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Research Paper

Therapeutic Evaluation of Methanolic Leaf and Stem Extracts of *Byrsocarpus Coccineus*

Christiana Jesumirhewe[1*], Raphael Nzubechukwu Raphael[2], Akibu Fajana[3]

[1]Department of Pharmaceutical Microbiology, College of Pharmacy, Igbinedion University Okada Edo state, Nigeria
[2]Department of Public Health, Glasgow Caledonian University Scotland, United Kingdom
[3] Department of Pharmacognosy, College of Pharmacy, Igbinedion University Okada Edo state, Nigeria

*Corresponding author: christiana.jesumirhewe@iuokada.edu.ng , +2348034648066

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Abstract—*Byrsocarpus coccineus* stem and leaves have been reported to have extensive ethno medicinal uses. The therapeutic evaluation of methanolic extracts obtained from the leaves and stem of *Byrsocarpus coccineus* was carried out to evaluate their antimicrobial potential and reported usefulness. The phytochemical screening of the powdered leaves and stem of *Byrsocarpus coccineus* were determined using previously described standard methods. The antimicrobial potency of the extracts on clinical isolates was determined using agar diffusion method. Phytochemical analysis of *Byrsocarpus coccineus* yielded important medicinal constituents which included glycosides, reducing sugars, tannins, alkaloids, saponins, phenols in both extracts. Result from the antimicrobial screening showed that the extract of the stem of *Byrsocarpus coccineus* had a significant inhibitory effect at 100mg/ml and 50mg/ml in comparison with the leaf extract. Gentamicin was used as a positive control. Results from this study validate the importance of *Byrsocarpus coccineus* especially stem extracts in the control of infections caused by microorganisms used in this study.

Keywords—*Byrsocarpus coccineus*, Phytochemical screening, Antimicrobial activity, antimicrobial susceptibility, methanolic extracts

1. Introduction

Since ancient times, plants have been the source of agents that have therapeutic potentials and up till today are important role in the basic health care system of a large percentage of the world's population [1]. In Nigeria, more than 70% of the estimated populations are rural dwellers, who depend entirely on traditional herbal medicines as a source of alternative therapy [2]. *Byrsocarpus coccineus* (Schum. and Thonn.) is a climbing plant found especially in west and central African countries including Nigeria [3]. *Byrsocarpus coccineus* has prominent and a large number of lenticules, pinnate leaves, 6-9 pairs of leaflets that are enlarged near rounded apex. Between January to March, *Byrsocarpus coccineus* usually has small white or pinkish scented flowers. *Brysocarpus coccineus* is referred to as "Tsamiyar kasa or kimbar maharba" by the Hausas in the Northern Nigeria. The Fulani people in the northern Nigeria call it "wangarabubi or yangara-bubihi", while the Bassange people also in the northern Nigeria call it "Kogi." In the southern Nigeria, the Yoruba people refer to *Brysocarpus coccineus* as "Oke abolo" or "Mybo-apepea" [4] while Kilba people in Adamawa State North eastern Nigeria refer to it as ''mblakiki''. A number of preparations of the plant using leaves, roots and whole plant have been useful as alternative therapeutic agents. In Nigeria a number of plants are available that have medicinal active agents which makes them useful alternative therapeutic agents in handling issues related to our health. Previously a number of research works have been carried out identifying a number of plant based bioactive compounds. Proper and extensive documentation is required in carrying out such works as this will affect the usefulness of such plants especially as drugs in African herbal medicine. In African herbal medicine, *Brysocarpus coccineus* has been used for the treatment of ailments including earache, gonorrhoea, impotency, jaundice, piles, diarrhoea, tumour and wounds [5, 6]. Identification of phytochemical constituents of pharmacological significance is important to determine their antimicrobial potentials and value as therapeutic agents. This present study seeks to evaluate the antimicrobial activity of stem and leaf extract of *Brysocarpus coccineus* and also validate its reported usefulness.

