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Original Research Article

Justicia secunda Vahl (Acanthaceae); Antibacterial and Antisickling Potential

*Tavs A. Abere[1], Nnamani N. Monica[2], Akwuruoha C. Charles[3]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. eseabere@yahoo.com*
[2]*Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Madonna University, Elele Campus, Nigeria. mnnenna.nnamani@gmail.com*
[3]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Madonna University, Elele Campus, Nigeria. ugwumbacharles87@gmail.com*

ABSRTACT

African traditional healers frequently utilize the Acanthaceae plant *Justicia secunda* Vahl for the treatment of various ailments, including sickle cell anemia. The primary objective of this study was to validate the assertions regarding the plant's antibacterial and antisickling properties. We explored the potential of utilizing whole and fractionated *Justicia secunda* leaves to prevent HbSS red blood cells from sickling when exposed to sodium metabisulphite. We conducted agar well diffusion tests to evaluate the efficacy of the *Justicia secunda* extract and fractions in inhibiting the growth of various bacteria; *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia*, *Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* among. When comparing *J. secunda* extract and fractions to normal saline as a control, a notable amount of sickling inhibition was observed ($P \leq 0.05$). Furthermore, different levels of inhibition on the growth of *K. pneumonia, S. aureus, B. subtilis,* and *E. coli* were shown by the extract and fractions. The antisickling and antibacterial properties of *J. secunda* leaves suggest that they might be helpful in the treatment of sickle cell diseases.

Keywords: *Justicia secunda*, Acanthaceae, Sickle cell disorder, Antimicrobial activity.

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Introduction

Healthcare providers in Africa are alarmed by the high occurrence of sickling diseases, including sickle cell disease (SCD), which has been identified as a significant contributor to mortality in the region.[1] A significant portion of the Nigerian population (25%), is known to have the sickle cell trait (HbAS).[2] Sickle Cell Disease (SCD) is a genetic blood disorder characterized by the presence of abnormal rigid sickle-shaped red blood cells, resulting in a reduced capacity to transport oxygen.

Based on observations in living organisms, sickled red blood cells cause a condition called stasis by closing off small blood vessels, which results in organs being deprived of essential oxygen and nutrients. This eventually leads to full tissue death and reduced organ function. Chronic clinical symptoms occur due to consistent changes in different stages of hemoglobin, specifically fetal hemoglobin (HbF).[3] Sickling reduces the flexibility of red blood cells, which raises the probability of several complications including sinus thrombosis,[4] retinopathy,[5] leukocytosis,[6] prolonged discomfort from blood vessel blockage,[7] tissue damage due to insufficient blood supply,[8] stroke,[9] priapism and penile tissue death.[10]
Amino acids, folic acid and hydroxyurea are essential components of the primary treatment protocol for sickle cell disease (SCD). The main goals of this protocol are to effectively manage complications, prevent infections, relieve pain and in some cases blood transfusion to ensure the patient's hemoglobin level is maintained.[11]

*Corresponding author. mail: eseabere@yahoo.com

Tel: 08023395616

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The use of hydroxyurea has been greatly restricted due to its negative effects on bone marrow function and its potential to cause mutations and cancer.[12] Research is currently ongoing to investigate the potential antisickling properties of medicinal plants, as conventional medications are associated with certain adverse effects. Researchers have discovered anti-sickling properties in various parts of plants that could potentially enhance retention of the biconcave shape of the red blood cells. These findings are a result of diligent pursuit of more cost-effective and less toxic alternatives to existing treatments. *Dennettia tripetala*, *Physalis angulata*, [13] *Aloe vera*, *Aloe arborescens*, [14, 15] *Woodfordia fruticosa*, [16] *Spavetta crassipes* and *Ziziphus mauritiana*[17] are plants that have demonstrated effective properties in preventing sickling of red blood cells. Medicinal plants have the ability to produce secondary plant metabolites, some of which have demonstrated significant impacts on human systems and could potentially be used for therapeutic purposes in the management and treatment of animal diseases. Traditional healers in many developing countries, especially in Africa, have a rich history of utilizing medicinal plants. There has been a surge in interest in these plants as potential sources of phytochemicals that can combat diseases. [18] There are over 2500 species and 250 genera in the Acanthaceae family. Many of these occur as shrubs and herbs that flourish in tropical regions. The United States of America, Australia and the Mediterranean region also possess medicinal plants of the Acanthaceae family. The Acanthaceae family is known for its wide range of chemical constituents, including diterpenoids, alkaloids, tannins, saponin and cyanogenetic glycosides.[19] According to reports, *Justicia* is recognized as one of the largest genera in the family. It is primarily found in tropical regions, covering a substantial portion of Asia, America, and Africa.[20]*Justicia secunda* Vahl, also known as the blood plant, is a herbaceous plant with leaves that contain various phytochemicals, including tannins, flavonoids, anthocyanins, and leuco-anthocyanins. These compounds are thought to possess medicinal properties and may have potential applications in the treatment of different conditions, such as anemia and hypertension.[21] Kitadi and his colleagues also discovered that different species of *Justicia* are frequently used in traditional medicine to treat gastrointestinal, inflammatory, and respiratory conditions. In addition, it is said to have potential applications as a treatment for depression,

