ASSESSMENT OF BIOACTIVE CONSTITUENTS, ACUTE TOXICITY, RADICAL SCAVENGING AND ANTIBACTERIAL ACTIVITIES OF THE METHANOL EXTRACT FROM *JUSTICIA SECONDA* VALH LEAVES CULTIVATED IN BENIN CITY, NIGERIA

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Abstract: Despite advances in modern healthcare delivery systems using Western medicines, medicinal plants still continue to play an essential and indispensable role in human and animal health. This study evaluated the phytochemical constituents, acute toxicity, antiradical, and antibacterial activities of the methanol extract of *Justicia secunda* leaves (JSL) using standard methods. Qualitative phytochemical screening of JSL revealed the presence of alkaloids, reducing sugars, saponins, phenolic compounds, terpenoids, and flavonoids. The total phenolic content was 12.83±0.97 mg of gallic acid equivalent/g of extract, while the total flavonoid content was 21.03±1.71 mg quercetin equivalent/g of extract. At the maximum dose of 5000 mg/kg, JSL extract was not toxic to adult albino Wistar rats. Histological examination of the kidneys of the rats in the control group and those administered JSL extract revealed no significant difference. However, there was dose-dependent vasodilation and increased blood flow in the kidneys. Antibacterial analysis revealed that *Pseudomonas aeruginosa* and *E. coli* were sensitive to JSL extract at concentrations of 25, 50, 75 and 100 mg/mL, while *P. vulgaris* was resistant at all four concentrations. *Staphylococcus aureus* was sensitive to three concentrations (25, 50 and 75 mg/mL) of the extract. An *in vitro* antiradical study using 2,2-diphenyl-2-picryl hydrazyl (DPPH) gave IC_{50} values of 0.23 µg/mL for ascorbic acid and 11.40 µg/mL for the extract. These experimental outcomes underscore the pharmacological potential of JSL.

Keywords: Histology, flavonoids, antibacterial, alkaloids, *Justicia secunda*, antioxidant

Introduction

Nature has provided an array of medicinal agents from plants for several years, and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the use of agents in traditional medicine. Plant-based, traditional medicine systems continue to play an essential role in health care, with approximately 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. This is mainly due to its affordability, accessibility and low cost (Owolabi *et al*., 2007). Medicinal plants are plants containing inherent active ingredients capable of curing diseases or relieving pain (Okigbo *et al*., 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (Sofowora *et al*., 2013). Interest in medicinal plants as a re-emerging health aid has fuelled by the rising cost of procuring drugs for the maintenance of personal health and well-being and the bioprospecting of new plant-derived drugs (Burton *et a*l., 2015). The ongoing increase in the recognition of medicinal plants is due to several reasons, including increasing faith in herbal medicine (Sofowora *et al*., 2013). In India, the ayurvedic tradition has outlined numerous medications derived from plants or plant-based materials. Analyzing their morphological, pharmacological, or pharmacognostic features can enhance our comprehension of their active components and how they function. The historical practice of utilizing plants to treat various human ailments dates back to ancient times. Various parts of plants such as leaves, stems, bark and roots are used to prevent, ameliorate symptoms or reverse abnormalities. Since the practice of "herbal remedies" does not adhere strictly to facts accrued using scientific approaches, orthodox medicine sees "herbal medicines" as "alternative medicine". However, most of the pharmaceutical products currently dispensed by physicians, including opium, aspirin, digitalis and quinine, have a long history of use as herbal remedies. Modern medicine today utilizes active compounds isolated from higher plants, and approximately 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and traditional uses.

Justicia seconda (also known as "Blood root" and "Sanguinaria" in Barbados and Venezuela) is a medicinal plant that belongs to the *Acanthaceae* family and is one of the many species of the *Justicia L*. genus that are commonly used in traditional African medicine. Blood leaves, "Blood tonic," and "*Hospital too far*" are some of its other names (Manda *et al*., 2011). The most common *Acanthaceae* genus is *Justicia*. In tropical regions of the world, this species is widespread, but is rare in temperate regions. There are species in Asia, America, and Africa that belong to the *Acanthaceae* genus *Justicia*. The height of the *Justicia secunda* ranges from 90 to 200 cm. Some of the functions of this plant include the treatment of stomach ache, anemia, and wound healing. To treat anaemia, Jehovah's Witnesses use leaf decoctions in Cote d'Ivoire and Congo. In Nigeria, a decoction of the leaves is reportedly used to treat anaemia (Mpiana *et al*., 2010). The herb is traditionally used as a hematinic to stimulate blood because the leaf decoction is known to generate a purplish/blood red colour. The anti- sickling, haematinic, antibacterial, and antihypertensive characteristics of *J. secunda* have been studied. (Mpiana *et al*., 2010; Kone *et al*., 2012).

