols and alkaloids were found to be significantly present in the fresh sample while only anthraquinones provided similar results with dried sample. Anthraquinones, flavonoids and tannins were moderately present in fresh samples relative to dried sample where they were fairly

**Mordanting and dyeing processes**

Using an analytical weighing scale, 2 g of the pre-mordanting mixture - cobalt chloride and lime were put into a 250 ml beaker containing 50 ml of water along with the cotton fabrics. The beaker was then placed in a water bath for 20 minutes. Following the mordanting process, the materials were taken out and 100 ml of each extracted dye sample was measured, poured into the beaker containing the mordanted fabrics, and the beaker was submerged in water for 30 minutes. The fabric was dyed, rinsed with water and allowed to air dry. For the dye that was removed from the dried sample, the process was repeated. In post-mordanting, the materials were mordanted after dyeing, whereas in simultaneous mordanting, the fabric, mordants, and dye extracts were all combined in the beaker utilizing the same method as previously described.

**Antibacterial and antifungal analysis**

Bacterial (*Bacillus* and *Streptococcus*) and fungi (*Candida albican*) species used for the tests were obtained from department of Applied Science, Osun State Polytechnic, Iree, Nigeria. Agar disc diffusion method was used where 0.1 ml of 1 in 100 dilutions of the overnight broth culture of each bacterium and fungi cells was added to sterile molten nutrient agar for bacteria and potatoes dextrose agar for fungi cell was maintained at 37° C. The plates were allowed to solidify, then 40 g/ml and 80 g/ml of aqueous extract of the *Justicia carnea* were added into appropriate wells (7 mm diameter) made in sealed plate which were incubated at 37° C for 24 hr.

**Colour fastness**

Dyed fabrics were evaluated for different colour fastness properties according to ISO standards such as ISO 105-B02 for perspiration (alkaline and acidic) and ISO 105 X 12 for rubbing (wet and dry) (Avinc *et al.*, 2013).

present. These bioactive components have been reported in the leaf extract of *Justicia carnea* (Ajuru *et al.*, 2022; Asiwe *et al.*, 2023; Andrew *et al.*, 2024) and these contributes to various physiologic and protective effects attributed to the plant (Shomali *et al.*, 2022). The presence of flavonoids and tannins among other phytochemicals are considered to be important characters for textile dyes (Sohini and Shyamala, 2017) to enhance better fastness. Flavonoids are the largest

group of plant phenols that are capable of providing flavour and colour to fruits and vegetables. This may explain the strong flavour and deep red colour despite

the greenish nature of *J. carnea* leaves when boiled for consumption (Orjiakor *et al.*, 2019).

**Table 1: Results of Phytochemical Screening for Bioactive Components in the Dye Extracts**

Parameter	Fresh	Dried	
Anthraquinones	++	+++	Steroids
	+	+	
Alkaloids		+++	Flavonoids
	++	++	
Phenols		+++	
Tannins	++	+	

**Key:**

+++ denotes significantly present

++ denotes moderately present

+ denotes present

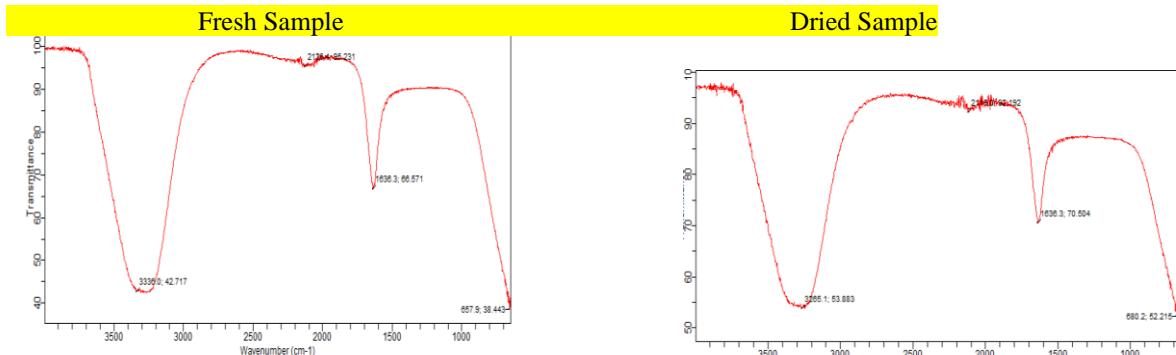
**Table 2: Antibacterial and Antifungal Activities of the Dye Extracts**

Organisms	Fresh	Dried
Bacteria		
<i>Bacillus</i> sp.	4 mm	10 mm
<i>Streptococcus</i> sp.	8 mm	6 mm
Fungi		
<i>Candida albican</i>	8 mm	Resistant

