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Phytochemical screening and *in-vitro* antibacterial activity of leaf extracts of *Justicia secunda* Vahl on selected clinical pathogens

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ABSTRACT: The phytochemical constituents and antibacterial activity of leaf extracts of *Justicia secunda* against five selected clinical pathogens were investigated. Leaves of *Justicia secunda* were obtained and authenticated, and subjected to drying at room temperature for thirty days. The dried leaves were blended into powder for solvent extraction. Aqueous, ethanol and methanol, extracts of the leaves were prepared and concentrated using a rotary evaporator. The phytochemical analysis was carried out using standard methods while the antibacterial activity of the extracts against the clinical isolates was determined using the agar well diffusion method. Saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, anthraquinones and alkaloids were all present in the methanol, ethanol and aqueous extracts, while cardiac glycosides and alkaloids were absent in the aqueous extract. All the extracts had antibacterial activity against the test bacteria at 150 mg/mL, with the exception of the aqueous and methanol extracts that had no activity against *E. coli*. The MIC of the aqueous extract for both *B. cereus* and *L. monocytogenes* was 37.5 mg/mL, 150 mg/mL for *S. aureus*, 18.75 mg/mL for *P. aeruginosa*, with no activity against *E. coli*. The methanol extract of had a minimum inhibitory concentration (MIC) of 18.75 mg/mL on all the tested bacteria, with the exception of *E. coli* with no activity, while the MIC of the ethanol extract on *P. aeruginosa* was 75 mg/mL, and 18.75 mg/mL for the other bacteria. *J. secunda* could be promising in the future design of antibacterial drugs due to its antibacterial properties as observed in this study.

Keywords: In-vitro activity; Phytochemical screening; Antibacterial activity; *Justicia secunda*; Minimum inhibitory concentration (MIC); Clinical pathogens.

1. INTRODUCTION

Acanthaceae is one of twenty-four families in the mint Order (Lamiales) of flowering plants. It contains approximately 220 genera and nearly 4,000 species of plants with *Justicia* L. being the largest genus consisting about 600 species. *Justicia* species are erect perennial herbs that are easily recognized by their

bilabial corolla, two lobed posterior lips, an anterior lip with three lobed, two stamens, a capsule with four seeds, and a basal sterile portion [1, 2].

Justicia secunda Vahl is an uncommon plant, and is used in traditional medicine by local dwellers in the rural communities. The red aqueous extracts of the leaves have been used in the treatment of several conditions including anaemia and hypertension in the Niger Delta region of Nigeria and other countries in Africa. Other local uses of the plant include wound healing, reduction of abdominal and menstrual pains, inducement of abortion, dilation and curettage after cases of miscarriage. The leaves have been used extensively in the treatment of cold, fever, malaria, measles, and whooping cough as reported in several studies [3-5].

The presence of bioactive compounds such as phytochemicals has been reported to be responsible for the pharmacological activities demonstrated by most species of plants [6-8]. Phytochemical screening of *Justicia secunda* has shown the presences of tannins, flavonoids, alkaloids, quinines and anthocyanins [9]. Anthocyanins extracted from *Justicia secunda* have been reported to play a key role in stabilizing the red blood cell membrane and inhibiting polymerization of the haemoglobin [10]. Calderon [11] reported the presence of peptidic alkaloids, phenylalanine derivatives, indoquinoleic alkaloids and triterpenes in the plant. The presence of these typical metabolites has been reported to be responsible for the hypoglycemic, antihyperglycaemic and other known therapeutic properties of the plant [6-8].

Despite the rapid improvement in the health care system and technological advancement in the production of orthodox drugs e.g. antibiotics; there is always one side effect or the other associated with the continuous usage of these drugs. More so, not all of these drugs are affordable to the common man. There is therefore a need to search for alternative sources of therapeutic drugs, in this era of geometric rise in drug resistance. Numerous studies have been carried out on *Justicia secunda*, but not much has been done on its antibacterial activities, hence the need for this study.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant

Justicia secunda plant used for this study was obtained from the Nursery of the Department of Botany, University of Ibadan. The identity of the plant was authenticated at the Herbarium of the same Department and assigned a Voucher Number (UIH-22860).

2.2. Preparation and extraction of leaf extracts of *J. secunda*.

The leaves were plucked from the stem and left to dry for thirty days at room temperature till they turned crispy. They were then crushed into powder using a clean kitchen blender. The sample was divided into three parts for extraction by the three solvents used (water, ethanol and methanol). The extracts were prepared by soaking 50 g of the dry powdered plant material in 1 L of each solvent at room temperature for 48 hours. At the end of the duration, the set-up was filtered, first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extract was concentrated using a rotary evaporator in a water bath set at 40°C.

