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Antimicrobial efficacy of *Justicia secunda* stem crude extracts

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ABSTRACT

Aim: This study was aimed to investigate antimicrobial efficacy of crude extracts from *Justicia secunda* stem.

Method and Materials: A total of seven pathogenic microbes, including *Staphylococcus aureus*, were subjected to testing against the extracts obtained using different solvents in their order of polarity.

Results: The results demonstrated selective sensitivity, with *Staphylococcus aureus* being the only microbe affected. Notably, methanol extracts exhibited the highest antimicrobial activity (10.5 mm zone of inhibition), followed by ethyl acetate extracts (8.0 mm zone of inhibition). The findings highlight the crucial role of solvent selection in obtaining bioactive compounds from *Justicia secunda* stem.

Conclusion: It was concluded that the potential of *Justicia secunda* as a source for developing new antimicrobial agents to combat *Staphylococcus aureus* infections, validating its traditional use in folk medicine.

Keywords: Antimicrobial resistance, *Justicia secunda*, crude extracts, pathogenic microbes, methanol extracts and ethyl acetate extracts, *Staphylococcus aureus*.

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Introduction

Antimicrobial resistance's growing threat has recently drawn attention on a global scale. Since pathogenic microorganisms including bacteria, fungi, and viruses have become resistant to common antimicrobial drugs, many current therapies are no longer effective. The ability to fight infectious diseases is compromised by this phenomenon, which also raises the risk of serious infections and treatment failures. This phenomenon represents a substantial problem for healthcare systems around the world (ECDC Antimicrobial Resistance and H, 2010). Researchers and scientists have stepped up their efforts to look for other sources of antimicrobial drugs in response to this urgent problem. Since natural plant products have a long history of usage in traditional medicine for treating a variety of disorders, including infectious diseases, they have shown significant promise in this respect.

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Medicinal plants could offer an alternative treatment option for non-severe cases of infectious diseases, and they may also serve as a viable source for potent antibiotics resistant to pathogen strains (Fabricant & Farnsworth, 2001). In developing nations, especially in Colombia, individuals with low incomes, such as farmers, residents of small isolated villages, and indigenous communities, rely on folk medicine as a remedy for common infections. They utilize various parts of plants, preparing them as decoctions, teas, or juices to address respiratory infections (Gonzalez, 1980). Native American tribes and local healers have long used the stems of *Justicia secunda* to treat infections and other illnesses. Due to these historical applications, scientists are now interested in exploring the plant's possible antibacterial properties. According to preliminary research, *Justicia secunda* stem extracts may contain bioactive substances with potent antibacterial activity (Irinmwinuwa *et al.*, 2023).

However, there hasn't been any thorough scientific investigation into the stem crude extracts of *Justicia secunda*, despite its historical use and early signs of antimicrobial activity. A thorough and methodical examination into the plant's antibacterial activity against a variety of pathogenic microbes is essential to determine its potential as a useful antimicrobial agent. This

study aims to fill this research gap and shed light on the antimicrobial efficacy of *Justicia secunda* stem crude extracts. By subjecting the plant extracts to various antimicrobial assays, including agar disc diffusion, broth dilution, and minimum inhibitory concentration (MIC) tests, the researchers seek to assess the extracts' ability to inhibit the growth of bacteria, fungi, and possibly viruses. The findings of this research could hold the promise of contributing to the growing body of knowledge on natural antimicrobial agents and have significant implications for the development of new antimicrobial agents. If *Justicia secunda* stem extracts demonstrate potent antimicrobial properties, they may serve as a valuable natural resource for the development of novel drugs or complementary treatments to combat infections, especially those caused by drug-resistant pathogens.

Materials and Methods

Sample Collection and Extraction

The stem of *Justicia secunda* was collected from their natural habitat in Wukari Local Government Area of Taraba State, Nigeria. The fresh stems of *Justicia secunda* were carefully washed with distilled water, then cut into smaller segments and air-dried for three weeks in a well-ventilated environment." The plant material was methodically pounded into a fine powder using a mortar and pestle when it had totally dried. The study collected and prepared the *Justicia secunda* stem for extraction using the cold maceration method as described by Ushie *et al.* (2019) and Bako *et al*. (2023). The plant material was successively extracted with solvents of increasing polarity (hexane, ethyl acetate, acetone and methanol) and the resulting extracts were stored for further testing and analysis.

Antimicrobial Activities of the Crude Extracts

The crude extracts were tested for antibacterial and antifungal activities using Minimum Inhibitory Concentration (MIC) described by Ushie *et al*. (2016) and Bako *et al*. (2023) with modification.

The antimicrobial activities of *Justicia secunda* stem crude extracts were assessed against various pathogenic microbes obtained from the Department of Medical Microbiology at Ahmadu Bello University Teaching Hospital, Zaria. *Justicia secunda* stem extracts were tested on seven (7) different test organisms, including *Staphylococcus aureus, Streptococcus Pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Candida albican*and *Aspergillus niger*. A 0.001 mg amount of each extract were dissolved in 10 ml DMSO to form a 100 µg/ml concentration. The diffusion method with Mueller Hinton agar was used to screen the compound's antimicrobial activities. After incubation at 37 °C for 24 hours, the growth inhibition zone was observed visually, measured and recorded in millimeters.