The antimicrobial sensitivity test (Table 2) of the aqueous extract of fresh and dried leaves of *Justicia carnea* showed (4 mm and 8 mm) and (10 mm and 6 mm) zones of inhibition for *Bacillus* spp and *Streptococcus* spp respectively. This indicates that *bacillus* and *streptococcus* spp are averagely resistant to dye extract from fresh leaves but only resistant to *streptococcus* spp using dye extract from dried leaves which means it is susceptible to *bacillus* spp. The sensitivity test against *Candida albican* (Fungi) showed 8 mm zone of inhibition against the extract from fresh leaves while it was completely resistant against dye extract from dried leaves. The difference in resistance of these microorganisms could be related to differences in their cell membranes (Abu-Zaid *et al.*, 2021). The results obtained indicate the presence of bioactive ingredients which are considered to serve as

antimicrobial agent as the tested organism related diseases are inhibited with extract from *Justicia carnea* leaves. Antimicrobial properties of dye extracts from bio sources have been reported (Imohiosen, 2023; Asiwe, *et al.*, 2023) and the mechanism of this behaviour among others could be attributed to hydrogen-bonding and hydrophobic reactions between the polyphenols from bio source and protein of the bacterial cell wall causing the leakage of cell fluids and ultimately killing the bacterial (Shafiq *et al.*, 2021). Tannins which are naturally astringent in nature along flavonoids and other bioactive compounds have been reported to have antibacterial property (Orjiakor *et al.*, 2019) which is an expected characteristic of dyed textiles in order to prevent damage to the surface of the fabric and prolong shelf life.





**Fig 1: The results of UV (up) and FTIR (down) spectroscopy analysis of dye extracts from fresh and dried leaves of *J. carnea* indicating important peaks and functional groups related to color formation.**

The absorption peaks of the UV analysis of the extracts from the fresh and dried leaves were shown on Fig 1. A broad band at 600 nm indicates weak absorbing species that resulted in low absorbance of 0.25 that can be attributed to the  $-C=C-$ . An absorbance of 0.68 at 300 nm substantiates the presence of low chromophoric OH component due to the absence of conjugation in the structure as revealed in the IR spectrum. Other spectrum values at 0.68, 0.9 and 1.2 showed that extracted dyes are mixtures of different compounds which may include phytochemicals such as tannins, phenols and flavonoids. The peaks within the range 200 -300 nm are related to tannin compounds while the peak at 320 nm and above indicate flavonoids derivatives.

The results of IR Spectra of dye extracts from the fresh and dried leaves of *J. carnea* also on (Figs 1) revealed identical spectra with two distinctive stretching vibrations each. The two dye extracts showed vibrations

at  $3300\text{ cm}^{-1}$  which is a broad absorption attributable to free  $-OH$  of hydrogen bonded alcohol. The second absorption at  $1638\text{ cm}^{-1}$  is a sharp stretching vibration that characterized aromatic  $-C=C-$  unsaturated group and  $C=O$  group.. The absorption at about  $3300\text{ cm}^{-1}$  is a bond stretching peak for  $N-H$ . While absorption between  $2200-2300\text{ cm}^{-1}$  indicated  $C-N$  or  $C-C$  triple bond. These functional groups,  $C=O$ ,  $C=C$ ,  $C-N$ ,  $OH$  are found in alkanoid compounds present in *J. carnea* such as Hordenine, Cytisine; flavonoids (which usually contained carbonyl and hydroxyl group) such as Isoquercetin, Rutin, Hesperidin and phenols such as ferulic acid (Obazeh, 2023). The spectral revealed a degree of purity owing to the absence of background interference absorptions as a result of impurity. The presence of these functional groups in the dye extracts which are electron rich species when in conjugation are very good chromophores and can enhance its application in dyeing of fabrics.

**Table 3: Rubbing Fastness of Dyed Cotton Fabrics using Selected Mordants and Mordanting ethods**

Mordanting Method	Mordant	Rubbing			
		Wet	Fresh	Wet	Dried
		Wet	Dry	Wet	Dry
PRM	Lime	3	3	3	3
SIM	Lime	4	3	3	3
POM	Lime	3	3	3	3
PRM	Cobalt Chloride	3	3	3	4

SIM	Cobalt Chloride	4	4	4	4
POM	Cobalt Chloride	4	3	4	3
	NM	1	2	2	2

Scales for the Tables (3 & 4): 5- excellent, 4- very good, 3- good, 2- moderate, 1- poor

Key:

PRM – pre mordanting

POM - post mordanting

SIM – simultaneous mordanting

NM - no mordanting

The results of fastness property due to rubbing (wet and dry) and perspiration (alkali and acid) were presented on Tables 3 and 4 which revealed that fastness due to perspiration and rubbing were improved by mordants and mordanting processes. The improved fastness of mordanted cotton fabrics (metallic and bio - source) has been reported (Asiagwu *et al.*, 2021) and could be explained in term of the fact that the dye was able to form complex with the fibre.

SIM process was found to produce better perspiration and rubbing fastness results than the POM and PRM processes. The rubbing fastness of the dyed cotton fabrics with lime and cobalt chloride as mordants and SIM process are presented on Table 3. The values of 4 (wet) and 3 (dry) were obtained for fabric dyed with extract from fresh leaf while a value of 4 was obtained for both rubbing fastness conditions (wet and dry) with dye extract from dried leaf. From these results, wet rubbing fastness gave better results for both dye extracts and may not require any after treatment (Jain, 2017). Chaudhari *et al.*, (2019) however reported overall good dry rubbing fastness properties for natural dyes

extracted from catechu plant. Rub fastness is a process related to the unfixed dye deposit on the fabric surface which could be removed easily by washing and this implies that the method of washing whether intensive or otherwise will affect the rubbing fastness performance.