Calderón *et al*. (2012) reported that a component of *J. secunda* stem has only weak acetylcholinesterase inhibitory activity in vitro, suggesting that it may facilitate muscle activation and contraction. By identifying glucosidase, Theiler *et al*. (2010) provided a scientific basis for understanding the anti-diabetic effects of the leaf extract by identifying inhibitory compounds in the extract.

Plate 1: *Justicia secunda* plant at the flowering stage

Although many different chemical compounds have been identified in the leaves of different *Justicia* species, research on the internal organ histology of animals fed with *JSL* extracts is scarce. In this study, the Wistar rat (*Rattus norvegious*), was used as an animal model to examine how the plant affects vital internal organs and to shed light on the phytochemicals that are responsible for the plant's antioxidant ability and other diverse medicinal therapeutic and pharmacological qualities.

Materials and Methods

Collection and identification of plant materials

J. secunda leaves were collected from a private garden at Ugbowo, Benin City, Nigeria (6˚23′11″N 5˚36′42″E). The plant was identified and authenticated in the Department of Plant Biology and Biotechnology (PBB), University of Benin, Ugbowo, and UBH-J386 was assigned as the voucher number.

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Preparation of extract

The leaves were rinsed with water and air- dried at room temperature for a period of two weeks under the shade and then pulverized to fine powder using an all-steel electric grinding machine. The powder was macerated in methanol (96% v/v) to get the extract according the procedure described by Aiwonegbe and Ativie, (2023).

Phytochemical screening

A mixture was made by adding 50 mL of methanol to 5 g of the powdered leaves of *J. secunda* in a 250 mL conical flask. The flask was gently agitated for 2 minutes and allowed to settle. Thereafter, the mixture was filtered and the filtrate was subjected to phytochemical screening using the methods described by Harborne, (1998); Trease and Evans, (2002).

Determination of total phenolic content (TPC)

The total phenolic content of the methanol extract of *Justicia secunda* leaves (JSL) was determined by the method described by Upadhya *et al*., (2015), with modifications by Aiwonegbe *et al*., (2022). The extract solution (0.5 mL), at a concentration of 1000 µg/mL was added to 4.5 mL of distilled water and 0.5 mL of Folin Ciocalteu reagent (previously diluted with water 1:10, v/v) which was then added to the solution. After mixing the tubes, they were maintained at room temperature for 5 minutes, followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. After mixing, the samples were incubated for 30 minutes at room temperature. The absorbance was measured with a spectrophotometer at 750 nm. The total phenolic content was recorded as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract). A standard curve was prepared with gallic acid in 6 different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Total flavonoid content (TFC)

The total flavonoid content was estimated using the method described by Ebrahimzadeh *et al*., (2008). Briefly, 0.5 mL of extract sample (0.2 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminum chloride was added, followed by 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured with a spectrophotometer at 415 nm. The results are expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared with quercetin at six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

In vitro Antioxidant Analysis

The scavenging effect of the crude methanol extract on the 2,2-diphenyl- 1-picrylhydrazyl (DPPH) radical was estimated with the method described by Jain and Jain (2011). A solution of 1 mM DPPH (2,2-dipheyl-1-picrylhydrazy) in methanol was prepared. A total of 1.0 mL of the prepared solution was mixed with 3.0 mL of extract in methanol containing 0.01 - 0.02 mg/mL of the extract. A micropipette was used to add equal volumes of the above reaction mixture to methanol at different concentrations (1, 2, 5, 10, 25, 50,100, or 200 mg/mL) in different test tubes. The reaction mixture was shaken vigorously and left in an incubator for 30 minutes. Afterwards, the absorbance at 517 nm was measured in a spectrophotometer. The percentage scavenging ability was calculated from equation 1.

$$\%\ \text{DPPH scavenging} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of blank}} \text{ x100} \quad \ldots\ldots.1$$

The percentage of DPPH radical scavenging was plotted against various concentrations and a standard curve was obtained. From the graphical plot, the 50% inhibition concentration (IC_{50}) of the extract was obtained. Ascorbic acid was used as a reference standard.

Antimicrobial analysis

Determination of the antibacterial activity of Justicia secunda leaf (JSL) extract

The test bacteria used were of clinical origin and included *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *protein vulgaris.* They were obtained from the Department of Microbiology, University of Benin, Nigeria.

Four concentrations (25, 50, 75 and 100 mg/mL) were prepared from the original extract of JSL. Mullen Hiton agar was weighed, dissolved, autoclave and poured into a petri dish and allowed to cool and solidify. After twenty-four hours of culture, test isolates of *Candida albicans* were streaked on plates using a sterile wire loop. A sterile cork borer was used to bore holes on the streaked agar plates, which were labeled according to the concentration of the extract prepared. The extract (0.1 – 1 mL) was transferred to the hole bored on the agar plate, ensuring that it did not get to underneath the plate. The plates were incubated at a temperature of 37°C for three days with the exception of potato dextrose agar (PDA) plates, which were at room temperature (25°C) for 3 to 5 days. Thereafter, a measuring ruler was used to determine the zone of inhibition, and the

minimum inhibitory concentration (MIC) was calculated and recorded. All the positive tubes or plates from the MIC test were used for minimum bactericidal concentration (MBC) test. The bacterial isolates at different concentrations (25, 50, 75 and 100 mg/mL) were inoculated on agar Petri dish. The Petri dish was then incubated at a temperature of 37°C for 24 - 48 hours. The minimum bactericidal concentration is the value after measurement or counting.