sedation, hallucination, epilepsy, and various psychiatric conditions.[21] *J. secunda* has been found to have potential applications in reducing inflammation, relieving pain, and scavenging free radicals according to Onoja and his co-workers.[22] In countries with limited economic development and a high prevalence of sickle cell disease, *J. secunda* has been widely utilized as an effective agent to prevent the sickling of red blood cells. Verifying this claim could be beneficial to the pharmaceutical sector in the development of alternative medicines for managing or treating various sickle cell illnesses. Despite the potential of *Justicia secunda* leaves in the management of sickle cell anaemia and its associated effects, from literature search, the antisickling and antibacterial activities of the ethanol crude extract of *J. secunda* has not been carried out, hence this study was aimed at investigating the antibacterial and antisickling properties of the crude ethanol extract and fractions of *Justicia secunda* leaves.

Materials and Methods

Collection and Identification process
In April 2022, the leaves of *Justicia secunda* were collected along Wire road in Benin City, Edo State, Nigeria, at coordinates 6.352036 and 5.619066. A voucher specimen with the Herbarium Number UBH-J66 was assigned and deposited in the herbarium. Prof. Akinnibosun H.A. of the Herbarium Unit, Faculty of Life Science (Plant Biology and Biotechnology Department), University of Benin, Edo State, verified its authenticity.

Plant Extraction
The leaves of *Justicia secunda* were separated, dried at room temperature for three days. Powdered *J. secunda* leaves (3.50 kg) were macerated in ethanol-water (50:50) (8 X 2L) for three days. Part of the solvent was removed using a water bath at 40 ºC, followed by oven drying, which yielded a crude extract of 0. 71 kg that was subsequently reconstituted in water and successively partitioned into ethyl acetate (6L), chloroform (6L) and n-butanol (6L).

Phytochemical screening
The phytochemicals found in *Justicia secunda* leaves were examined using standard techniques as outlined by Trease and Evans. Alkaloids, tannins and different classes of Glycosides (saponins, Anthracene, cyanogenetic and cardiac) were among the phytochemicals that were assessed.[19]

HbSS Blood Samples for Antisickling Screening
Venipuncture was utilized at MAU Teaching Hospital in Elele, Rivers State, Nigeria to obtain blood samples from sickle cell patients who had not recently received an HbAA transfusion and were not in a life-threatening situation (Ethical Certificate Number MAU/SREC/A/18).

Assessment of antisickling activity

The serum from sickle cell patients was centrifuged from EDTA vials housing the blood sample. Following each wash in normal saline, the erythrocytes underwent three rounds of centrifugation to remove the supernatant. Antisickling tests were conducted on the crude extract of *Justicia secunda* and fractions. [23] In the test tubes, a precise amount of blood cells and the appropriate concentration of the extract or fractions were combined. A solution containing 5 mg/ml of PABA in normal saline was utilized as the positive control, while normal saline served as the negative control. Samples were taken from the different mixtures and the remaining portions of the mixtures incubated for three hours, shaking occasionally. For deoxygenation, each mixture was treated with 0.5 ml of a 5% sodium metabisulphite solution and securely sealed with liquid paraffin. Each mix was sampled in five replicates at time 0 minute, followed by seven additional readings taken at 30-minute intervals. The materials were carefully spread over a microscope slide, fixed with ethanol, and then stained with Giemsa dye. Each slide was meticulously analyzed using an oil immersion microscope to ascertain the quantity of red blood cells. By utilizing the counts of sickled and unsickled cells, we successfully calculated the ratio of non-sickled red blood cells.