Using SIM and lime as mordant, the fastness due to perspiration (Table 4) of dyed cotton fabric using fresh dye extract was 4 (very good) and 3/4 (good/ very good) in alkaline and acidic conditions respectively while it was 4 (very good) in both conditions with dye extract from dried leaf. With SIM and cobalt chloride as mordant, the perspiration fastness value of 4 (very good) was obtained for alkaline and acidic conditions with dye extract from fresh leaf while it was 4 -4/5 (very good and very good/excellent) with extract from dried leaf. The synthetic mordant (cobalt chloride) and natural mordants (lime) exhibit similar rubbing and perspiration fastness which differs from the submission of Alam *et al.*, (2022) that synthetic mordanted fabric has less colour fastness to rubbing than bio- mordanted fabric.

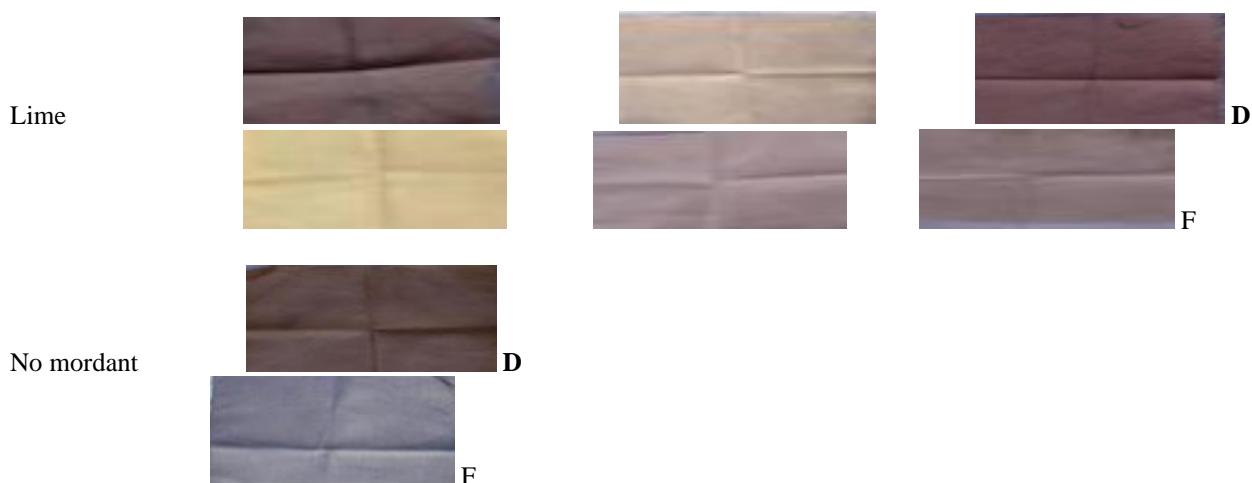
**Table 4: Perspiration Fastness of Dyed Cotton Fabrics using Selected Mordants and Mordanting Methods**

Mordanting Method	Mordant	Perspiration			
		Fresh		Dried	
		Acid	Alkali	Acid	Alkali
PRM	Lime	3	1	2	2
SIM	Lime	3/4	4	4	4
POM	Lime	3	3	3	2
PRM3	Cobalt Chloride	4	3/4	3	3
SIM 3	Cobalt Chloride	4	4	4/5	4
	Cobalt Chloride	3	3	3	3
	NM	1	1	1	1

Cobalt Chloride  
D



F



**Plate 2: Cotton fabrics dyed with aqueous dye extracts of *J. carnea* using different mordants and mordanting methods where F and D designate Fresh and dried leaves respectively.**

From plate 2, it was observed that cotton fabrics could be dyed directly from the extracted dyes without mordants but more variety shades of colours were obtained using chemical and natural mordants. This is in line with the work of Kumar *et al.*, (2019) and Alam *et al.*, (2022) that reported different colours and shades with the use of mordants. The influence of mordanting techniques on colour formation despite using the same mordant was also established. The ability of the extracted dyes to produce colours with the cotton fabric without mordants could be attributed to the presence of bioactive polyphenols in the dyes that acts as natural mordants to sustain colouring matters (Win *et al.*, 2020). These polyphenolic compounds especially tannins are naturally occurring water soluble materials containing phenolic hydroxyl group which enable them to form effective crosslink's (Win *et al.*, 2020) and in this case between the dye and fiber.

#### Conclusion

The aqueous extracts from fresh and dried leaves of *J. carnea* produced different colours on the cotton fabric with the help of mordants. This result indicates the presence of bioactive ingredients which serves as antimicrobial agent against microorganism as the tested organism related diseases were inhibited with the *Justicia carnea* dye extracts. The mordants and mordanting methods produced better choice for cotton fabric dyeing because of the improvement in fastness properties. SIM process was found to produce better perspiration and rubbing fastness results than the POM and PRM processes.

Conflict of interest: None

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