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Original Research Article

PHARMACOGNOSTIC STANDARDIZATION AND ANTIMICROBIAL EVALUATION OF *BYRSOCARPUS COCCINEUS* SCHUM. AND THONN. (CONNARACEAE)

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ABSTRACT

The plant *Byrsocarpus coccineus* Schum and Thonn is used traditionally for treating bacterial and fungal illnesses. This study investigated the ethnobotanical use of *B. coccineus*, pharmacognostic standard procedures and the antimicrobial properties of the leaf. A semi-structured questionnaire was employed, following the Logit model. Microscopy, macroscopy, physical constants, phytochemical screening, thin layer chromatography and antimicrobial activities (using the agar diffusion and micro broth dilution assays on *Bacillus subtilis, Staphylococcus aureus,* Multi resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans and Tricophytum rubrum*) were evaluated. The results showed that the ethnobotanical uses include treatment of typhoid, men impotency, gonorrhoea, dysentery, cancer and urinary problems (highest with 30%). Microscopy showed the presence of jigsaw-shaped epidermal cells with numerous anomocytic stomata. Phytochemical screening revealed the presence of carbohydrates, glycosides, alkaloids, saponins, tannins, flavonoids and terpenoids, but steroids were absent. Eight (8) spots were observed for the *n*-hexane and methanol extract development on TLC in a solvent mixture of *n*-hexane: ethyl acetate (4:1). All microorganisms were susceptible to *n*-hexane extract at all serial dilutions (12.5 -50 mg/mL), highest susceptibility to *B. subtilis* at the 16 mm inhibition zone with gentamicin (positive control) at 16 mm inhibition zone as well. Also found was the lowest minimum inhibitory activity (MIC) on *P. aeruginosa* (0.1953 mg/mL) and minimum bactericidal concentration (MBC) of 12.5 mg/mL, 6.25 mg/ml and 12.5 mg/mL against *S. aureus*, *B. subtilis* and *E. coli*, respectively, with gentamicin (positive control) having 5 µg/mL, 5 µg/mL and 10 µg/mL, in that order. The aqueous extract showed the highest susceptibility on *S. aureus*, *E. coli* and *P. aeruginosa*, the lowest MIC against *B. subtilis* (0.78125 mg/mL) and MBC against *T. rubrum* (12.5 mg/mL). The plant extract showed good antibacterial and antifungal activity and thus can be explored as a new antimicrobial agent or source for new drug discovery.

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INTRODUCTION

The use of medicinal plants in therapeutics has grown significantly in the past few decades due to the increasing interest in natural substances, medicinal plants still play a significant role in primary healthcare today for over 80% of the people living in impoverished communities in developing nations [1-3]. Standardization is an important measure for ensuring the quality, purity and authenticity of crude drugs. It

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entails a number of procedures that uncover and compile a collection of innate, peculiar qualities, such as precise, qualitative and quantitative values or particular, distinctive features, on the basis of which analogous herbal medications that are purportedly identical can be contrasted for authenticity, efficacy, purity, repeatability and general quality assurance [4].

Byrsocarpus coccineus Schum. and Thonn. (Connaraceae) is a common plant in West and Tropical Africa. A scandent shrub of secondary jungle and savanna thickets, it has exquisite pink-tinged foliage and sweet-smelling flowers [5][6]. It local names in Nigeria include “tsaamiyar-kasa” (Hausa, North), “oke abolo” (Igbo, East), and “orikoteni” (Yoruba, Southwest) [5]. The plant's leaves have been used as an abortifacient, for skin and oral conditions, German measles, jaundice, gonorrhoea, urinary issues, impotence, anaemia, primary and secondary sterility, blennorrhagia and tachycardia. [5]. The plant has also been used as an emetic, for bleeding, and for swellings and tumours. Previous research examined and documented the plant's leaf aqueous extract's *in vivo* analgesic, antidiarrhoeal, antipyretic, and anxiolytic/sedative properties [5,6].

This study evaluated the pharmacognostic standardization and antimicrobial activity of *Byrsocarpus coccineus* leaves as mentioned in ethnomedicine.

METHODS

Ethnobotanical Survey

Study Area

The investigation was carried out at Itoku herbal medicine market in Abeokuta, Ogun State, Nigeria (7.1555° N, 3.3420° E). Although the Egba were the first residents of the old city, the Yoruba now make up the majority ethnic group in the region. In the region, there are dialectal groups, such as Ijebu, Egba, Remo, Oyo (Owu), Awori, Ikale and Ilaje. The folks are well-known for their traditional sculpture and carving. Abeokuta has a population of roughly a million, and it is encircled by a sizable pile of rocks.

Materials and Method of Data Collection

The study used a questionnaire, a camera, and a recorder to collect data from the Itoku herbal market, a major market for traditional medicine sellers in the capital city. The primary data were collected through semi-structured interviews with 50 respondents, including traditional medicine sellers and buyers of medicinal herbs. The interviews were conducted in the native language to prevent information distortion and to improve understanding. The questionnaire provided demographic information, as well as identification and knowledge of the medicinal use of *B. coccineus*.

Pharmacognostic Standard Procedure

Plant Collection

The *Byrsocarpus coccineus* Schum. and Thonn (Connaraceae) plant was collected at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The plant was identified and authenticated by Mr Egunjobi at Forest Herbarium, Ibadan (FHI), with voucher number 113723. A duplicate of the verified plant sample was placed at the University of Ibadan's Department of Pharmacognosy Herbarium (DPHUI).

Macroscopy and Microscopy

The macroscopic and microscopic evaluations followed standard procedures [8-10]. The process of examining the epidermal layers of the leaf involved scraping the abaxial surface to obtain a translucent layer, then clearing the surface with a 5% sodium hypochlorite solution, rinsing and staining with Safranin O briefly. The layers were then transferred into 50%, 70% & 100% ethanol and mounted with glycerol. By cutting through the midrib, a piece of lamina and a thin segment that had been bleached and stained were retrieved, together with the transverse section of the leaf. Clear cameras were used to take photomicrographs. After mounting the powdered material in various reagents, cellular diagnostic and diagnostic cell inclusions were seen. Additionally, quantitative microscopy of the leaf was carried out [9,10].

Fluorescence Analysis for the Qualitative Detection of *B. coccineus* Leaf

After being processed with various solvents, the powdered sample of *Bysocarpus coccineus* leaf was examined under three different light settings: natural daylight, short ultraviolet (254 nm), and long ultraviolet (365 nm). The samples were created using the following solvents: distilled water, 50% H_2SO_4, 50% HCl, 50% acetic acid and ferric chloride [10].

Physico-chemical Analysis

The physicochemical examination of the powdered leaf was carried out in accordance with WHO recommendations [9]. With the aid of the loss on drying/weight difference method, the contents of ash and moisture were ascertained. By measuring the crucible's weight loss and its contents upon lighting, the fibre content was measured. Also measured were the extractive value, moisture content, total ash, water-soluble ash and acid-insoluble ash.

