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PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF ETHANOL EXTRACTS OF SIX DYE PLANTS

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ABSTRACT

Ethanol extracts of six dye plants viz. *Pterocarpus erinaceus, Zingiber officinale, Zanthoxylum zanthoxyloides, Morinda lucida, Bixa orellana* and *Sorghum caudatum,* were screened for secondary metabolites and antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The ethanol extracts of *Z. zanthoxyloides, Z. officinale* and *M. lucida* were able to inhibit the growth of the three indicator bacteria with the zones of inhibition between 0.50-11.6mm. *P. erinaceous* inhibited *Staphylococcus aureus* and *E. coli; B. orellana* inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa* while *S. caudatum* inhibited *Pseudomonas aeruginosa* only. All the dye extracts show the presence of at least three secondary metabolites, and these might be respondible for their antibacterial properties and their usefulness as medicinal plants. It may also enhance the stability of the plants colurants as added additives to polymer substrates.

INTRODUCTION

Trees and shrubs are the source of many products beside timber. Their extracts have been used to combat several diseases[1]. Africans from earlier times had learned to extract natural colours from trees and shrubs for a number of end uses such as cosmetics and small scale textile handicraft.

Bixa orellana popularly called "Annatto" of the family *Bixaceae* is a tropical plant of a small tree or shrub. The seed yielded the orange dye used for colouring foodstuffs, soaps and various fabrics[2]. *Morinda lucida,* family *Rubiaceae*, is a medium sized tree grown in tropical Africa. Its wood produce a yellow dye of high molar absorptivity[3]. *Zanthoxylum zanthoxyloides* belongs to the family *Rutaceae* and its stem/root bark yields red dyes. The colouring potential of the dye on cotton fabric had been reported[4]. *Zingiber officinale* (Ginger) is a perennial tropical herb with underground branching stems called rhizomes. It belongs to the *Zingiberaceae* family. The rhizomes yield the yellow dye which had been characterised[5]. Popoola *et al.,* had found the colourant useful in textile and non textile applications. *Pterocarpus erinaceous* belong to the family *Papilionaceae.* It is one of the various species of red wood known as Barwood[7]. *P. erinaceous* is the common species in Western Nigeria and is popularly known as African Rose wood. The wood also yields a red dye. *Sorghum caudatum* is of the most important cereals after rice and maize in the tropics. It belongs to the family *Gramineae* and its leaves yields a red dye[7].

The present work reports the phytochemical and antibacterial properties of these six dye plants' extracts.

MATERIALS AND METHODS

Source & Extraction

The plants part (Table 1) were purchased at 'Oja Oba' market at Akure, Ondo State and identified. The samples were cleansed, dried in an oven at 105°C for 6hr and pulverised. Each sample was extracted at a solute-solvent ratio of 1:25 for 6hr in a Soxhlet extractor.

Phytochemical screening

The extracts were evaluated for the presence of alkaloids, glycosides, reducing sugars, saponins, tannins, flavonoids and phlobatanins[8].

Table 1 : Dye Plants evaluated for phytochemical and antimicrobial activity

Dye Plants	Parts used
Morinda lucida	Bark
Sorghum caudatum	Leaves/Stem
Bixa orellana	Seed
Pterocarpus erinaceous	Heartwood
Zingiber officinale	Rhizome
Zanthoxylum zanthoxyloide	Bark

Alkaloids

About 0.2g of the extracts were warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragencloff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Glycosides

The extract was hydrolysed with dilute HCl solution and neutralized with sodium hydroxide solution. A drop of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

Reducing sugars

The extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for two minutes. An orange red precipitate indicates the presence of reducing sugars.

Saponins

About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Flavonoids

Extract (0.2 g) was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Phlobatanins

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of Phlobatanins.

Antibacterial Screening

Preparation of Medium

Nutrient Agar (LAB M) used for the antagonistic test was prepared according to Manufacturer's instruction and sterilized at 121°C for 15 minutes.

Indicator Bacteria

Stock culture of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Table 2 : Inhibition of bacterial growth by the dye plants extracts
Zone of Inhibition (mm)

Indicator bacteria	*M. lucida*	*S. caudatum*	*B. orellana*	*P. erinaceous*	*Z.officinale*	*Z. zanthoxyloide*
S. aureus	9.0	NI	9.1	1.2	2.2	11.6
E. coli	4.0	NI	NI	2.1	3.1	10.20
P. aeruginosa	10.9	10.0	8.9	NI	3.6	0.5

NI : No Inhibition

Table 3 : Phytochemical analysis of the ethanol extract of the dye plants

Dye plants	Alkaloids	Glycosides	Reducing sugars	Saponins	Tannins	Flavonoids	Phlot-anins
M. lucida	+	+	+	+	+	+	-
S. caudatum	-	+	+	+	-	-	+
B. orellana	-	+	+	+	-	-	-
P. erinaceous	+	+	+	+	-	-	-
Z. officinale	-	+	+	+	+	+	-
Z. zanthoxyloide	+	+	+	+	+	+	+

+ Present & - Absent

were obtained from the Microbiology Department, Federal University of Technology, Akure. The cultures were maintained throughout the duration of the work on agar slant.

Antibacterial Assay

The agar well diffusion for antibacterial test described by Schillinger and Lucke was adopted[9]. Overnight broth culture of the indicator bacteria were used to seed agar before pouring into plates. This was done in triplicate for each of the indicator bacteria. Two wells were made on the seeded agar plate with the aid of sterile cork borer of diameter 12mm. One well which contain sterile ethanol serves as control while the other was filled with the ethanol extract of the plant.

RESULTS AND DISCUSSION

The antibacterial assay showed that the ethanol extract of *Z. zanthoxyloides, Zingiber officinale* and *Morinda lucida* were able to inhibit the growth of the three indicator bacteria with the zone of inhibition between 0.50-11.6mm (Table 2). The extract of *Sorghum caudatum, Bixa orellana* and *Pterocarpus erinaceous* could not inhibit all the bacteria. Extract of *Zanthoxylum zanthoxyloides* was found to be more active against *Staphylococcus aureus* with a zone of inhibition of 11.6mm.

The result of the phytochemical screening reveals that the dye extracts contain either two or one of tannins and flavonoids. This may be responsible for their antibacterial properties[10]. Pamplona Roger[11] had earlier reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections.

In addition, inhibitory properties of these dye extracts may also enhance the stability of these plants colourants as added additives to polymer substrate.

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