Antimicrobial assessment
Clinical isolates of *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis*, and *Staphylococcus aureus* were utilized in the antimicrobial evaluation. The culture was examined for integrity using conventional cultural, biochemical, and morphological methods prior to employing the organisms obtained from the Department of Microbiology, MAU Teaching Hospital, Elele, Rivers State, Nigeria. [24] The microbial cultures were kept at 4 ºC in Nutrient Agar.
Preparation of the inoculum To create a microbiological suspension with turbidity equal to 0.5 McFarland's standard, an overnight culture was employed.[24]
Diffusion method using Agar wells The media were sterilized for 15 minutes at 121 degrees Celsius. Each plate was prepared with 10 mm (diameter) wells that held 30 ml of nutrient agar seeded with bacterial culture and left to harden. After filling the wells with the crude extract and fractions at concentrations ranging from 20 mg/ml to 100 mg/ml, they were placed in an incubator set at 37 ºC for 24 hours. Three iterations of the trials were conducted. Measurements and comparisons of the inhibition zone diameter (IZD) with ciprofloxacin were made.[25]

Analytical statistics Data are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) was used to look at the changes in the means. We used P values less than 0.05 to show that the differences between the sets of data were statistically significant.

Results and Discussion

A phytochemical examination of *Justicia secunda* leaves detected cardiac, cyanogenetic glycosides, flavonoids, alkaloids and tannins (Table 1). Table 2 showed that *J. secunda* leaf extract and fractions effectively prevented sickling ($p < 0.05$). Researchers have linked the presence of secondary plant metabolites to medicinal plants' antibacterial and antisickling capabilities. Phytochemical analysis of *J. secunda* revealed tannins, flavonoids, alkaloids, cyanogenetic and cardiac glycosides. While tannins, flavonoids, and alkaloids have well-established antibacterial characteristics, [26, 27] polyphenols, also known as anthocyanins, have shown significant efficacy with a high normalization rate in sickle cell types.[28] Ajawaron, whose major constituent is *Cissus populnea* L, is an herbal product that is being sold in Nigeria. Its antisickling properties are claimed to be due to the presence of cardiac glycosides in *Cissus populnea* L.[23]

Table 1: Phytochemical constituents of *J. secunda* leaves

Class of secondary metabolites	Inference
Tannins	+
Flavonoids	+
Alkaloids	+
Anthracene derivatives	-
Cardiac glycosides	+
Saponin glycosides	-
Cyanogenetic glycosides	+

Key: - = absent, while + = present

Despite the availability of multiple antisickling medications that act at different phases of the sickling process; medicinal herbs have been demonstrated to be particularly useful in the management and treatment of sickle cell anemia. This can be partly related to the potential toxicity of majority of orthodox drugs. *Fagara zanthoxyloides* Lam was one of the earliest medicinal plants whose antisickling properties were investigated. [29] Some other species whose antisickling activities have been investigated are *Boscia angustifolia, Kigelia Africana, Mitragyna innermis, Ipomea batatas,* and *Calotropis procera*. [30] These plants' antisickling abilities may be ascribed to their

ability to prevent hemoglobin from polymerizing *in vitro* or to structural changes caused by the various extracts' interactions with hemoglobin. [1] Mixing *J. secunda* crude extract and fractions with SS blood resulted in most sickle-shaped erythrocytes reverting to a normal biconcave shape. This demonstrated that *J. secunda*, which grows in Nigeria, possesses antisickling properties. This validates the use of *J. secunda* leaves to treat sickle cell anemia in traditional Congolese medicine. [31] Several species of *Justicia*, including *J. tenella*, *J. gendarussa*, and *J. insularis* have been claimed to have antisickling properties. [28]

The crude extract and fractions of *J. secunda* showed broad-spectrum bactericidal activity against both gram-positive and gram-negative bacteria (Tables 3 and 4). Whereas Nirmairaj and co-workers claimed that *J. gendarussa* has a broad range of activity against both Gram-positive and Gram-negative pathogens, [32] Herrera-Mata and co found that *J. secunda* was ineffective against Gram-negative bacteria. [33] It should be noted that Gram-negative bacteria have more complex cellular wall structures than Gram-positive bacteria. Gram-negative bacteria, unlike the gram-positive, have an inflexible peptidoglycan structure and a double cellular membrane.[33]

Table 2: Sickling inhibition activities of *J. secunda* crude extracts and fractions