Ash Value Determination

Total Ash

A nickel crucible that had been tarred was heated to 600ºC for approximately 15 minutes in a muffle furnace. After cooling it for an hour in a desiccator, the crucible was weighed to determine its starting weight (W1). The weights of the crucible and sample were then determined after five grams of the powdered material was added to the nickel crucible and heated slowly until all of the moisture had been removed from the plant material and it was completely burned (W2). At 600ºC, the sample turned grey as the heat was gradually increased until the carbon had evaporated and the residue had been liberated from the carbon (white ash). With the use of crucible tongs, the crucible was taken out, allowed

to cool in a desiccator, and then reweighed (W3). The proportion of ash content was calculated using Eqn 1.

$$\% TA = \frac{(W3) - (W1)}{(W2) - (W1))} \times 100$$ ----------Eqn 1

where:
TA = Total Ash
W2 =Weight of sample & crucible
W1 = Initial weight of crucible
W3 = Final weight of crucible

Water Insoluble Ash Determination
After emptying the crucible's contents into a beaker, 25 mL of water was added, and the mixture was allowed to boil for five minutes. After passing the combination through ashless filter paper, the residue and the filter paper were both baked to dryness. The residue-containing ashless filter paper was crushed into the crucible and heated to 600ºC until the ashless paper was removed. After weighing the crucible again (W3), the discrepancies were computed as follows (Eqn 2 and Eqn 3):

$$\% WIA = \frac{(W2 - W1)}{W3} X\ 100$$ ----------Eqn 2

$$\% WSA = \% TA - \% WIA$$ ----------Eqn. 3

where:
WIA = Water Insoluble Ash
WSA = Water Soluble Ash
TA = Total Ash
W2 = Weight of sample & crucible
W1 = Initial weight of crucible
W3 = Final weight of crucible

Acid Insoluble Ash
After being separated from the entire ash, the ash was put into a beaker with 25 mL of diluted hydrochloric acid and allowed to boil for five minutes. Next, the insoluble material was gathered using ash-free filter paper and a sintered crucible. Hot water was used to rinse the crucible and beaker through the filter paper several times until there was no more acid present. After being placed in a crucible, the filter paper was burned in a muffle furnace at 600 ºC until it was carbon-free. After being cooled in a desiccator, the crucible and its contents were weighed. The air-dried material was used as a reference to compute the percentage of acid-insoluble ash using Eqn 4:

$$\% AIA = \frac{AIAW}{WS} X\ 100$$ ----------Eqn. 4

where:
AIA = Acid Insoluble Ash
AIAW = Acid Insoluble Ash Weight
WS = Weight of Sample

Determination of Extractive Values
Alcohol Soluble Extractive Value
Five grams of the leaf material was precisely weighed, and added to a conical flask with a cork, and the flask's cap was securely replaced after 100 mL of 90% alcohol was poured. After allowing the flask and its contents to macerate further for 18 hours, the mixture was mechanically agitated for approximately 72 hours before filtering. Following the collection and drying of the filtrate, the residue was dried to a consistent weight at 105ºC in the oven. Percentage ash (% Ash) was calculated using Eqn. 5:

$$\% ASH = \frac{(WA) - (WC)}{(WC\ \ WS) - (WC)} \times 100$$ ----------Eqn. 5

where:
WA = Ashed Weight
WC = Crucible Weight
WC + WS = Crucible weight + Sample Weight

Water Soluble Extractive Value
After precisely weighing five grams of the leaf material, it was added to a stoppered conical flask. After adding 100 millilitres of chloroform-water, the conical flask's cork was securely reinstalled. After giving the flask and its contents a 6-hour mechanical shake and letting them macerate for 18 hours, the mixture was mechanically agitated for approximately 72 hours before filtering. After gathering the filtrate and evaporating it until it was dry, the residue was dried at 105ºC to a consistent weight was calculated using Eqn. 6:

$$\% WSE = \frac{WE}{WS} \times 100$$ ----------Eqn. 6

where:
WSE = Water Soluble Extractive
WE = Weight of Extract
WS = Weight of Sample

***n*-hexane Extractive Value**
After precisely weighing five grams of the leaf material, 100 mL of *n*-hexane was poured into it, and the conical flask's cap was securely reinstalled. After being manually shaken for around six hours, the flask's contents were left to macerate for eighteen hours before being filtered. After gathering the filtrate and evaporating it until it was dry, the residue was baked at 105ºC until it reached a consistent weight. Three duplicates of each extraction value were completed was calculated using Eqn. 7:

$$\% HSE - = \frac{WE}{WS} \times 100$$ ----------Eqn. 7
where:
HSE = n-hexane Soluble Extractive
WE = Weight of Extract
WS = Weight of Sample

Determination of Moisture Content

Weighing a warmed, tarred porcelain crucible with a cover allowed us to record its weight (W1). After adding two grams of the dry sample to the crucible and reweighing it (W2), the sample was heated to 650°C for 12 hours, with 6-, 3-, and 1-hour intervals until the weight remained constant. After cooling it in a desiccator, the sample was weighed again. W3 and the constant weight were observed. The following relationship (Eqn. 8) was used to compute the moisture percentage:

$$\% MC = (W2) - \frac{(W3)}{(W2)} - (W1) \times 100$$ ----------Eqn. 8

where:
MC = Moisture Content
W1 = Weight of crucible
W2 = Weight of Sample in crucible
W3 = Constant Weight
W2-W1 = weight of sample
W2-W3 = weight of moisture in the Sample

Determination of Dry Matter

Dry matter was calculated by weighing the fresh weight, which was air-dried and then weighed using a sensitive scale after the weight constancy had been carried out according to WHO guidelines [9].

The dry matter was calculated as shown below (Eqn 9 and Eqn 10):

$$\%MC = \frac{FW-DW}{FW} \times 100$$ ----------Eqn. 9

$$\%DM = 100 - \%MC$$ ----------Eqn.10

where:
MC = Moisture content
FW = Fresh weight
DW = Dry weight
DM = Dry Matter

Qualitative Phytochemical Analysis of the Crude Extract

With the aid of standard protocols, a qualitative phytochemical test was conducted to identify the presence of several secondary metabolites in the crude extract [11-13].

Thin Layer Chromatographic (TLC) Fingerprint Profile

Through the use of TLC pre-coated plates 60F254, 7X6 cm (Merck, Germany), thin-layer chromatography was performed on all samples with a one-way ascending approach. Before the huge TLC plate was cut to the proper size with scissors and marked with a pencil around 1 centimetre from the bottom of the plate, it was first activated in the oven at a high temperature for a while. Every *Byrsocarpus coccineus* extract sample was ready. Aqueous crude extract was dissolved in 1 mL of methanol: water solvent in a ratio of 7:3; 0.01 g alcohol extract was dissolved in 1 mL of methanol; and 0.01 g of the n-hexane extract was dissolved in 1 mL of *n*-hexane solvent. The dissolved samples were evenly applied to the plates using capillary tubes, and they were then left to dry. Several solvent solutions were used in a chromatographic tank to create the plates. The produced TLC plate was allowed to air-dry before being seen under a UV lamp set to 365 nm (long wavelength) and 254 nm (short wavelength). A picture of the chromatograph was taken.