Acute oral toxicity studies

Experimental Animals

The lethal dose of the methanol extract of JSL was determined via an acute oral toxicity test. Young adult albino Wistar rats, weighing 135 –180 g were used for this experiment according to the protocol described by Akhila *et al*., (2007). The animals were sheltered in polypropylene cages (40 by 20 by 17 cm) with sawdust litter, and the temperature was maintained at 25±2°C. Each cage had a label showing the cage number, dose level, number and weight of the animals it contained and the route of administration. The animals were fed pelleted food and water and handled according to protocols for laboratory use of animals set by the Faculty of Life Sciences Ethical Committee, University of Benin, Nigeria. The ethical approval number given for the study was LS21311.

Experimental Protocol

The animals were randomly classified into six groups, each containing 3 rats. Picric acid (yellow stain) was used to mark the animals for ease of identification. The control group was orally treated with 10 mL of distilled water and the treatment groups received 100, 1000, 1600, 2900, or 5000 mg/kg of methanol, as described above, from JSL.

Mode of Administration and Symptoms Recorded During Study

The acute oral toxicity studies for young adult albino Wistar rats were performed in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines. Young adult albino Wistar rats selected by a random sampling technique were used in this study. The animals were weighed, and plant extract was orally administered to them at doses of 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg for cages I, II, III, IV and V, respectively. Cage VI (control) received 1 mL of distilled water. Thereafter, the animals were left under similar conditions, and observed for general behavioral changes continuously for 30 minutes, every hour during the first 24 hours and once per day for 14 days after the administration of the extract. Observations were focused on parameters such as piloerection, sensitivity to sound and touch, locomotion, aggressiveness, the appearance of feces, salivation, urination, convulsions, coma and death. The rate of food and water intake by the animals also supervised. The number of survivors was noted after 24 hours. The weights of the animals were measured at 0, 7 and 14 days (Malik *et al*., 2022). At the end of the study, all surviving animals were sacrificed and the kidneys were removed and weighed. A gross pathological examination of the kidneys was also performed.

Method of sacrifice

During sacrifice, the final weights of the rats were measured using a compact electrical scale. After weighing, the rats were placed an enclosed container with cotton wool soaked in approximately 50 mL of chloroform for 20 seconds for anaesthesia. After anaesthetizing the rat, it was placed on a dissecting table in the supine position for dissection. An abdominal incision was made with dissecting scissors to expose the abdominal viscera. Blood was collected from the inferior vena cava for analysis after opening the abdominal cavity.

Thereafter, the kidneys of individual rats were harvested and fixed with 10% formalin in sample bottles for histological analysis. All histological slides were stained with hematoxylin and eosin (H&E) and examined under a medium-power microscope at magnifications of 100× and 400×. The choice of staining and magnification enabled comprehensive visualization and assessment of histological structures, facilitating the detection of subtle morphological changes within the kidney tissues.

Results and Discussion

Phytochemical screening

The results of the phytochemical screening of JSL are displayed in Table 1. The extract contains alkaloids, reducing sugars, saponins, phenolic compounds, terpenoids, and flavonoids. However, tannins and cardiac glycosides were absent.

Table 1: Qualitative phytochemical screening of the methanol extract of *Justicia secunda* leaves

Phytochemical Constituent	Observation
Alkaloids	++
Reducing sugar	++
Saponins	++
Phenolic compounds	++
Terpenoids	++
Flavonoids	++
Tannins	-
Cardiac glycosides	-

Key: + = present ++ = largely present - = absent

The qualitative phytochemical screening of *JSL* extracts revealed the presence of saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars and alkaloids. However, cardiac glycosides and tannins were absent. The presence of saponins, flavonoids, phenolic compounds and alkaloids in *JSL* is consistent with the findings of studies carried out by Koffi *et al*., (2013) and Mpiana *et al*., (2010). However, the absence of cardiac glycosides in the aqueous extract is in sharp contrast with the findings of Koffi *et al*., (2013). Phytochemicals are known to demonstrate a variety of biological activities. The literature suggests that plants that are rich in flavonoids and alkaloids exhibit antimicrobial, anti-inflammatory and antioxidant effects (Anyasor *et al*., 2019). Terpenoids possess unique antioxidant activity in their interactions with free radicals, while steroids play significant roles as anticancer agents, antihormones, antimicrobials agents, and cardiovascular agents, amongst others (Onoja *et al*., 2017).