2.3. Phytochemical analysis of extract of *Justicia secunda* leaves

The phytochemical screening was carried out using standard methods at the Pharmaceutical Chemistry Laboratory, Department of Pharmacy, University of Ibadan, Nigeria. Each of the extracts was screened for the presence of secondary metabolites such as saponins, tannins, flavonoids, cardiac glycosides, terpenoids,

steroids, anthraquinones and alkaloids using standard methods [12-16]. The procedures for the determination of the presence of each metabolite are highlighted as follows:

2.3.1. Test for saponins

Distilled water (5 mL) was added to 0.5 g of sample and shaken vigorously and thereafter observed for a stable persistent frothing which indicated the presence of saponins.

2.3.2. Test for tannins

Distilled water (10 mL) was added to 0.5 g of sample, boiled and filtered. A few drops of ferric chloride solution were added to the filtrate and this was observed for a blue-black colour which indicated the presence of tannin.

2.3.3. Test for flavonoids

Distilled water (5 mL) was added to the sample (0.5 g) and filtered. To the filtrate, 5 mL of ammonium hydroxide was added. A yellow color disappearing on standing with the introduction of concentrated H₂SO₄ (1 mL) indicated the presence of flavonoids.

2.3.4. Test for cardiac glycosides

Distilled water (5 mL) was added to the sample and filtered. This is followed by the addition of 2 mL of ferric chloride solution contained in acetic acid to the filtrate and concentrated H₂SO₄ (1 mL). The observation of a brown colouration at the interface indicated the presence of cardiac glycosides.

2.3.5. Test for terpenes

There was addition of chloroform (2 mL) to 0.5 g of sample, followed by filtration. To the filtrate, concentrated H₂SO₄ (3 mL) was added carefully to form a layer and this was observed for a reddish-brown colouration at the interface.

2.3.6. Test for steroids

Chloroform was added to the sample and filtered. Acetic anhydride was thereafter added to the filtrate and mixed. This was followed by the addition of H₂SO₄. The observation of a green colour indicated the presence of steroids.

2.3.7. Test for anthraquinones

Leaf extract (0.5 g) was boiled and 10 mL of concentrated H₂SO₄ was added. This was filtered while still very hot. The filtrate was then shaken with 5 mL of chloroform and allowed to partition. The chloroform layer was collected using a pipette into another test tube. 1 mL of ammonium hydroxide was added and observed for a colour change which indicated the presence of anthraquinones.

2.3.8. Test for alkaloid

Sample (0.5 g) was diluted in 10 mL acid-alcohol combination (ethanol and HCl) and filtered. To 5 mL of the filtrate, 2 mL of ammonium hydroxide (2 mL) was added followed by 5 mL of chloroform. The solution was shaken and allowed to partition. The chloroform layer was collected in another test tube after which Dragendoff reagent was added and the set-up observed for a reddish-brown precipitate.

2.4. Test bacteria

The test bacteria used were of clinical origin and included: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. They were obtained from the Department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Nigeria and a personal culture collection in the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria. The isolates were resuscitated on nutrient agar (Oxoid, UK) for the determination of the antibacterial activity of the leaf extracts.

2.5. Determination of the antibacterial activity of leaf extracts of *J. secunda*

The media used was Mueller Hinton agar (Oxoid, England). It was prepared according to manufacturer's instruction and sterilized at 121°C for 15 minutes in an autoclave after which it was allowed to cool to around 45°C before pouring into plates. A suspension of the bacteria was prepared by introducing one or two colonies of each organism (18-24 hour old) into a test tube containing sterile normal saline. The turbidity was adjusted to an already prepared 0.5 McFarland standard. The standardized inoculum was introduced into already prepared Mueller Hinton agar plates by swabbing using sterile swab sticks. Wells were then bored in the plates using a 6 mm cork borer and the 150 mg/mL concentration of the three extracts were introduced into the wells using a sterile pipette. The set-up was incubated at 35±2°C for 18-24 hours after which the zones of inhibition were measured using a graduated ruler. Ciprofloxacin and streptomycin were used as positive controls.

2.6. Determination of the minimum inhibitory concentration (MIC) of the extracts on the test bacteria

Four concentrations (150 mg/mL, 75 mg/mL, 37.5 mg/mL and 18.75 mg/mL) of the aqueous, ethanol and methanol extracts of the leaves of the plants were prepared by standard dilution. Wells were made on already prepared Mueller Hinton agar plate seeded with the test bacteria (as described previously). The different concentrations of the leaf extracts were thereafter applied to the wells using sterile pipette. The set-up was incubated at 35±2°C for 18-24 hours after which the zones of inhibition were observed and the diameter measured. The least concentration showing inhibitory activity against the test bacteria was taken as the MIC.