The R_f (Retardation) value for each spot was calculated using the following formula using Eqn 11:

$$Rf = \frac{a}{b}$$ ----------Eqn. 11

where a = the distance between the point of application and the centre of the spot being examined; b = the distance between the point of application and the solvent front.

Antimicrobial Assay

Preparation of Plant Extract

The plant extracts were made at three different quantities (50 mg/mL, 25 mg/mL and 12.5 mg/mL), using the serial dilution method. To make a total volume of 3 ml per extract preparation, 0.15 g of the plant extract was dissolved in 1.5 mL of dimethyl sulfoxide and 1.5 mL of sterile water to create the stock solution. This procedure was done for the two fractions of the plant extract (water, methanol) for the *n*-hexane extract. Owing to the low yield, the stock solution was prepared with 0.1 g of extract diluted in 1 mL of diethyl acetate and 1 mL of sterile water to make a total volume of 2 mL [14].

Preparation of Microorganisms for Antimicrobial Screening

Clinical isolates (*S. aureus*- ATCC29523, *E. coli*-ATCC29522, *P. aeruginosa*-ATCC27853 *K. pneumoniae*-ATCC700603 from the Department of Medical Microbiology, University College Hospital, Ibadan; and *E. coli* ATCC 35218, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633, *Candida spp.* and *T. aeruginosa*, clinical stains from the Department of Pharmaceutical Microbiology, University of Ibadan) were subcultured and incubated on nutrient agar for 24 hours at 37°C. There was no control organism for MRSA and the *Candida spp.* microorganisms. This was diluted further to 10^{-3} of sterile distilled water after which it was inoculated using a special spread plate method.

Microorganisms Used

Two non-resistant strains and one resistant strain of Gram-positive (*Staphylococcus aureus and Bacillus subtilis*) were collected from the Department of Pharmaceutical Microbiology, University of Ibadan and the Department of Medical Microbiology, University College Hospital (UCH), Ibadan. Three non-resistant strains of Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) were collected from the Department of Pharmaceutical Microbiology, University of Ibadan and the

Department of Medical Microbiology, University College Hospital (UCH), Ibadan.

Antimicrobial Sensitivity Test (AST)

Antimicrobial sensitivity testing or antimicrobial susceptibility testing was done using the Agar Diffusion method [15,16]. The plant extract was given to the microorganisms in varying concentrations. It assessed how big the regions free of bacterial growth were.

In this process, 20 mL of sterilized Muller Hilton Agar (MHA) was aseptically poured into sterile petri plates. The agar was allowed to be set for around 10 minutes, after which the petri dishes holding the agar were dried in a hot air oven to eliminate moisture from the cover of the plates and from the surface of the agar, using a swab. Following this, wells were drilled into the infected agar, using a cock borer with a 6 mm diameter that had been flame-broken and left to cool. The various plant extract concentrations that had previously been made were put into the bored wells along with the proper labels. Gentamicin (10 µg/mL) and ketoconazole (1%) were the positive controls in assay I, and they were each put in a separate bored well. After allowing the extracts to properly diffuse for approximately an hour, the plates were incubated for a full day at 37°C. The zone of inhibition was determined for every extract concentration and positive control after 24 hours [15,16].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Procedure

Microbial Assay I

Through the use of the micro broth dilution test, the extracts' MIC was ascertained. Mueller Hilton Broth (MHB) was used to create a stock solution of 50 mg/mL from the two-fold dilution of the plant extracts. This stock solution was then serially diluted to get 25 mg/mL and 12.5 mg/mL. In particular, the MIC was determined by making further serial dilutions from 12.5 mg/ml to 6.25 mg/mL, 3.125 mg/mL, 1.5625 mg/mL 0.78125 mg/mL, 0.39062 mg/mL and 0.1953 mg/mL. Ten µL of standardized inoculum was added to every well, and 100 µL of each extract concentration was added to the wells holding 100 µL of MHB. Gentamicin and ketoconazole were used as the positive control, whereas MHB and the inoculum alone constituted the negative control well. For twenty-four hours, the plates were incubated at 37°C.

After twenty-four hours, the turbidity of the wells was assessed, and the minimum inhibitory concentration (MIC) was determined by mixing 10 µL of p-iodonitrotetrazolium chloride, containing 0.2 mg/mL, with the contents of the wells. When live bacterial cells were present, the colour changed to pink. The MIC was determined to be the lowest concentration at which no growth occurred. Mueller Hilton Agar was infected with a loop full of the inoculum from the wells that did not exhibit any growth, and it was then incubated for 24 hours at 37°C. The MBC was determined to be the lowest concentration that exhibited no growth.

Microbial Assay II

The plant extracts were diluted twice using Mueller Hilton Broth (NHB) to create a stock solution of 50 mg/mL. This solution was then serially diluted to yield 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL and 1.562 mg/mL, using the micro broth dilution assay. Ten microlitres of a standardized inoculum was put to each well, and 100 µL of each extract concentration was added to the wells holding 100 µL of MHB. The positive control consisted of ceftriaxone 10 µg, amoxiclav 10 µg and fluconazole 25 mg, whereas the negative control consisted of the well containing just MHB and the inoculum.

Method of Data Analysis

Descriptive statistics, like mean, percentages and frequency, were used to examine the data. The socioeconomic aspects impacting the general public's understanding of the use of *B. coccineus* in the research region were also investigated using the logit model.

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RESULTS

Socio-Economic Factors Influencing General Knowledge on Use of *B. coccineus*

The study revealed that age had a negative impact on the general understanding of *B. coccineus* use in the study region, but gender, years of experience, marital status, and knowledge had a favorable impact. These socio-economic factors were among the respondents (Table 1, 2).

Uses of *B. coccineus* Plant by Respondents in the Study Area

Table 3 reveals that 78% of the respondents were not aware that *B. coccineus* can be used for the cure of cancer. Table 2 shows other uses of *B. coccineus* by the respondents, which include urinary problems (30%), typhoid (20%), men impotency (16%), gonorrhoea (6%), and dysentery (14%). The part used however varies as 18% use leaves alone, 2% use the stems and branches, 12% use the roots, 28% use whole plants, 38% use the leaves and roots and 2% use the leaves, stems and the root (Table 4).