2. Related Work

The anxiolytic, sedative, anti-inflammatory, anti-proliferative, antidiarrheal, antimicrobial, anagelsic and uterotonic properties of various extracts of *Brysocarpus coccineus* have

also been previously reported [6, 7, 8, 9, 10, 11, 12, 13]., The presence of biologically active constituents that had effects comparable to oxytocin on the uterus have been detected in ethanol leaves extract of *Byrsocarpus coccineus* showing uterotonic potentials of the plant which could be harnessed for possible application in humans [7]. The presence of secondary metabolites of medicinal value such as flavonoids, saponins, tannins in extracts of *Byrsocarpus coccineus* has been linked with the antimicrobial potentials and activity of the plant [8].Most of the effects and activities previously demonstrated by extracts of *Byrsocarpus coccineus* showed a dose dependent effect as the higher the concentration of the extract used gave a better antagonistic effect [6, 13]. A previous study on the antioxidant and toxicological evaluation of ethanol extract from root bark of *Brysocarpus coccineus* also suggested it has a good antioxidant potentials with no assocated toxic signs or symptoms in acute and sub-chronic oral toxicity [14]. Several literatures reported the medicinal importance of *Byrsocarpus coccineus* that include therapeutic activities on the head, ear, mouth, skin, urogenital tract and blood [7, 8, 10, 15, 16]. Plants based extracts do not only have important phytochemical constituents but also have been reported to contain nutrients also that are important also for humans [17]

3. Methodology

Plant Identification and authentication
The fresh leaves and stem of *Byrsocarpus coccineus* (BC) were collected from Forest Research Institute of Nigeria {F.R.I.N} Ibadan, Oyo state. The plants were also authenticated at the Forest Research Institute of Nigeria {F.R.I.N} Ibadan, Oyo state. A voucher specimen of the plant with reference number FHI 112941 has been deposited in the FRIN herbarium for further reference. The fresh leaves and stem of *Byrsocarpus coccineus* were carefully selected and left to dry at room temperature after which they were grinded with a mechanical grinder to obtain coarse powder. The powders were stored in separate sterile air tight containers for extraction.

Extraction
25g of the leaf and stem of BC respectively were macerated in 250ml of methanol for 72 hours with occasional stirring during the period of the extraction. Extracts obtained after filtering and concentration on a water bath (70°C) were stored in sterile glass containers and refrigerated at 4°C till further used.

Phytochemical analysis
The presence of phytochemical constituents of medicinal value were determined as described by previously described standard methods with slight modifications [18, 19]
Test for Saponins

A volume of 0.5ml of the extracts was placed in a test tube and 5ml distilled water was added to it. The test tube was shaken vigorously. The presence of persistent frothing indicates a positive test for saponins.

Test for Flavonoids
A volume of 2ml of dilute ammonium solution was added to 2ml of each extract sample. The presence of yellowish color indicates a positive test for flavonoids.

Test for Glycoside and Reducing sugars
A volume of 1ml of Fehling solution A and 1ml of Fehling solution B was added to 2ml of each samples and heated for 15 minutes in a water bath. A brick red coloration indicates the presence of glycosides while a green coloration indicates the presence of reducing sugars.

Test for Alkaloids: Wagner's test
A volume of 2ml of Wagner's reagent was added to 2ml of each sample. A reddish-brown precipitate indicates a positive result for Alkaloids.

Dragendorff's test
A volume of 2ml Dragendorff's reagent was added to 2ml of each sample. An orange colored precipitate indicates a positive result for alkaloids.

Mayer's test
A volume of 2ml of Mayer's reagent was added to 2ml of each sample. A cream colored precipitate indicates a positive result for Alkaloids.

Test for Phlobotannins
A volume of 2ml of 1% HCL was added to the sample and heated in a water bath for 15 minutes. A red colored residue at the base of the test tube indicates the presence of phlobotannins.

Test for Phenolic compounds
A volume of 2ml of 1% ferric chloride was added to 2ml of each sample. A blue-black coloration indicates the presence of idoizable tannins while a brownish green coloration indicates the presence of condensed tannins.

Test for Terpenoids
A volume of 2ml of chloroform was added to 2ml of each sample and 1ml of concentrated sulphuric acid was gently introduced gradually unto the side of the test tube. A reddish-brown ring at the interface of the two liquids indicates the presence of terpenoids.

Micro-organism used
Gram positive and negative bacteria {*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*} from different sources {urine, swab and aspirate samples} were collected from the University of Benin Teaching Hospital, Benin City (UBTH) Edo state and used to evaluate the antimicrobial potency of the extracts. All the isolates used in the study were maintained on nutrient agar slants at 4^{O}C.