Incubation Time	Percentage inhibition (%)								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
0	29.4±0.11	42.2±1.32	75.6±0.14	72.3±0.98	75.4±0.12	55.3±1.02	59.4±0.38	68.3±1.56	56.7±0.04
30	29.0±1.52	49.0±0.44	70.2±1.22	73.4±1.16	74.8±1.15	56.7±0.05	61.2±1.24	66.2±1.08	54.2±0.26
60	31.2±0.25	52.4±0.21	73.7±1.16	74.0±0.45	74.7±0.14	54.0±1.16	58.3±1.08	71.5±1.14	55.4±1.16
90	33.0±1.22	59.1±1.03	76.5±0.48	76.2±0.02	75.6±0.32	52.8±1.64	56.7±0.02	71.6±0.36	53.8±1.04
120	33.6±0.18	59.4±0.19	78.0±1.74	79.4±1.14	77.3±1.36	51.9±0.08	57.8±1.74	70.4±0.05	54.3±0.08
150	35.0±0.62	55.6±0.12	74.1±0.56	82.6±0.62	75.2±1.18	52.4±1.12	56.2±0.54	69.2±1.68	55.9±0.18
180	34.4±1.06	55.0±1.54	72.4±0.74	80.2±0.04	70.7±0.02	51.0±0.06	57.7±1.08	67.0±0.74	53.5±1.14

A = blood + normal saline + sodium metabisulphite
B = blood + PABA + sodium metabisulphite
C = blood + crude extract of *J. secunda* leaf at 100 mg/ml + sodium metabisulphite
D = blood + crude extract of *J. secunda* leaf at 200 mg/ml + sodium metabisulphite
E = blood + crude extract of *J. secunda* leaf at 300 mg/ml + sodium metabisulphite
F = blood + ethylacetate fraction of *J. secunda* leaf + sodium metabisulphite
G = blood + chloroform fraction of *J. secunda* leaf + sodium metabisulphite
H = blood + n-butanol fraction of *J. secunda* leaf + sodium metabisulphite
I = blood + aqueous fraction of *J. secunda* leaf + sodium metabisulphite

Table 3: Antibacterial activity of *J. secunda* leaf extract

Test Organisms	Diameter of zones of inhibition (mm)				
	10 mg/ml	**50 mg/ml**	**100 mg/ml**	**Cp (10 µg/ml)**	**DMSO**
E. coli	13±0.25	13±0.12	20±0.01	31±0.03	G
S. typi	G	G	G	17±0.24	G
K. pneumonia	10±0.01	11±0.35	14±0.52	15±0.06	G
P. aeruginosa	G	G	G	26±0.08	G
S. aureus	14±0.01	16±0.42	19±0.06	21±0.02	G
B. subtilis	16±0.04	21±0.04	22±0.08	24±0.05	G

The values are presented as mean ± standard error of mean; (G) stands for no inhibition zone; (ND) for not determined; and (Cp) for ciprofloxacin P<0.05.

Table 4: Antibacterial activity of 50 mg/ml of the fractions of *J. secunda* leaf extract

Test Organisms	Diameter of zones of inhibition (mm)					
	Ethylacetate fraction	**Chloroform fraction**	**n-butanol fraction**	**Aqueous fraction**	**Cp (10 µg/ml)**	**DMSO**
E. coli	7.0±0.25	7.0±0.36	7.0±0.12	6.0±0.02	31±0.03	G
S. typi	G	G	G	G	17±0.24	G
K. pneumonia	5.0±0.70	7.0±1.25	6.0±0.24	6.0±0.05	15±0.06	G
P. aeruginosa	G	G	G	G	26±0.08	G
S. aureus	5.0±1.06	6.0±0.15	4.0±0.08	5.0±0.06	21±0.02	G
B. subtilis	9.0±0.42	9.0±1.04	6.0±0.15	4±0.22	24±0.05	G

The values are presented as mean ± SEM; G stands for no inhibition zone; ND for not determined; Cp for ciprofloxacin; and P<0.05 for significant differences.

Conclusion

The study has demonstrated the antisickling and antibacterial properties of *J. secunda* leaves, supporting its use in the treatment of SCD and related illnesses. The existence of intrinsic phytoconstituents in the plant may be the cause of these actions. In order to evaluate the efficacy and ascertain the safety profile of *J. secunda* and encourage its use by medical professionals, additional study aimed at isolating the active constituent(s) implicated in the observed activities, including toxicity tests, should be conducted.

Contribution of authorship: Conceptualization and design of study-TAA, Sample collection and data analysis-NNM, Antibacterial study-AC.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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