Total phenolic content (TPC)

The standard curve used to determine the total phenolic content of the methanol extract of *JSL* is shown in Figure 1.

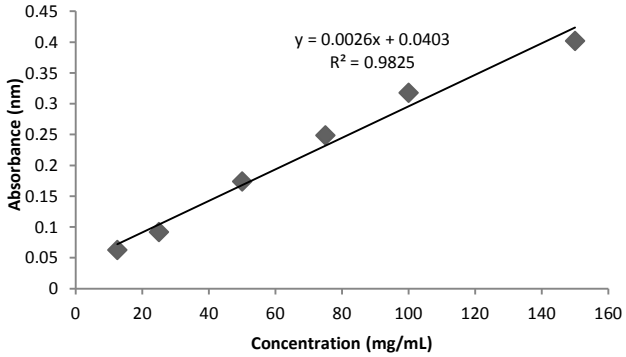


Figure 1: Calibration curve for the total phenolic content of methanol extract of *JSL* expressed in milligrams (mg) of gallic equivalent (GAE) per milliliter of the extract.

The total phenolic content of the plant extract was 12.83 ± 0.97 mg GAE/mL

Total flavonoid content (TFC)

The calibration curve for the determination of the total flavonoid content of the *JSL* methanol extract is displayed in Figure 2.

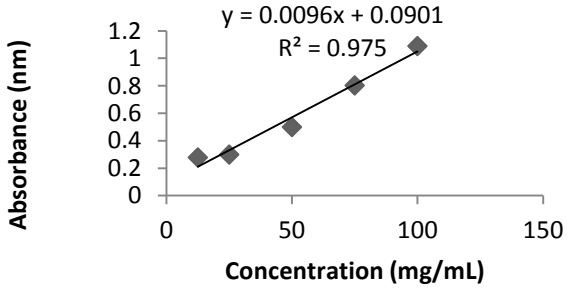


Figure 2: Calibration curve for total flavonoid content expressed as milligram quercetin equivalent (mgq) per gram of extract.

The total flavonoid content of the *JSL* methanol extract was 21.03 ± 0.71 mg QE/g extract. This shows that the plant extract is rich in phenolic and flavonoid compounds.

Phenolic compounds such as phenolic acids are well-known for their antioxidant properties and have been reported to exhibit numerous health benefits, including anti-inflammatory, anticancer, and neuroprotective effects (Gnwali *et al*., 2013). Flavonoids are also known for their antioxidant, anti-inflammatory, antiviral, and cardioprotective properties. The phenolic content identified in this study may contribute significantly to the pharmacological activities of *Justicia secunda*. These results are consistent with previous studies reporting the phytochemical composition of *Justicia secunda* (Koffi *et al*., 2013). However, it is essential to acknowledge potential variations in phytochemical composition due to factors such as geographical location, plant maturity, extraction method, and analytical techniques employed.

In vitro antiradical study

Figure 3 shows a graphical representation of the DPPH scavenging activities of the methanol extracts of JSL and ascorbic acid (the standard). The assessment of antioxidant activity through in vitro assays plays a crucial role in the identification and evaluation of potential therapeutic agents derived from natural sources. The amount of extract required to inhibit 50% of the DPPH radicals, the IC_{50}, was calculated to be 11.40 µg/mL while that of the standard (ascorbic acid) was 0.23 µg/mL.

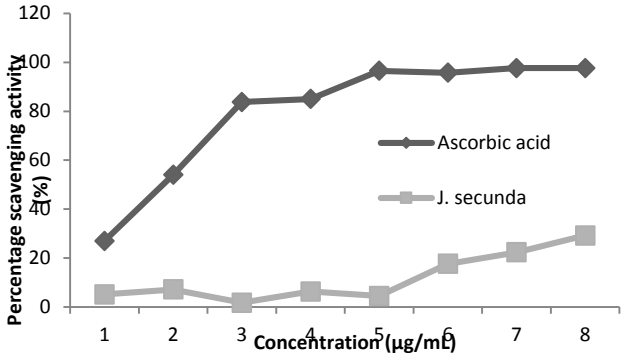


Figure 3: DPPH radical scavenging activity of methanol extract of *Justicia secunda* leaves

The results showed that the *JSL* extract had lower antioxidant scavenging activity than ascorbic acid. This could be attributed to the low concentrations of flavonoids and phenols in the leaf extracts of the plants. Although the efficacy of the methanol extract was inferior to that of ascorbic acid, the observed antiradical activity suggested the presence of bioactive compounds within the extract capable of scavenging free radicals. This underscores the complexity of natural product-derived antioxidants, which often comprise a multitude of phytochemicals with diverse chemical structures and mechanisms of action (Aiwonegbe *et al*., 2022).