Preparation of Media, Extracts and Standards
All media were prepared according to the manufacturer's directions. For susceptibility testing, 0.5 g of the extracts was weighed into respective universal bottles. 5 mL of dimethylsulphoxide (DMSO) was added in the bottles to

dissolve the extracts giving a concentration of 100mg/ml as stock concentration. A two-fold serial dilution of the stock concentration was carried out to obtain the following concentrations: 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml. A volume of 1 mL of DMSO was introduced into 9mL of sterile distilled water to make a concentration of 10% DMSO which was used as the negative control. The positive control was prepared by firstly making 400 µg/mL of gentamicin. This was prepared by introducing 1 mL of gentamicin (80 mg/2mL) into 99 mL of sterile distilled water. A volume of 1 mL of the 400 µg/mL was introduced into 79 mL of sterile distilled water to make a final use concentration of 5µg/ml.

Evaluation of extracts for Antibacterial activity

The extracts were evaluated for antibacterial activity using the agar diffusion method with slight modifications [20]. A dilution of 10^{-2} was prepared for the isolates. 0.1 mL of the prepared dilution of each isolate was surface plated on pre-prepared Muller Hinton Agar plates using sterile swab sticks. Equidistant holes were made on the plates using a sterile cork borer {8 mm diameter}. A volume of 0.1 mL of the different concentrations including the positive and negative control were used to fill up the holes on the agar plates. The experiment was carried out in duplicates.

4. Results and Discussion

Table 1 summarizes the qualitative phytochemical analyses of *B. coccineus*. The extracts were found to contain constituents of medicinal value which included flavonoids, saponins, alkaloids, phlobatannins, glycoside and reducing sugar, condensed tannins, phenols in both extracts while terpenoids were not found in any of the extracts.

Table 1 Phytochemical constituent of *Brysocarpus coccineus*

	STEM	**LEAF**
Flavonoids	+	+
Saponin	+	+
Glycosides and reducing sugars	+	+
Alkaloids	+	+
Phlobatannins	+	+
Condensed tannins	+	+
Terpenoids	-	-
Phenols	+	+

Antibacterial Activity

Susceptibility of the tested clinical isolates to the extracts of *B. coccineus* is given in Table 2. The stem extract and the positive control (gentamicin) were found to have significant activity on the isolates especially at a concentration of 100mg/ml and 50mg/ml compared to the negative control (10% DMSO). The stem extract at lower concentrations (25mg/ml- 3.125mg/ml) were found to have no activity on the tested clinical isolates. The results showed that the stem extract had activity that was concentration dependent as at higher concentrations better inhibitory activity was observed. The leaf extract showed no inhibitory effect to the clinical isolates at the different concentration used

Table 2 Antibacterial activities of the methanolic stem extract of *B. coccineus*

Concentra-tions → organisms ↓	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**3.125mg/ml**	**Positive control**	**Negative control**
Staphylococcus aureus	S	S	R	R	R	R	S	R
Pseudomonasaeruginosa 1	S	S	R	R	R	R	S	R
Pseudomonasaeruginosa 2	S	S	R	R	R	R	S	R
Klebsiella pneumonia	S	S	R	R	R	R	S	R

