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Research Article

Antibacterial Activity of Some Medicinal Plants Against Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* and *Klebsiella pneumonia* in Khartoum State – Sudan

Nashwa Azhari[1], Mohamed H. Arbab[2*], Ahmed Khidir[3], Mohamed M. Babeker[3] and Sahar Mohammed Sedahmed[3]

[1] *Sudan International University - Faculty of Medical Laboratory Sciences - Department of Medical Microbiology - Khartoum, Sudan.*

[2] *Associate Professor – Medical Microbiology Faculty of Medical Laboratory Sciences - Omdurman Ahlia University, Khartoum, Sudan.*

[3] *Faculty of Medical Laboratory Sciences - Omdurman Ahlia University, Khartoum, Sudan.*

***Corresponding Author:** Mohamed H. Arbab, Associate Professor, Medical Microbiology Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan Khartoum, Sudan.*

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Abstract

The rising threat of antimicrobial resistance, particularly among Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria, has spurred interest in alternative sources of antibacterial agents. This study evaluates the antibacterial efficacy of three medicinal plants—*Acacia nilotica (Fabaceae), Syzygium aromaticum (Myrtaceae),* and *Ziziphus spina-christi (Rhamnaceae*) against *ESBL*-producing *Escherichia coli* and *Klebsiella pneumoniae*. Clinical isolates of these pathogens were obtained from hospitals in Khartoum, Sudan. The plants' active compounds were extracted using 80% ethanol, and their antibacterial activity was assessed via the cup-plate agar diffusion method. Additionally, the Minimum Inhibitory Concentration (MIC) of each extract was determined. The results indicated that *A. nilotica* and S. *aromaticum* extracts exhibited significant antibacterial activity, while *Z. spina-christi* demonstrated no effect against the tested strains. These findings underscore the potential of *A*. *nilotica* and *S*. *aromaticum* as sources for developing novel antibacterial agents targeting ESBL-producing pathogens.

Keywords: *Antimicrobial resistance, ESBL, Escherichia coli, Klebsiella pneumoniae, Medicinal plants, Antibacterial activity.*

Introduction

The global rise of antimicrobial resistance poses a severe threat to public health, particularly with the proliferation of *ESBL*-producing pathogens like *Escherichia coli* and *Klebsiella pneumoniae*. These bacteria are commonly implicated in a wide range of infections, including urinary tract infections, pneumonia, and septicemia, particularly in healthcare settings. The emergence of resistance to multiple antibiotics in these organisms has created an urgent need for alternative therapeutic options. In many parts of the world, including Sudan, traditional medicine remains a primary source of healthcare. Medicinal plants are integral to this tradition, often used to treat various ailments due to their bioactive compounds, including tannins, alkaloids, *flavonoids*, and *saponins*, which have been shown to possess significant antimicrobial properties (2-5), Among the most widely used medicinal plants in Sudan are *Acacia nilotica*, *Syzygium aromaticum*, and *Ziziphus spina-christi*, each known for its broad spectrum of therapeutic applications (6-9). *Acacia nilotica*, commonly referred to as the Egyptian thorn or gum Arabic tree, belongs to the *Fabaceae* family. Traditionally, it has been used in the treatment of a variety of conditions (10-15), including gastrointestinal disorders, respiratory infections, and skin diseases. *Syzygium aromaticum*, or clove, from the *Myrtaceae* family, is well known for its analgesic, anti-inflammatory (16), and antimicrobial properties. It is widely used in dental care as an antiseptic and for its ability to alleviate pain.

Ziziphus spina-christi, from the *Rhamnaceae* family, also known as Christ's thorn Jujube (17 - 20), has been utilized in traditional medicine for treating wounds, ulcers, and inflammatory conditions. Given the increasing resistance of bacteria to conventional antibiotics, this study aims to explore the potential of these three plants as alternative sources of antibacterial agents against ESBL-producing *E. coli* and *K. pneumoniae* (20-23).

Materials and Methods

Collection of Plant Materials: The plant materials were collected from different regions across Sudan. *Acacia nilotica* pods, *Syzygium aromaticum* flower buds, and *Ziziphus spina-christi* leaves were authenticated by a botanist at the National Herbarium in Khartoum. After collection, the materials were air-dried under shade, ground into a fine powder using a mechanical grinder, and stored in airtight containers until extraction

Preparation of Plant Extracts: Ethanolic extraction was performed using 80% ethanol (v/v) by cold maceration. Approximately 100 grams of each powdered plant material were soaked in 500 ml of ethanol for 72 hours with occasional stirring. The extracts were then filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C to obtain the crude extracts. The yields were calculated based on the initial dry weight of the plant material.

Identification of Clinical Isolates: A total of 50 clinical isolates of *ESBL*-producing *E. coli* and *K. pneumoniae* were collected from patients at Soba University Hospital, Fedail Hospital, and Sahiroon Specialized Hospital in Khartoum, Sudan. The bacteria were identified and confirmed as ESBL producers using standard microbiological techniques, including the double-disk synergy test (DDST).