Minimum Inhibitory Concentration

The MIC was evaluated on plant extracts that showed antimicrobial activity. This test was performed at four concentrations of each extract (6.25, 12.5, 25, 50, 100 µg/ml) employing the broth dilution method as described by Ushie*et al.,* (2019). Two-fold serial dilution of the compound was done in the sterile broth to obtain the concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml. The initial concentration was obtained by dissolving 0.001 mg of the compound in 10 ml of the sterile broth. Having obtained the different concentrations of the compound in the sterile broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37 ^{o}C for 24 hr, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the compound in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration.

Results and Discussion

Antimicrobial Activities of *Justicia secunda* stem crude extracts and control (Table 1) and minimum inhibitory concentration (MIC) of *Justicia secunda* stem crude extracts (Table 2).

The antimicrobial activity testing of crude extracts from *Justicia secunda* stem (Table 1) revealed sensitivity only against *Staphylococcus aureus* among the tested microbes. The methanol extracts exhibited the highest activity with a zone diameter of inhibition of 10.5 mm, followed by the ethyl acetate extracts with 8.0 mm for *Staphylococcus aureus*. The selective activity against *Staphylococcus aureus* suggests specific interactions with its cellular structures. The observed difference in antimicrobial activity among the extracts obtained using different solvents implies that the choice of solvent influences the extraction of bioactive compounds from the plant material.

Table 1. Antimicrobial activities of *Justicia secunda* stem crude extracts and control

Test Organism	JSSHE	JSSEE	JSSAE	JSSME	Streptomycin	Ketokonazole
Staphylococcus aureus	**R**	**S**	**R**	**S**	**S**	**R**
Streptococcus Pneumoniae	**R**	**R**	**R**	**R**	**S**	**R**
Pseudomonas aeruginosa	**R**	**R**	**R**	**R**	**S**	**R**
Escherichia coli	**R**	**R**	**R**	**R**	**S**	**R**
Salmonella typhi	**R**	**R**	**R**	**R**	**S**	**R**
Candida albican	**R**	**R**	**R**	**R**	**S**	**S**
Aspergillus niger	**R**	**R**	**R**	**R**	**R**	**S**

Keywords abv: R = Resistance **S =** Sensitive **JSSHE**: *Justicia secunda*Stem Hexane Extracts, **JSSEE**: *Justicia secunda* Stem Ethyl Acetate Extracts, **JSSAE**: *Justicia secunda* Stem Acetone Extracts, **JSSME**: *Justicia secunda* Stem Methanol Extracts

Table 2. Minimum Inhibitory Concentration (MIC) of *Justicia secunda* stem crude extracts

Test Organism	JSSHE	JSSEE	JSSAE	JSSME
Staphylococcus aureus	000	400	000	400
Streptococcus Pneumoniae	000	000	000	000
Pseudomonas aeruginosa	000	000	000	000
Escherichia coli	000	000	000	000
Salmonella typhi	000	000	000	000
Candida albican	000	000	000	000
Aspergillus niger	000	000	000	000

Keywords abv: JSSHE: *Justicia secunda* Stem Hexane Extracts, **JSSEE**: *Justicia secunda* Stem Ethyl Acetate Extracts, **JSSAE**: *Justicia secunda* Stem Acetone Extracts, **JSSME**: *Justicia secunda* Stem Methanol Extracts

In this case, methanol appeared to be the most effective solvent for extracting compounds with potent antimicrobial properties against *Staphylococcus aureus*. *Staphylococcus aureus* is a notorious pathogen responsible for a wide range of infections, including skin and soft tissue infections, pneumonia, and bloodstream infections (Ayodele et al., 2020). Overall, the findings from our study align with and complement the existing body of research on the antimicrobial properties of *Justicia secunda* stem extracts, supporting its potential as a promising source for developing new antimicrobial agents to combat *Staphylococcus aureus* infections (Irinmwinuwa et al., 2023).

The traditional use of *Justicia secunda* in folk medicine for treating infections might be validated by these findings, specifically for the treatment of *Staphylococcus aureus* related infections. It highlights the importance of exploring traditional medicinal practices and the potential of natural sources for modern drug development. The validation of traditional medicinal use and the importance of exploring natural sources for drug development further strengthen the relevance and significance of this research in the field of antimicrobial studies. (Suleiman *et al.*, 2019; Ayodele *et al.*, 2020; Świątek *et al.*, 2023) Therefore, it indicates the potential of *Justicia secunda* as a source for developing new antimicrobial agents against *Staphylococcus aureus* infections, warranting further investigation to identify and isolate the active compounds for future drug development and validation of traditional medicinal use.

Conclusion

It was concluded that antimicrobial activity testing of crude extracts from *Justicia secunda* stem revealed selective sensitivity against *Staphylococcus aureus*, with methanol extracts displaying the highest activity. The findings suggest the potential of *Justicia secunda* as a source for developing new antimicrobial agents to combat *Staphylococcus aureus* infections, validating its traditional use in folk medicine. Further investigation to identify and isolate the active compounds is warranted for potential drug development and clinical applications.

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