3. RESULTS

Table 1 is showing the qualitative analysis of phytochemicals present in the aqueous, ethanol and methanol leaf extracts of *Justicia secunda*. Saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, anthraquinones and alkaloids were all present in the aqueous, ethanol and methanol extracts, the only exception being cardiac glycosides and alkaloids which were absent in the aqueous leaf extract.

Table 2 is showing the diameter of the zones of inhibition of leaf extracts of *Justicia secunda* on the test bacteria at 150 mg/mL. The three extracts had antibacterial activity on *Bacillus cereus*, with the highest zone of inhibition observed with the methanol extract (12.0 mm). The values were however lower compared to the 22.0 mm and 16.5 mm inhibition diameter for ciprofloxacin and streptomycin respectively. All the extracts had antibacterial activity on *S. aureus*, with the highest diameter for the zone of inhibition recorded for the aqueous extract (13.0 mm). *S. aureus* was resistant to streptomycin, while there was activity against the organism by ciprofloxacin (19.5 mm). The diameter of the zone of inhibition for the aqueous, ethanol and methanol extracts against *L. monocytogenes* were 14.0 mm, 14.0 mm and 10.0 mm respectively. The inhibition zones for the antibiotics were higher than the zones observed for the extracts. There was activity

against *P. aeruginosa* by the three extracts with the least zone observed with the ethanol extract (8.5 mm). Ciprofloxacin and streptomycin had 27.0 mm and 9.5 mm diameter of inhibition respectively. The aqueous and methanol extracts of *J. secunda* had no antibacterial activity against the test bacteria, but the ethanol extract had a zone of inhibition of 8.0 mm.

Table 1. Phytochemical constituents of methanol, ethanol and aqueous leaf extracts of *J. secunda*.

Test	Methanol	Ethanol	Aqueous
Saponins	+	+	+++
Tannins	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	++	++	-
Terpenoids	+	+	+
Steroids	+	+	+
Anthraquinones	+	++	+
Alkaloids	++	+	-

Key: -: Absent; +: Present; ++: Highly present; +++: Abundant.

Table 2. Diameter of zones of inhibition (mm) of the extracts of *Justicia secunda* against test bacteria at 150 mg/mL concentration.

Test bacteria	Aqueous extract	Ethanol extract	Methanol extract	CIP	STR
<i>Bacillus cereus</i>	11.0±0.02	11.5±0.03	12.0±0.01	22.0±0.03	16.5±0.01
<i>Staphylococcus aureus</i>	13.0±0.01	8.0±0.02	12.5±0.02	19.5±0.01	R
<i>Listeria monocytogenes</i>	14.0±0.03	14.0±0.01	10.0±0.01	23.5±0.02	17.5±0.01
<i>Pseudomonas aeruginosa</i>	11.5±0.01	8.5±0.03	9.0±0.01	27.0±0.02	9.5±0.03
<i>Escherichia coli</i>	NA	8.0±0.01	NA	R	12.0±0.01

Note: Each value is a mean of three replicates. Key: NA: No activity; R: Resistant; CIP: Ciprofloxacin; STR: Streptomycin.

Table 3. Minimum inhibitory concentration (MIC) of the extracts of *Justicia secunda* on selected test bacteria (mg/mL).

Test bacteria	Aqueous extract	Ethanol extract	Methanol extract
<i>Bacillus cereus</i>	37.5	18.75	18.75
<i>Staphylococcus aureus</i>	150	18.75	18.75
<i>Listeria monocytogenes</i>	37.5	18.75	18.75
<i>Pseudomonas aeruginosa</i>	18.75	75	18.75
<i>Escherichia coli</i>	NA	18.75	NA

Key: NA: No activity.

Table 3 is showing the minimum inhibitory concentration (MIC) of the leaf extracts of *J. secunda* at four different concentrations (150 mg/mL, 75 mg/mL, 37.5 mg/mL and 18.75 mg/mL) on the test bacteria. The MIC of the aqueous extract for both *B. cereus* and *L. monocytogenes* was 37.5 mg/mL, while it was 150 mg/mL for *S. aureus* and 18.75 mg/mL for *P. aeruginosa*. There was no activity by the aqueous extract on *E. coli*. The methanol leaf extract of *J. secunda* had an MIC of 18.75 mg/mL on all the tested bacteria, with the

exception of *E. coli* to which the extract had no activity, while the MIC of the ethanol extract on *P. aeruginosa* was 75 mg/mL, with the MIC for the other bacteria observed at 18.75 mg/mL.