The differential antioxidant efficacy observed between ascorbic acid and the methanol extract of *JSL* may be attributed to several factors, including the composition and concentration of bioactive constituents present in the plant extract. *Justicia secunda*, which belongs to the *Acanthaceae* family, is known to contain a variety of phytochemicals, such as phenolics, flavonoids, and alkaloids, which are associated with its antioxidant properties (Onoja *et al*., 2017). The synergistic or antagonistic interactions among these phytochemicals could influence the overall antioxidant capacity of the extract, contributing to the observed differences in activity compared to that of the individual compound, ascorbic acid. Variations in extraction methods, solvent polarity, geographical location, and environmental factors can influence the phytochemical profile and consequently, the antioxidant potency of plant extracts

(Upadhya *et al*., 2015). The optimization of extraction protocols and further phytochemical characterization of *JSL* may provide insights into enhancing the antiradical activity of the extract.

Antibacterial activity of JSL extract against selected bacteria

Four bacterial isolates, namely; *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were utilized in this study. The respective agar plates are shown in Plates 2 and 3.

Table:2: Minimum inhibitory concentration (MIC) of the methanol extract of *Justicia secunda* leaves on selected bacteria

Bacteria isolates (mm)	*Justicia secunda* leaves extract (mg/mL)			
	25	50	75	100
P. vulgaris	8.3	7.8	6.9	5.8
P. aeruginosa	17.1	15.3	12.6	10.8
S. aureus	12.0	11.5	10.7	9.5
E. coli	18.0	15.0	11.0	10.5

Key: >10 = Sensitive, <10 = Resistance

The minimum inhibitory concentration of the *JSL* extract for the bacterial isolates is shown in Table 2. The methanol extract of *JSL* had no bactericidal effect on *P. vulgaris* at any of the four tested concentrations (25, 50, 75, and 100 mg/mL) as the organism was completely resistant. *Pseudomonas aeruginosa* and *Escherichia coli* were sensitive to the four concentrations. *Staphylococcus aureus* was also sensitive to 25, 50, and 75 mg/mL of the extract but was resistant to 100 mg/mL.

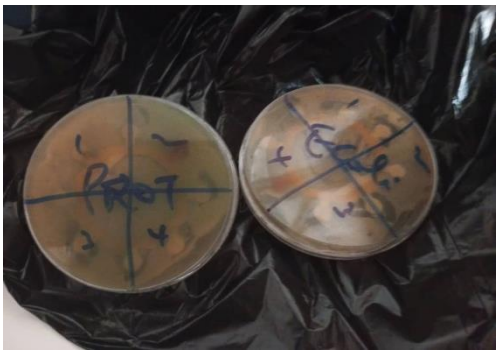


Plate 2: Agar plate containing *Proteus vulgaris* and *Escherichia coli*

Plate 3: Agar plate containing *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Table 3: Minimum bactericidal concentration (MBC) of *JSL* methanol extract

	Justicia secunda leaves extract (mg/mL)			
Bacteria isolates (mm)	25	50	75	100
P. vulgaris	6.5	5.3	5.0	4.6
P. aeruginosa	12.0	10.6	9.3	7.5
S. aureus	9.6	9.3	7.4	6.4
E. coli	5.3	11.2	8.6	9.2

Key: >10 =Sensitive, <10 =Resistance

Table 6: Antibiotic Sensitivity Test of *JSL* methanol extract

	Potency	**Growth of organisms(mm)**			
Antibiotics	**(µg/mL)**	***S. aureus***	***E. coli***	*P. aeruginosa*	*P. vulgaris*
Ofloxacin	5	12	8	17	13
Augmentin	30	13	11	12	10
Gentamicin	10	10	5	11	9
Ciprofloxacin	5	8	6	5	0
Cefuroxime	30	0	5	0	0
Nitrofurantoin	30	5	0	6	0

Table 5 shows the results for the minimum bactericidal concentration (MBC) of the *JSL* methanol extract. These results were compared with the potencies of the various antibiotics used, as shown in Table 6. The minimum bactericidal concentration (MBC) is normally used to evaluate potency when there is a possibility of having multiple antimicrobial agents. This approach addresses the challenges of masking and active ingredient in an antimicrobial formulation with other ingredients in the formulation. The methanol extract of *JSL* did not exhibit antibacterial activity against *P. vulgaris* or *S. aureus*. *P. aeruginosa* was sensitive to the extract only at 25 and 50 mg/mL, while *E. coli was* resistant at all concentrations of the extract except at 50 mg/mL. This contrasts with the findings of Bako *et al*., (2023) in which *P. aeruginosa* and *S. aureus*, were sensitive to the methanol extract of *JSL*. Herrera-Mata *et al*., (2002) reported the sensitivity of *P. aeruginosa* to the aqueous leaf extract of *J. secunda*, with *E. coli* showing resistance. The antibacterial activity of the *JSL* extract could be linked to the presence of secondary metabolites such as flavonoids, saponins, tannins and terpenoids (Ayodele *et al*., 2020). Reports have shown that antimicrobial compounds in plants act by forming a protective cover, inhibiting the action of microbial enzymes, aiding the disruption of the plasma membrane and preventing the substrates required for growth by organisms (Takó *et al*., 2020).