Macroscopy and Microscopy

Macroscopy and microscopy have been used to identify the diagnostic value of *Byrsocarpus coccineus*. The plant is a scandent or climbing shrub with petiolate leaves (1 -2 cm) having an average length and width of 1 – 3 cm. The leaves were seen to be smooth, whole, dark green, and arranged sub oppositely. The leaf surface is areolate, and veins are

Table 1: Socio-economic characteristics of respondents

Variables	Frequency	Percentage (%)
Age		
21-30	6	12.0
31-40	9	18.0
41-50	19	38.0
51-60	12	24.0
>60	4	8.0
Gender		
Female	32	64.0
Male	18	36.0
Marital status		
Single	5	10.0
Married	36	72.0
Divorced/Separated	5	10.0
Widow(er)	4	8.0
Experience		
1 – 5	5	10.0
6 – 10	10	20.0
11 – 15	11	22.0
16 – 20	9	18.0
>20	15	30.0

Table 2: Socio-economic factors influencing general knowledge on the use of *B. coccineus*

Variables	Co-efficient	Standard error	T-ratio	Marginal effect
Sex	0.12662	0.74017	0.17	0.02105
Age	-0.05088	0.05714	-0.89	-0.00837
Experience	0.08097	0.06545	1.24	0.01332
Marital status	0.65912	0.79955	0.82	0.10274
Knowledge	0.13666	0.73906	0.18	0.02252
Constant	-0.94775	1.8611	-0.51	
Number of observation	50			
LR chi^2 (5)	2.32			
Prob> Chi^2	0.8031			
Pseudo R^2	0.0441			
Log likelihood	-25.18452 ***			

****Coefficients significant at p<0.001, **Coefficients significant at 5%, *Coefficients significant at 10%*

Table 3: Medicinal uses of *B. coccineus*

S/N	Ailment	Frequency	Percentage (%)
1	Gonorrhea	3	6.0
2	Male impotency	8	16.0
3	Jaundice	7	14.0
4	Urinary problem	15	30.0
5	Typhoid	10	20.0
6	Cancer	11	22.0
7	Dysentery	7	14.0

Table 4: Parts of *B. coccineus* used by respondents in the study area

S/N	Part used	Frequency	Percentage (%)
1	Leaves	9	18.0
2	Stem/branch	1	2.0
3	Root	6	12.0
4	Whole plant	14	28.0
5	Leaf and root	19	38.0
6	Leaf, stem and root	1	2.0

Table 5: Macroscopy of *B. coccineus* Leaf

Macroscopy	*B. coccineus* leaf
Habit	Scandent
Leaf Apex	Rectuse
Leaf Base	Oblique
Leaf Margin	Entire
Leaf Shape	Orbicular/Oval
Leaf Colour	Dark-green
Leaf Surface texture	Glabrous
Leaf Arrangement on Stem	Imparipinnate, sub opposite
Leaf Venation	Reticulate
Leaf Petiole	Petiolate (sub sessile)
Petiole length	1-2 cm
Leaf width	2 -3cm
Leaf length	2 -5 cm
Stipule	Stipulate

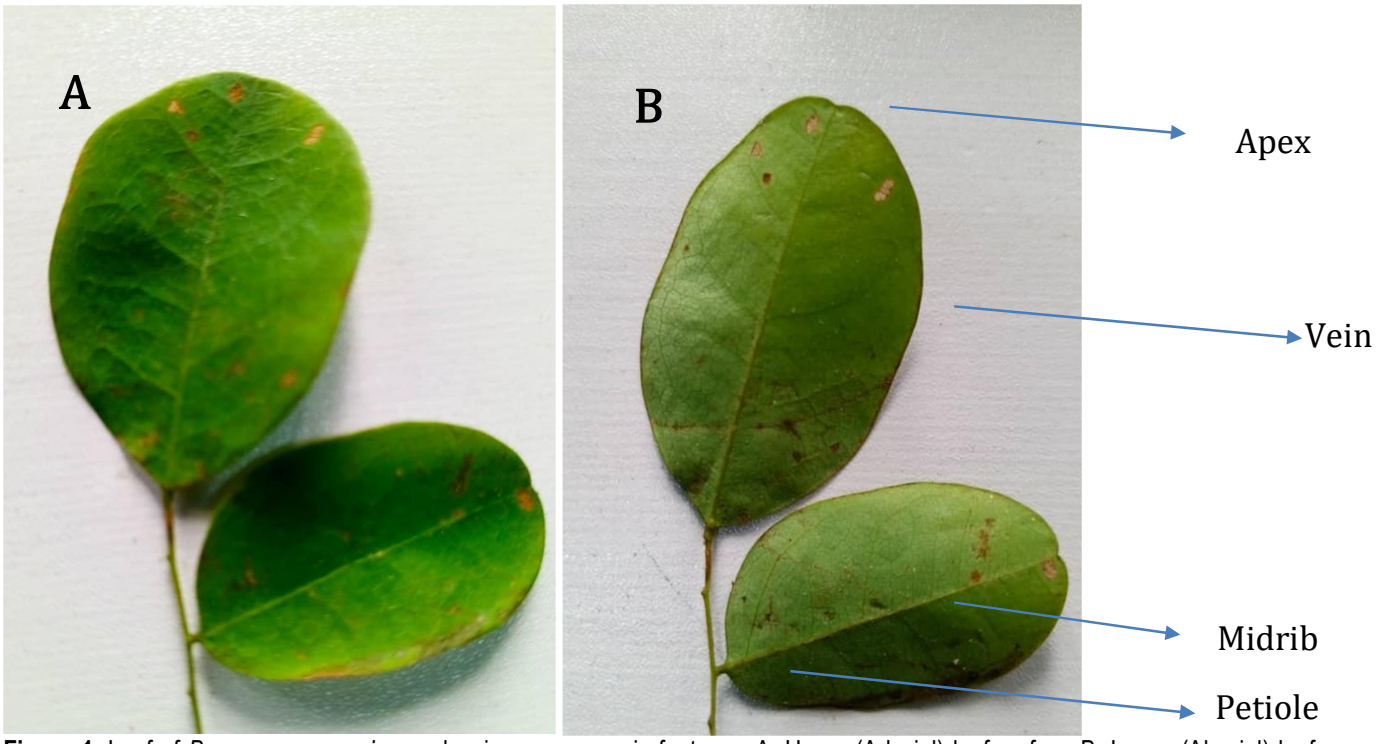

Figure 1: Leaf of *Byrsocarpus coccineus* showing macroscopic features. A. Upper (Adaxial) leaf surface B. Lower (Abaxial) leaf surface

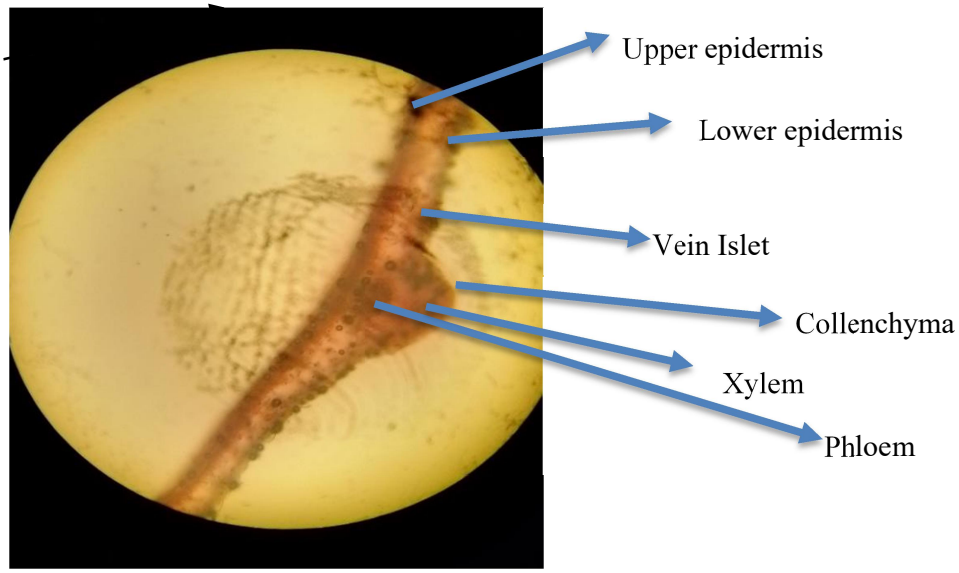

Figure 2: Photomicrograph of *Byrsocarpus coccineus* showing the epidermal features of the transverse leaf section at X100 magnification