4. DISCUSSION

Justicia secunda is a largely uncultivated plant known locally for its use in traditional medicinal preparations. The plant has been used in the treatment of wounds, anaemia and abdominal pain [4, 17]. The anti-sickling, haematinic, antimicrobial and anti-hypertensive activities of *Justicia secunda* have been reported by different authors [10, 18]. The phytochemical screening of this plant by several researchers has reported the presence of tannins, saponins, steroids, alkaloids, anthocyanins and other flavonoids [5, 10, 19]. These findings showed that *Justicia secunda* is a promising medicinal plant that could be of a good use in the search for alternative to synthetic drugs.

In this study, the qualitative phytochemical screening of *Justicia secunda* leaf extracts showed that saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, anthraquinones and alkaloids were present in the ethanol and methanol extracts of the plant, however cardiac glycosides and alkaloids were absent in the aqueous extract. The presence of saponins, tannins, flavonoids, steroids and alkaloids in *Justicia secunda* leaf is consistent with the studies carried out by Koffi et al. [5], Mpiana et al. [10] and Osioma and Amarachee [19], while the presence of terpenoids and cardiac glycosides in the aqueous leaf extract of *Justicia secunda* is in accordance with the work of Rojas *et al.* [20]. However, the absence of cardiac glycosides in the aqueous extract is in sharp contrast with their findings. Anthraquinones and alkaloids were present in the ethanol extract of *Justicia secunda* as documented by Akibou et al. [21]. The presence of alkaloid in the aqueous extract is however contrary to the report of Mea et al. [22] while the relative abundance of saponins in the aqueous extract is in agreement with their study. These variations could be the adduced to several environmental factors which could have directly or indirectly influence the bioactive components in the plant in different regions.

Phytochemicals are known to demonstrate a variety of biological activities. Literature suggests that plants that are rich in flavonoids and alkaloids exhibit antimicrobial, anti-inflammatory and antioxidant effects [23]. Terpenoids possess unique antioxidant activity in their interactions with free radicals while steroids play significant roles as anti-cancer agent [24], anti-hormones [25], antimicrobials [26], and cardiovascular agents [27] amongst others. Cardiac glycosides have been reported to possess anticancer properties while saponins on the other hand, have also been reported to possess antibacterial, anti-inflammatory, anti-ulcer properties [28]. Anthraquinones have been found to inhibit the growth and proliferation of various cancer cells as reported by Meggie et al. [29] and Chen et al. [30].

The ethanol extract of *Justicia secunda* exhibited antibacterial activity on all the tested bacteria in this study, which is in contrast to the findings of Rojas et al. [20], in which the ethanol extracts of *Justicia secunda* was ineffective against *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The sensitivity of *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* to the aqueous extracts of *Justicia secunda*, to which *Escherichia coli* was resistant to agrees with the study of Herrera et al. [31], where they reported the sensitivity of *Bacillus* sp. and *Staphylococcus aureus* to the aqueous leaf extracts of *J. secunda*, with *Escherichia coli* showing resistance. However, the resistance of *Escherichia coli* to the aqueous extract is not in agreement with the observation of Rojas et al. [20] who reported antibacterial activity of the aqueous extract on *Escherichia coli* in their study.

The antibacterial activity of *Justicia secunda* could be linked to the presence of secondary metabolites such as flavonoids, saponins, tannins and terpenoids. Reports have it that antimicrobial compounds in the

plants act by forming a protective cover, inhibiting the action of microbial enzymes [32] aiding the disruption of the plasma membrane and deprivation of substrates required for growth by the organisms [33, 34]. Despite the fact that the antibacterial activity of the leaf extracts of *Justicia secunda* is lower in comparison to commercial or synthetic antibiotics, it could still offer a promise in the development of future antibacterial agents.

5. CONCLUSION

This study showed that the aqueous, ethanol and methanol extracts of *Justicia secunda* exhibited varying degree of antibacterial activity on most of the tested clinical bacteria. The ethanol leaf extract was most effective on the test organisms as no resistance was observed to it. The leaves of *Justicia secunda* could be a potential source of natural antibiotics for drug development based on the antibacterial activities observed in this study.

Authors Contributions: AEA designed the study. AOA and OIO performed the laboratory experiments and acquired the data. AEA and AOA wrote the first draft of the manuscript. AOA and OIO did the typesetting of the first draft. All the authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflicts of interest.

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