Acute toxicity analysis

No signs of lethality or morbidity were observed in rats administered varying doses of the methanol extract of *Justicia secuda* leaves (JSL), up to 5000 mg/kg, over a two-week period. This finding suggested that the median lethal dose (LD_{50}) of the methanol extract of JSL exceeded 5000 mg/kg (Sani *et al*., 2020). The absence of lethality or adverse effects within the tested dose range indicates a wide margin of safety for the methanol extract of JSL in rats (Ahmed, 2015). This finding aligns with the traditional use of *Justicia secuda* in herbal medicine without reported incidents of acute toxicity.

Relative organ and body weight study

The body weight changes of rats given graded doses of the methanol extract of *J. secunda* are displayed in Table 1. Administration of the methanol extract of *JSL* at different doses (100, 1000, 1600 and 2900, 5000 mg/kg) resulted in significant changes in the body weight of the rats fed the methanol extract of *JSL* compared with that of the control group. This finding shows that the methanol extract of JSL may have the capacity to increase muscle mass and/or bone density, when fed to experimental rats.

The organ weights of rats given graded doses of the methanol extract of *JSL* are shown in Table 2. The changes in body weights were recorded after 24 hours, 48 hours, 7 days and 14 days, while the organs (kidneys) were weighed using a standard weighing balance to calculate the relative organ weight for the different sets on the sacrifice day. The weights of the organs of the rats treated with the methanol extract of *JSL* were not significantly different from those of the control group.

$$\text{Relative organ weight (\%)} = \left(\frac{\text{Absolute weight of organ (g)}}{\text{weight of rat on sacrifice day (g)}}\right) \times 100 \quad \ldots\ldots 2$$

Table 4: Changes in the body weights of rats following treatment with different doses of the methanol extract of *Justicia secunda* leaves

Treatment	Dose (mg/kg)	Body weight (g)				
		Initial	After 24 hours	After 48 hours	After 7 days	After 14 days
Control	DW	156.55±04.03	158.55±05.08	157.55±06.03	156.55±06.02	155.85±07.04
Methanol extract of *Justicia secunda* leaves	100	157.67±08.84	187.00±05.14	188.66±05.60	182.00±03.28	165.67±06.62
	1000	158.67±08.29	191.67±07.17	195.67±08.26	191.00±09.54	173.33±08.95
	1600	150.33±04.06	168.33±06.28	167.00±09.85	180.33±09.96	178.67±09.13
	2900	145.67±08.69	172.67±05.32	169.33±03.62	183.67±06.91	166.00±06.56
	5000	167.33±06.98	186.00±03.21	185.33±05.24	195.33±05.17	180.67±03.84

DW= distilled water, values are presented as the mean ±SEM

Table 5: Kidney weight of rats treated with different doses of the methanol extract of *J. secunda*

Treatment	Dose (mg/kg)	kidney weight(g)	
		Left	Right
Control	DW	0.60±0.10	0.60±0.00
Methanol extract of *Justicia secunda* leaves	100	0.50±0.00	0.53±0.03
	1000	0.60±0.06	0.57±0.07
	1600	0.60±0.06	0.57±0.09
	2900	0.53±0.09	0.53±0.09
	5000	0.53±0.03	0.47±0.03

DW = distilled water, values are presented as the mean ±SEM

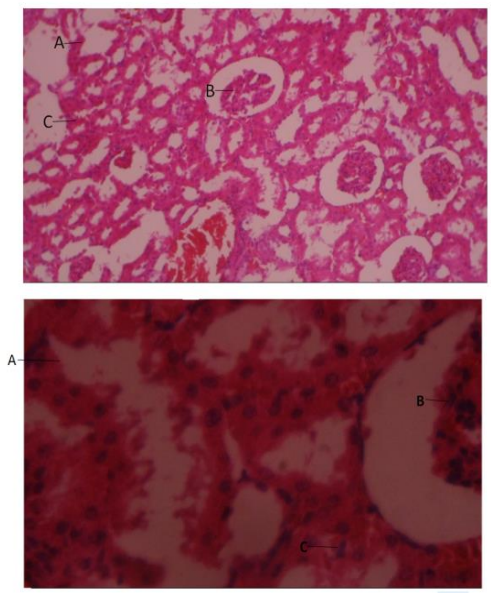
Histological evaluation

Histological evaluation serves as a crucial tool for assessing the potential toxicological effects of bioactive compounds or extracts on vital organs. Histological examination of the kidneys revealed no significant differences between the control group (Plate 4) and the groups administered *JSL* extract at doses up to 5000 mg/kg (Plates 5, 6, 7 and 8). This absence of discernible histopathological alterations suggested that the administration of JSL extract at the tested doses did not induce overt toxicological effects on renal architecture (Onochie *et al*., 2020). These results are indicative of the safety of JSL extract within the dosage range investigated in this study.