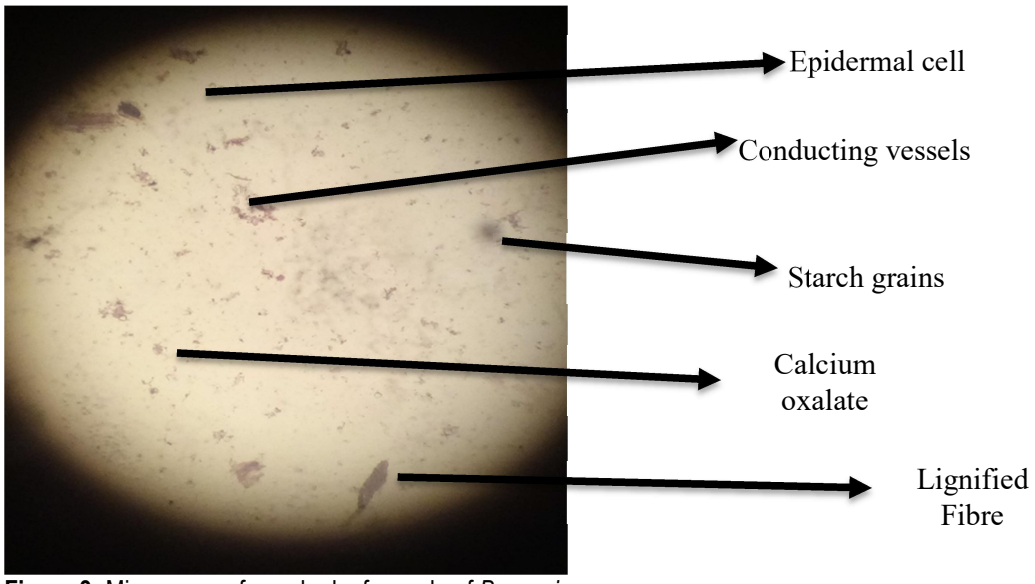

Figure 3: Microscopy of powder leaf sample of *B. coccineus*

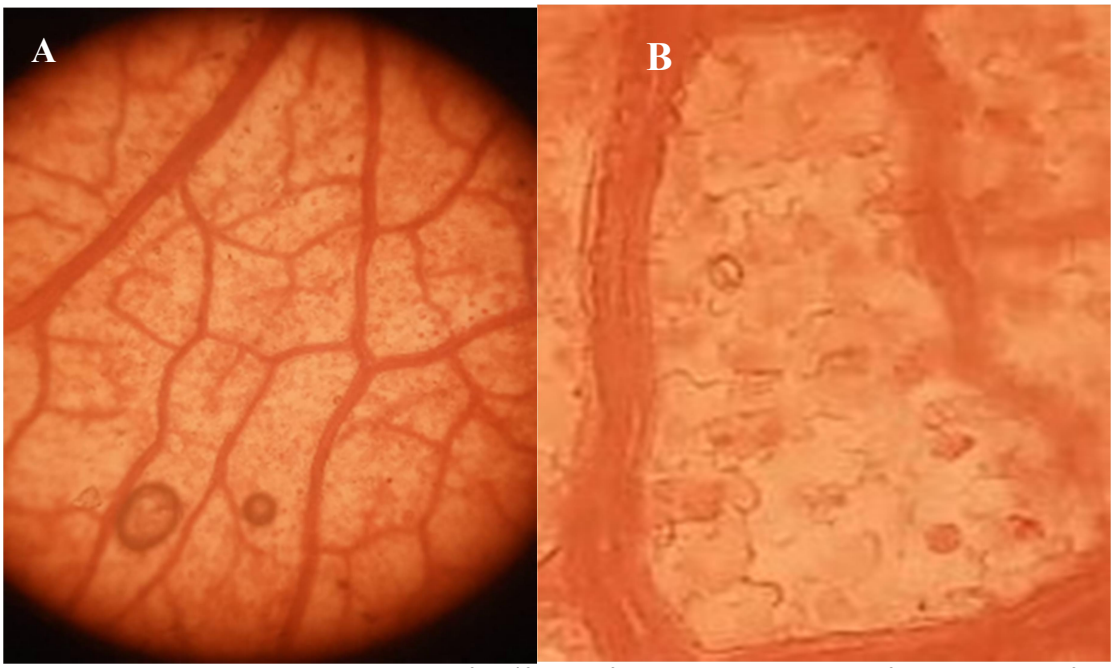

Figure 4: A: Adaxial view *B. coccineus* leaf × 40 magnification. **B**: Adaxial view of the epidermis of *B. coccineus* showing numerous stomata

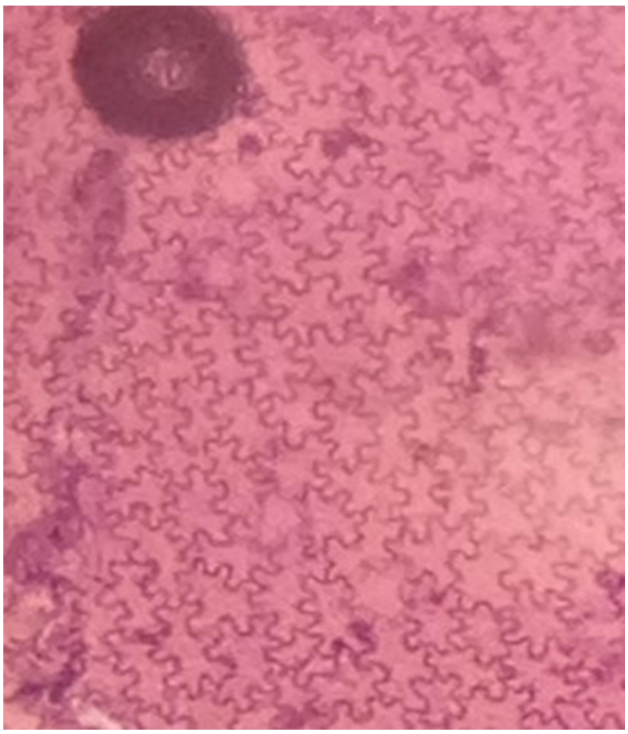

Figure 5: Abaxial view of *B. coccineus* leaf showing the jigsaw epidermal cells x100 magnification

Table 6: -Physical constants for the leaf of *B. coccineus*

Test	Amount of sample used (g)	Average yield (g)	Value (%)
Total ash	5	0.285± 0.02	5.70 ± 0.01
Water soluble ash	0.285	0.055± 0.02	1.11 ± 0.01
Acid insoluble ash	0.285	0.227± 0.04	4.54 ± 0.02
Alcohol soluble extract	5	0.352± 0.08	7.05 ± 0.06
n-hexane soluble extract	5	0.053± 0.09	1.05 ± 0.06
Water soluble extract	5	0.574± 0.13	11.48 ± 0.11
Moisture content	5	0.119± 0.06	5.95 ± 0.03

Table 7: Phytochemical tests on leaf powder.