Key S- Sensitive R-Resistant

There is a rise in the demand for more drugs from medicinal plants as a result of antimicrobial resistance to certain drugs as well as their associated side effects in the consumption of such drugs [21]. As a result of a large reliance of a large global population (80%) on traditional medicines as an alternative therapy in the treatment of common infections, screening of medicinal plants is required and necessary to determine their potency as well as their safety [22]. There is an increase in the number of research works on screening and analysis of medicinal plants to determine possible antimicrobial effects and phytochemical constituents responsible for their antimicrobial properties [23, 24]. The therapeutic use of plants in herbal medicine is as a result of phytochemical compounds with antimicrobial activity present in them [25]. The phytochemical screening of *Brysocarpus coccineus* in this study shows alkaloids, saponins, flavonoids, phenolic compounds, reducing sugar, tannins were present in the plant leaves and stem extract. This shows that the plant extracts contain phytochemical constituents of medicinal significance. For example Flavonoids are potent water soluble anti-oxidants and free radical scavengers, which prevents oxidative cell damage and possess strong anti-cancer activity [26]. As anti-oxidants, flavonoids from these plants provide anti-inflammatory activities. The results of the phytochemical screening correlates with previous results obtained by some other researchers like Chukwuemeka et al., [27] who reported the presence of saponins, glycosides, flavonoids alongside other phytochemical components in methanolic leaf extract of *Byrsocarpus coccineus*. Hamid and Aiyelaagbe, [28] also reported previously the presence of saponins, reducing sugars, glycosides, flavonoids alongside other phytochemical constituents in methanolic stem extract of *Byrsocarpus coccineus.* The antimicrobial screening of *Brysocarpus coccineus* showed that the stem extracts at 100mg/ml concentration had the highest antimicrobial activity compared to the other concentrations. There was also activity of the methanolic stem extract at 50mg/ml. This correlates with the report of Hamid and Aiyelaagbe, [29] that showed clinical bacteria strains to be sensitive to hexane, ethylacetate and methanol extracts of *Byrsocarpus coccineus* at concentrations between 12.5 and 200mg/ml. The antimicrobial activity of the extracts on *Klebsiellae pneumonae* was observed very low in the study and the antimicrobial activity of the extracts were determined to be concentration dependent with their activity being higher at increased concentrations of the extracts. Significantly, the leaf extract in this study showed no activity on the isolates. This result contrasts with previous study by Chukwuemeka et al., [27] that reported antibacterial activity of methanolic *Brysocarpus coccineus* leaf extract. The Leaf extract in their study had no activity on *K. pneumoniae* and *Salmonella typhi*. Hamid and Aiyelaagbe, [29] also reported inhibitory activity of methanolic leaf extract of *Byrsocarpus coccineus* especially at high concentrations of 200 and 100mg/ml which contrast also with results from this study. The stem extracts were seen to have better inhibitory effects compared to the leaf extract. The activity of the leaf extract on the isolates may be possible due to the concentration used. Higher concentrations of the leaf extract may be required to possibly have inhibitory effects on the isolates as the phytochemical screening show that they contain active phytochemical components.

5. Conclusion and Future Scope

This study provides a scientific justification for the therapeutic use of the plant extracts especially *Byrsocapus coccineus* stem extracts in treating ailments associated with organisms used in the study as indicated by the bacteriostatic effect of the stem extract on the tested bacteria. It is important to recommend that further studies should be carried out to isolate the bioactive constituents of pharmacological significance in this plant to produce more potent and less toxic drugs which can be used for therapeutic purposes in infections caused by the organisms.

Conflict of Interest

Authors declare that they do not have conflict of interest

Authors contributions

Conceptualization: C. Jesumirhewe.
Investigation: C. Jesumirhewe, R. N. Raphael
Methodology: C. Jesumirhewe, R. N. Raphael
Supervision: C. Jesumirhewe
Writing – original draft: C. Jesumirhewe.
Writing – review & editing: C. Jesumirhewe, A. Fajana

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AUTHORS PROFILE

Christiana Jesumirhewe is a Senior Lecturer in the Department of Pharmaceutical Microbiology, Prof Dora Akunyili College of Pharmacy, Igbinedion University Okada Edo state Nigeria. She got her BSc, MSc and PhD from the University of Ibadan, Ibadan Oyo state Nigeria in 2004, 2008 and 2014 respectively. She is a pharmaceutical microbiologist. Her main research interests are Microbial Genetics: Bacteria Drug resistance in relation to Public health, Therapeutic evaluation of potential products exhibiting antimicrobial activity. She has published articles in reputable local and international journals. She has attended and presented papers in conferences. She has 12 years of teaching and undergraduate students' supervision experiences.

Raphael N Raphael is a graduate of Department of Pharmaceutical microbiology Prof Dora Akunyili, College of Pharmacy Igbinedion University Okada Edo state Nigeria where he obtained his BPharm in 2020. He is currently a Masters student in the Department of Public health Glasgow Caledonian University Scotland, United Kingdom.

Akibu Fajana is a Lecturer I in the Department of Pharmacognosy, Prof Dora Akunyili College of Pharmacy, Igbinedion University Okada Edo state Nigeria. He got his BSc and MSc from Ekiti state University and University of Lagos in 1994 and 2006 respectively; with PhD in view. He is a Pharmacognosist. His main research interest is Cancer research. He has published articles in reputable local and international journals. He has 12 years of teaching and undergraduate students' supervision experiences.

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