The examination of the histological slides also revealed additional features in the JSL extract-fed groups (Plates 5, 6, 7, 8, and 9) compared to those in the control group. Plates 5, 6, and 7 exhibited signs of vasodilation and increased blood flow, indicating of potential physiological responses to the administration of JSL extract. The observed vasodilation and increased blood flow may suggest a regulatory effect of the extract on renal vasculature, possibly mediated through vasodilatory mechanisms of its bioactive constituents (Radajewska *et al*., 2023).

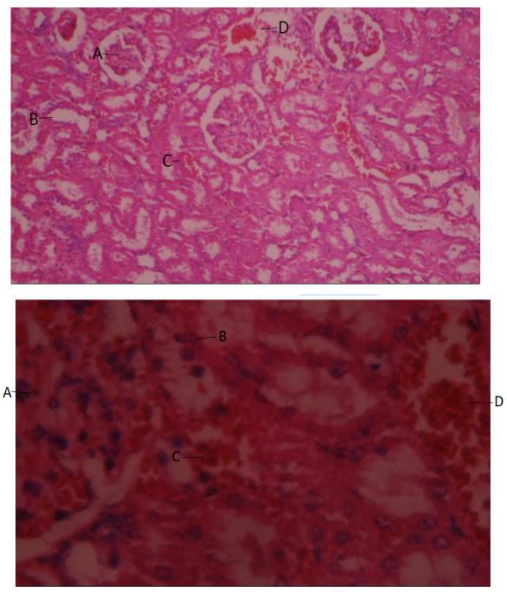
Interestingly, the degree of vasodilation and increased blood flow appeared to decrease with increasing doses of *JSL* extract, indicating a dose-dependent response. This observation raises intriguing questions regarding the dose-response relationship and the pharmacokinetic properties of *JSL* extract constituents.

Additionally, Plates 8 and 9 exhibited some degree of interstitial congestion, suggesting alterations in renal interstitial fluid dynamics following JSL extract administration. Although mild, interstitial congestion may reflect transient physiological changes or adaptive responses to the bioactive components present in the extract (Ashtiyani *et al*., 2013). Further studies are necessary to delineate the nature and implications of interstitial congestion observed in these groups.



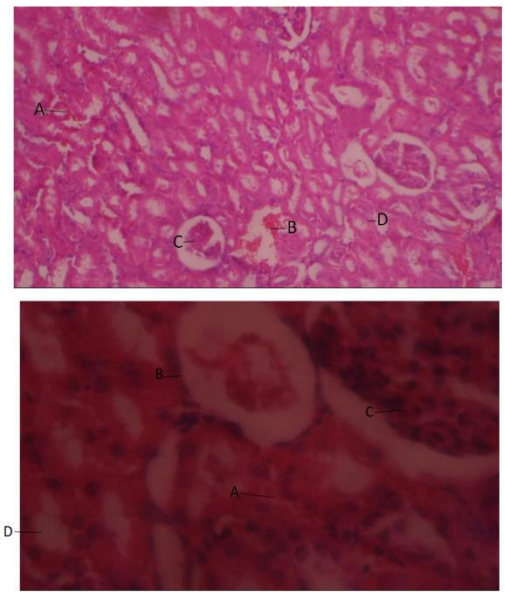
(a) magnification 100×
(b) magnification 400×

Plate 4: Rat kidney from the control group composed of normal architecture A= tubules, B = glomeruli, C = interstitial space



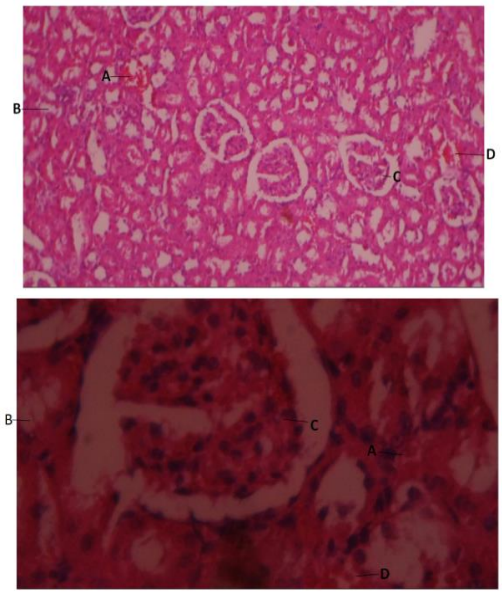
(a) magnification 100×
(b) magnification 400×

Plate 5: Rat kidney from the group given 100 mg of the methanol extract of *JSL* showing normal architecture
A =glomeruli, B = tubules, C = active interstitial congestion, D = vasodilatation