Tests (leaf powder)	Results
Carbohydrate	+
Glycoside	+
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids	-
Terpenoids	+
Gums and mucilage	+

Key: (-): Absent: (+): Present

Table 8: Fluorescence Analysis

Powdered Leaf Sample	Daylight	UV (254 nm)	UV (365 nm)
Acetic acid	Cream	Olive green	Black (no fluorescence)
Ferric chloride	Dark brown	Dark green	Deep violet fluorescence
50% HCl	Light brown	Olive green	Deep violet fluorescence
50% H_2SO_4	Olive green	Army green	Black (no fluorescence)

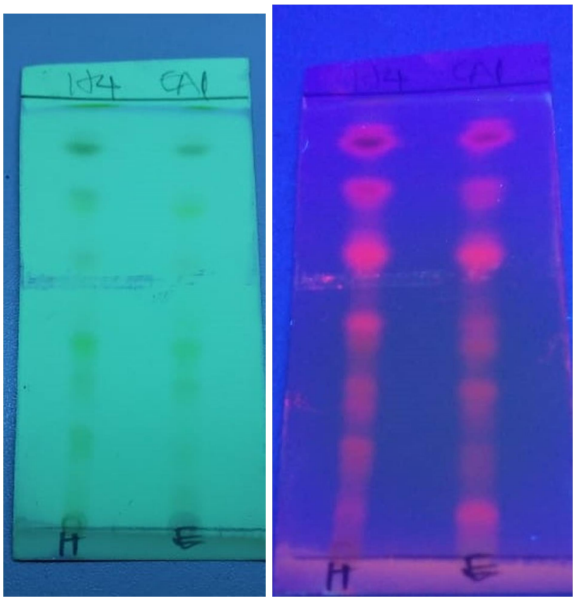

Figure 6: The TLC Plate for the *n*-hexane extract and alcohol extract at 254nm and 365nm (Using *n*-hexane 4:1 Ethyl acetate solvent system)

Table 9: Visualization of alcohol extract on TLC Plate

TLC Spot	R_F Values	Daylight	254 nm	365 nm	Vanillin/H_2SO_4 Spray	Dragendorff Spray	Inference
A1	0.11	Yellow	Yellow	Pink	Red	Yellow	amines, aldehydes, ketones, steroids terpenoids present
A2	0.19	Black	Black	Pink	Red	Yellow	amines, aldehydes, ketones, steroids terpenoids present
A3	0.28	Green	Green	Pink	Red	Light yellow	amines, aldehydes, ketones, steroids terpenoids present
A4	0.31	Yellow	Yellow	Pink	White	Light yellow	amines, aldehydes, ketones, steroids terpenoids present
A5	0.37	Yellow	Yellow	Pink	White	Red	Alkaloids present
A6	0.46	Green	Green	Red	White	Red	Alkaloids present
A7	0.61	Yellow	Green	Red	White	Fuschia	Compound unknown
A8	0.76	Green	Green	Red	white	Red Violet	Compound unknown

Table 10: Visualization of *n*-hexane extract on TLC

TLC Spot	R_F Values	Daylight Colour	254 nm Colour	365 nm Colour	Vanillin/H_2SO_4 Spray	Dragendorff Spray	Inference
H1	0.11	Yellow	Yellow	Pink	Purple	Light Yellow	Terpenoids present
H2	0.19	Black	Black	Pink	Purple	Light Yellow	Terpenoids present
H3	0.28	Green	Green	Pink	Purple	Light yellow	Terpenoids present
H4	0.32	Yellow	Yellow	Pink	Purple	Light yellow	Terpenoids present
H5	0.39	Yellow	Yellow	Pink	Pink	Red	Phenols, Alkaloids present
H6	0.50	Green	Green	Red	Red	Red	amines, aldehydes, ketones, steroids Alkaloids present
H7	0.63	Yellow	Green	Red	White	Fuchsia	Compound unknown
H8	0.78	Green	Green	Red	Red	Violet.	amines, aldehydes, ketones, steroids present

Table 11: Antimicrobial Susceptibility Test (AST) I

Sample code/ Conc	*n*-Hexane			Methanol			Aqueous			Control Positive	Control Negative
	50	25	12.5	50	25	12.5	50	25	12.5	GENT	KET
	Mg/ml									[10 µg/mL]	[1%]
S. a	14	10	8	14	12	8	20	16	10	16	NA
B. s	16	12	8	12	10	8	12	10	8	16	NA
E .c	12	10	8	14	12	10	16	12	0	18	NA
Ps. a	12	8	8	12	10	8	16	12	10	0	NA
C .a	14	10	10	14	12	10	12	0	0	NA	18
T. r	12	10	10	0	0	0	10	8	0	NA	0

Code: CONC: Concentration, S. a: Staphylococcus aureus, B. s: Bacillus subtilis, E. c: Escherichia coli, Ps. a: Pseudomonas aeruginosa, C. a: Candida albicans, T. r: Tricophytum rubrum, GENT: Gentamicin, KET: - Ketoconazole

Table 12: Antimicrobial Sensitivity Testing (AST) II

Sample code	Aqueous extract (Inhibition)			Methanol extract (Inhibition)			*n*-Hexane extract (Inhibition)			CRO	AMC	FLU
	50	25	12.5	50	25	12.5	50	25	12.5	10	10	25
					mg/ml					µg/mL	µg/mL	mg/ml
E.c	12 mm	0	0	0	0	0	0	0	0	17 mm	0	NA
S.a	12 mm	8 mm	0	0	0	0	0	0	0	25 mm	0	NA
MRSA	10 mm	9 mm	0	0	0	0	0	0	0	0	0	NA
Ps.a	11 mm	0	0	0	0	0	0	0	0	0	0	NA
C.a 1	25 mm	20 mm	18 mm	0	0	0	0	0	0	NA	NA	20 mm
C.a 2	30 mm	24 mm	20 mm	0	0	0	0	0	0	NA	NA	21 mm

KEYS: *E. c: Escherichia coli, Ps. a: Pseudomonas aeruginosa, S. a: Staphylococcus aureus, MRSA: Multi resistant Staphylococcus aureus, C. a 1: Candida albicans strain 1, C. a 2: Candida albicans strain 2, CRO: Ceftriaxone, AMC: Amoxiclav, FLU: Fluconazole, 0: no diffusion or no inhibition or Microbial Resistance, NA: Not applicable, mm: millimeter*

Table 13: **MIC and MBC in Microbroth Dilution Assay I**

Sample Code	*n*-Hexane mg/mL		Methanol mg/mL		Aqueous mg/mL		Broth Only	Broth And Oil	Broth and Isolates	GENT (µg/mL)		KET (%)	
	MIC	MBC	MIC	MBC	MIC	MBC				MIC	MBC	MIC	MBC
S. a	6.25	12.5	1.5625	25	25	25	-	-	+	5	5	NA	NA
B .s	1.5625	6.25	3.125	6.25	0.78125	12.5	-	-	+	5	10	NA	NA
E. c	1.5625	12.5	0.1953	25	0.1953	12.5	-	-	+	10	10	NA	NA
Ps. A	0.1953	25	1.5625	12.5	0.1953	25	-	-	+	>10	>10	NA	NA
C.a	1.5625	12.5	0.1953	3.125	1.5625	3.125	-	-	+	NA	NA	1%	1%
T. r	6.25	25	1.5625	25	0.3906	12.5	-	-	+	NA	NA	0.25%	5%

Key: *- CONC-: Concentration, Sa: - Staphylococcus aureus, B.s: - Bacillus subtilis, E. c: - Escherichia coli, Ps. a: -- Pseudomonas aeruginosa, Ca: - Candida albicans, C. a1: - Candida albicans strain 1, C.a 2: - Candida albicans strain 2, T. r: - Tricophytum rubrum, GENT: - Gentamicin, KET: - Ketoconazole. NA: - not applicable*

Table 14: MIC AND MBC in micro broth dilution assay II

Microorganism (Sample code)	Aqueous extract (mg/mL)	
	MIC	MBC
E. c	25	50
S. a	3.13	25
MRSA	3.13	25
Ps. A	6.3	50
K. p	25	25
C .a 1	1.6	3.13
C. a 2	1.6	3.13

Key: *S. a: Staphylococcus aureus, MRSA: Multi resistant Staphylococcus aureus, E. c: Escherichia coli, Ps. a: Pseudomonas aeruginosa, K. p: Klebsiella pneumoniae, C. a1: Candida albicans strain 1, C.a 2: Candida albicans strain 2.*

visible on the surface. The leaf is orbicular to oval in shape, rounded at the apex and the base and has an entire margin, wet appearance. It has a spicy aroma, making it known as thyme leaf. Microscopic evaluation of the leaf includes aspects such as collenchyma, vascular bundles, xylem, phloem, palisade cells, and upper and lower epidermal cells.

The powder sample showed lignified vessels, calcium oxalate, starch grains, and conducting vessels. The adaxial layer of *B. coccineus* has reticulate venation, and the epidermal cells show numerous stomata. The adaxial layer has a profound jigsaw arrangement of epidermal cells, while the lower epidermal abaxial layer shows numerous stomata

within the reticulate venation of the leaf. The jigsaw-shaped epidermal cells are more clearly seen.

Quantitative Microscopy

The quantitative microscopic evaluation showed that the stomatal index (per mm^2) and palisade ratio (per mm^2) of the upper layer of the leaf of *B. coccineus* are 23.01 ± 0.40 mm^2 and 13.98 ± 0.64 mm^2 respectively. In the *Byrsocarpus coccineus* leaves, trichomes are absent. Thus, we have a smooth leaf surface (adaxial and abaxial).

DISCUSSION

The study reveals that there were more females in the sales of herbal materials in the study areas (Table 1). This is in agreement with Patrick et al. [18] who found out that the female folk dominated the trade in trado-medicinal ingredients. Among the socio-economic characteristics of the respondents, gender, years of experience, marital status, and knowledge were found to have a positive effect on the general knowledge on the use of *B. coccineus* while age has a negative effect on the general knowledge on the use of *B. coccineus* in the study area (Table 2) which is in agreement with the findings carried out by Ikenna et al. [19]. The logit model is used to determine the socio-economic factors influencing the general knowledge of the use of *B. coccineus* in the study area which was significant at $p \leq 0.01$. Sex, years of experience, marital status, and knowledge have been discovered to have a positive effect on the general knowledge of the use of *B. coccineus* while age harms the general knowledge of the use of *B. coccineus* (Table 2). Table 3 reveals that 78% of the respondents were not aware that *B. coccineus* can be used for the cure of cancer. Table 2 shows other uses of *B. coccineus* by the respondents, which include urinary problems (30%), typhoid (20%), men impotency (16%), gonorrhoea (6%), and dysentery (14%). The part used however varies as 18% use leaves alone, 2% use the stems and branches, 12% use the roots, 28% use whole plants, 38% use the leaves and roots and 2% use the leaves, stems and the root (Table 4). The study also revealed the use of *B. coccineus* in the treatment of cancer (Table 3). This is in accordance the findings of Odewo et.al. [20] that also discovered that *B. coccineus* can be used for the treatment of prostate enlargement and tumor through the process of fermentation. From the survey carried out, the use of *B. coccineus* for the treatment of dysentery, urinary tract infection, gonorrhea and jaundice collaborates with the findings of Sunday et. al [21] that reported that *B. coccineus* can be used for the cure of jaundice, pile, gonorrhea, impotence, dysentery and urinary problems (Table 3). The different parts of *B. coccineus* were used in ethnomedicine according to the survey carried out (Table 4).

Physicochemical analysis is a method of evaluating and expressing the overall composition or value of an herbal sample [18]. The fresh sample of *B. coccineus* was easily identified by all respondents; this might be because fresh plant samples still retain their color, and fruit or flowers can also be identified easily [20] (Table 5). Macroscopy and microscopy are used to identify the diagnostic value of *B. coccineus*. The plant is a scandent or climbing shrub with petiolate leaves (1 -2 cm) having an average length and width of 1 – 3 cm. The leaves were observed to be dark green, entire, and smooth with a sub-opposite arrangement. The leaf surface is areolate, and veins are visible on the surface. The leaf is orbicular to oval in shape, rounded at the apex and base, and has an entire margin, wet appearance. It has a spicy aroma, making it known as thyme leaf. The results of the macroscopic evaluation of the leaf of *B. coccineus* (Figure 1) were similar to the findings of Ikenna et al. [19]. Microscopic evaluation of the leaf includes aspects such as collenchyma, vascular bundles, xylem, phloem, palisade cells, and upper and lower epidermal cells. The powder sample (Figure 2 and 3) showed lignified vessels, calcium oxalate, starch grains, and conducting vessels. The adaxial layer of *B. coccineus* has reticulate venation, and the epidermal cells show numerous stomata. The adaxial layer has a profound jigsaw arrangement of epidermal cells, while the lower epidermal abaxial layer shows numerous stomata within the reticulate venation of the leaf. In the *B. coccineus* leaves, trichomes are absent. Thus, there is a smooth leaf surface (adaxial and abaxial) (Figure 4A and 4B). In Figure 5 at x1000 magnification, the stomata show anomocytic arrangement with guard cells and chloroplast cells present. The jigsaw-shaped epidermal cells are more clearly seen. The microscopical study show the presence of stomata on the lower surface of the leaf which is of similar stomata index and palisade ratio values. In Figures 2 and 3, trichomes are confirmed absent in the leaf resulting in a smooth surface of the leaf which agrees with the study carried out by Watts and Kariyat [22].