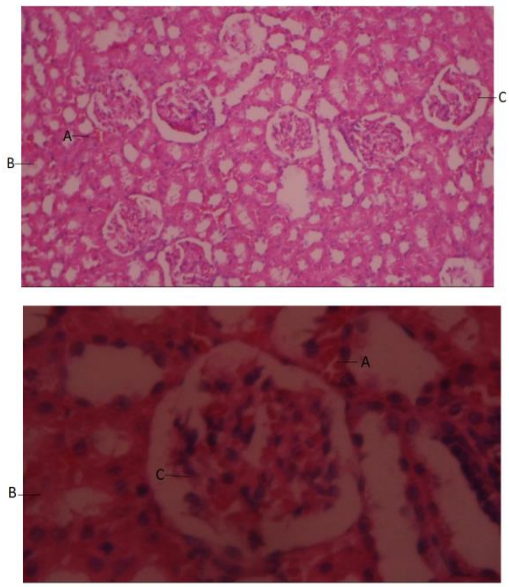
(a) magnification 100×
(b) magnification 400×

Plate 6: Rat kidney from the group given 1000 mg of methanol extract from *JSL* showing normal architecture
A = active interstitial congestion, B = vasodilatation, C = glomeruli, D = tubules



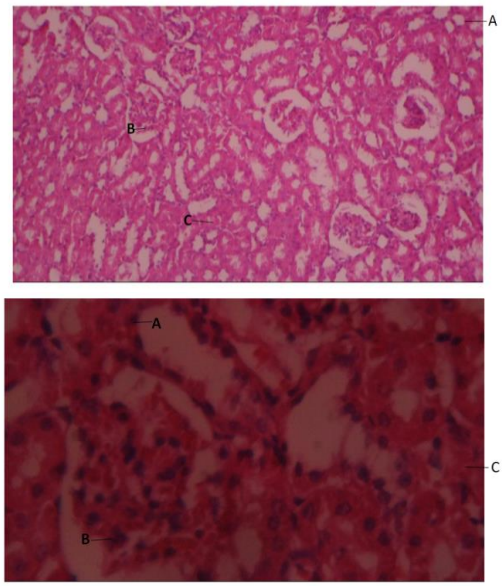
(a) magnification 100×
(b) magnification 400×

Plate 7: Rat kidneys from the group given 1600 mg of the methanol extract of *JSL* showing normal architecture
A = active interstitial congestion, B = tubules, C = glomeruli, D = vasodilatation



(a) magnification 100×
(b) magnification 400×

Plate 8: Rat kidney from the group given 2900 mg/kg of methanol extract of *JSL* showing normal architecture
A = active interstitial congestion, B = tubules,
C = glomeruli



(a) magnification 100×
(b) magnification 400×

Plate 9: Rat kidney from the group given 5000 mg/kg of methanol extract of *JSL* showing normal architecture
A = tubules, B = glomeruli, C = active interstitial congestion

Information regarding the toxic effects of the methanol extract of *JSL* in health care settings exists in research archives. To guarantee the quality of *JSL* for human consumption, a methodical toxicity assessment is needed to estimate the dangers of toxicity and to provide a basis for safe dose selection and scientific data in humans. The acute toxicity study revealed that there were no signs of morbidity or death after two weeks of treatment. The rats were able to tolerate higher doses of *JSL* extract. Therefore, the lethal dose of *JSL* extract should be greater than 5000 mg/kg body weight when taken orally. Consequently, the *JSL* extract may be considered a class 5 drug based on the OECD 423 guidelines (OECD 423, 2001) adopted worldwide synchronized categorization system (GSH) for chemical materials and concoctions. In summary, oral treatment with the methanol extract of *JSL* had no striking negative effects on the body weights or relative organ weights of the fed set.

Conclusion

This study confirmed that the methanol extract of *JSL* contains alkaloids, reducing sugars, saponins, phenolic compounds, terpenoids, and flavonoids. The leaves of *J. secunda* may serve as a significant source of natural antioxidants and antimicrobial agents, which may be helpful in preventing the progression of various oxidative stresses. Although the antibacterial activity of the extract of *JSL* is lower than that of commercial or synthetic antibiotics, it could still offer promise for the development of future antibacterial agents. This study demonstrated the tolerability of *JSL* methanol extract administered at doses up to 500 mg/kg. The nontoxic nature of the methanol extract prepared from *J. secunda* plant was confirmed by an acute oral toxicity test conducted according to the OECD guidelines. The normal behavour of the animals during the 14-day observation period suggested the safe and harmless nature of the methanol extract, even at concentrations up to 5000 mg/kg body weight.

However, it is essential to interpret these results cautiously and recognize the limitations of acute toxicity studies in predicting chronic toxicity or long-term effects. Further investigations, including subchronic and chronic toxicity studies, are warranted to comprehensively evaluate the safety profile of the methanol extract of JSL for potential therapeutic applications. Additionally, future studies should explore the safety profile of JSL extracts in other animal models and assess its potential toxicity in human subjects through clinical trials or observational studies. Such comprehensive safety evaluations are imperative for the responsible development and utilization of herbal medicines derived from *J. secunda*.

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