Further evaluation shows that *B. coccineus* leaf has numerous anomocytic stomata on the adaxial (Figure 4A), and epidermal cells with straight to wavy walls, jigsaw puzzle in shape). Epidermal cells, vein islets, vessel element, fibre, cellulose, starch, lignin, mucilage and vascular bundles are present in the leaf (Figure 2 and 3) which agrees with Ikenna et al. [21]. The quantitative microscopic evaluation shows that the stomatal index (per mm2) and palisade ratio (per mm2) of the upper layer of the leaf of *B. coccineus* are 23.01 ± 0.40 mm2 and 13.98 ± 0.64 mm2 respectively. Table 6 expresses the physical constants for *B. coccineus* which shows that the ash values obtained (Eqn 1, 2, 3, and 4) from this study fall within the required specifications [9]. The n-hexane extract has a low yield at 1.05 ± 0.06 % (Eqn 7). This may be due to its non-polar nature it will extract more of the lipophilic, non-polar metabolites in the crude extract. Thus, we may infer that there are very few lipophilic compounds in the plant. The water-soluble extract yield at 11.48 ± 0.11% (Eqn 6) and methanol extract at 7.05± 0.06 % (Eqn 5) yield show that there are more polar compounds or secondary metabolites in the plant sample. This agrees with the research carried out by Musa et al. [23]. The moisture content is low at 5.95 ± 0.03 %

(Eqn. 8, 9, and 10) which is preferable for crude drugs as it helps the stability of crude samples [24].

Table 7 indicates the presence of various compounds such as carbohydrates, glycosides, saponins, tannins flavonoids, and terpenoids in agreement with Ahmadu et al. [5]. Alkaloids are present in this study, and this is in contrast to their findings. The phytochemical tests on the powder leaf sample indicate the presence of carbohydrates, glycosides, alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, gums and mucilage. Steroids are absent.

Table 8 which indicates the fluorescence analysis show different color changes at different wavelengths which may be indicative of fluorescent compounds as reported by Gabor et al. [25].

Tables 9, 10, and Figure 6 show the presence of specific secondary compounds like amines, ketones, steroids using the visualization of the n-hexane and alcohol extract on the TLC plates utilizing different wavelengths under different sprays using the specified solvent system (n-hexane 4:1 ethyl acetate) with retardation factors calculated using equation 11.

The antimicrobial sensitivity test (AST) determines a microbe's vulnerability to antimicrobial drugs by exposing a standardized concentration of organism to specific concentrations of antimicrobial drugs. Susceptibility testing can be done for bacteria, fungi, and viruses. This term is used when microbes such as bacteria and fungi are unable to grow in the presence of one or more antimicrobial drugs [14]. Results of AST are essential for the selection of definitive antimicrobial chemotherapy for bacterial infections diagnosed in individual patients and to know the drugs the strains are resistant to. minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions. The difference between MIC (Minimum Inhibition Concentration) and MBC is that MIC is defined as the lowest concentration of antimicrobial or drug that will inhibit the visible growth of bacteria after overnight incubation, while MBC is the lowest concentration of antibacterial agent required to kill a particular bacterium. The MIC test is done by the use of broth dilution assay [17]. The antimicrobial sensitivity test I (Table 11) show that the aqueous extract at 50 mg/mL has the highest antimicrobial activity against *B. subtilis* at 20 mm zone of inhibition.

Table 12 shows the antimicrobial sensitivity test II which revealed that the alcohol and n-hexane extract did not diffuse nor show activity on the agar. This may be due to the following factors: the concentration gradient, membrane permeability, temperature, pressure, surface area of the membrane, or pH differences [26].

Table 13 evaluates the highest inhibitory action of the aqueous plant extract which was against *C. albicans* at the lowest concentration of 1.56 µg/mL and lowest fungicidal concentration of 3.13 mg. This further elucidates that the plant *B. coccineus* has antimicrobial properties and even stronger antifungal activity [5]. For Gram-positive bacteria, the lowest MIC of 0.78 µg/mL in the aqueous extract and MBC of 6.25 µg/mL were obtained in the n-hexane and methanol extract of *B. coccineus* against *B. subtillis*, while for Gram-negative bacteria the lowest MIC of 0.20 µg/mL were obtained in both the aqueous extract against *E. coli* and *P. aeruginosa* and both n-hexane and aqueous extract against *P. aeruginosa*. The lowest MBC was 12.5 µg/mL in both hexane and aqueous extract against *E. coli* and methanol extract against *P. aeruginosa*. The aqueous extract has the lowest inhibitory concentration (MIC) against the fungi *T. rubrum* at 0.39 µg/mL while both the aqueous and methanol extract have the lowest fungicidal concentration (MBC) against *C. albicans* at 3.13 µg/mL. In the micro broth dilution assay II (Table 14), the aqueous extract has the lowest MIC of 3.13 µg/mL against both Gram-positive *S. aureus* and MRSA and MBC of 25 µg/mL. Tables 13 and 14 show that all the extracts of *B. coccineus* leaf showed anti-microbial activity against fungi, Gram-positive and Gram-negative microorganisms following the study of Ahmadu et al. [5] who confirmed the plant's antimicrobial activity against *E. coli* [5]. The lowest MIC for Gram negative organisms was 3.13 µg/mL against *P. aeruginosa* while the lowest MBC was against *K. pneumonia* at 25 µg/mL. It is important to note that the resistant strain of *S. aureus* (MRSA) is susceptible to the aqueous extract of the plant. This positive outcome is an indication to explore for treatment infections caused by resistant microorganisms.

CONCLUSION

Pharmacognostic standardization methods are crucial as they provide accurate and reproducible results, ensuring the reliability of results. By confirming the antimicrobial activity of *B. coccineus*, the study not only validates traditional knowledge but also opens possibilities for new antimicrobial agents or drug discovery from the plant. The ethno-survey highlighted the extensive use of *B. coccineus* in treating various ailments, suggesting its potential as an antimicrobial plant. The subsequent pharmacognostic evaluations and antimicrobial studies confirmed its efficacy, underscoring the importance of standardization procedures. This comprehensive approach not only justifies the ethnomedicinal uses of *B. coccineus* but also paves the way for future research and development of new antimicrobial agents derived from this plant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION
JOM designed the work; TKO carried out the study, TKO and TOA drafted the manuscript; JOM and TOA supervised the study; JOM and TOA revised the manuscript. All authors read, made comments, and agreed on the final manuscript.

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