Antibacterial Activity Assay: The antibacterial activity of the crude extracts was evaluated using the cup-plate agar diffusion method. Muller-Hinton agar plates were inoculated with standardized bacterial suspensions (0.5 McFarland standard), and wells were cut into the agar using a sterile cork borer. Each well was filled with 100 µl of the plant extract at a concentration of 100 mg/ml. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC): The MIC of each extract was determined using the broth micro dilution method. The plant extracts were serially diluted in Mueller-Hinton broth to obtain concentrations ranging from 100 mg/ml to 0.78 mg/ml. The bacterial inoculums were added to each well, and the plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth.

Results

Yields of Extraction: The yields of the crude ethanol extracts were 18.88% for *Syzygium aromaticum*, 10.23% for *Acacia nilotica*, and 8.55% for *Ziziphus spina-christi*. These yields reflect the varying concentrations of bioactive compounds within each plant material. Antibacterial Activity of Crude Extracts: The ethanolic extracts of *Acacia nilotica* and *Syzygium aromaticum* exhibited notable antibacterial activity against both *E. coli* and *K. pneumoniae*. The zones of inhibition ranged from 20 to 39 mm for *A. nilotica* and 18 to 30 mm for *S. aromaticum*. However, the *Ziziphus spina-christi* extract showed no significant antibacterial activity against the tested strains, with inhibition zones measuring less than 10 mm, suggesting minimal or no activity.

Minimum Inhibitory Concentration (MIC): The MIC values for *Acacia nilotica*and *Syzygium aromaticum* were found to be ≤12.5 mg/ml, indicating strong antibacterial activity. In contrast, the MIC for *Ziziphus spina-christi* exceeded 100 mg/ml, corroborating its lack of significant antibacterial effect.

Table 1

No.	*A. nilotica*		*S. aromaticum*		*Z. spina-christi*	
	E. Coli	*K. pneumonia*	*E. Coli*	*K. pneumonia*	*E. Coli*	*K. pneumonia*
MDIZ* (mm) ± SD						
1.	30 ±0.01	29 ±0.03	28 ±0.05	28 ±0.02	-	-
2.	25 ±0.05	22 ±0.02	24 ±0.01	23 ±0.07	-	-
3.	25 ±0.07	40 ±0.01	30 ±0.03	24 ±0.08	-	-
4.	25 ±0.03	35 ±0.02	18 ±0.02	28 ±0.04	-	-
5.	39 ±0.01	30 ±0.09	28 ±0.05	25 ±0.01	-	-
6.	36 ±0.05	30 ±0.03	21 ±0.02	21 ±0.06	-	-
7.	39 ±0.01	35 ±0.08	22 ±0.01	18 ±0.09	-	-
8.	31 ±0.04	33 ±0.03	18 ±0.01	24 ±0.03	-	-
9.	28 ±0.02	30 ±0.05	20 ±0.06	28 ±0.07	-	-
10.	30 ±0.01	37 ±0.09	23 ±0.02	30 ±0.08	-	-
11.	25 ±0.07	38 ±0.06	25 ±0.01	25 ±0.04	-	-
12.	30 ±0.02	39 ±0.05	24 ±0.03	18 ±0.05	-	-
13.	28 ±0.01	35 ±0.03	25 ±0.04	23 ±0.07	-	-
14.	30 ±0.02	39 ±0.06	24 ±0.03	24 ±0.05	-	-
15.	25 ±0.05	38 ±0.01	22 ±0.06	22 ±0.02	-	-
16.	27 ±0.04	36 ±0.02	21 ±0.03	20 ±0.07	-	-
17.	31 ±0.01	39 ±0.09	18 ±0.09	24 ±0.02	-	-
18.	25 ±0.07	31 ±0.08	24 ±0.07	25 ±0.01	-	-
19.	26 ±0.03	35 ±0.06	28 ±0.05	20 ±0.03	-	-
20.	28 ±0.01	31 ±0.07	25 ±0.01	22 ±0.04	-	-
21.	22 ±0.04	39 ±0.01	24 ±0.03	24 ±0.08	-	-
22.	34 ±0.02	30 ±0.06	21 ±0.05	21±0.06	-	-
23.	22 ±0.01	39 ±0.03	25±0.01	28 ±0.04	-	-
24.	20 ±0.05	36 ±0.04	18±0.08	18 ±0.03	-	-
25.	27 ±0.01	31 ±0.07	30 ±0.07	24 ±0.05	-	-

Key: MDIZ* (mm) = Mean diameter of growth inhibition zone in mm. **Interpretation of results**: MDIZ (mm): < 9 mm zone was considered as inactive; 9-12 mm as partially active; while13-18 mm as active and >18 mm as very active (28), (-): No inhibition zone, concentration used 100 mg/ml of 0.1ml/cup for extracts.

Discussion

The purpose of this study was to determine the antibacterial activity of some natural plant extracts against *ESBL*-producing *E. Coli* and *K. pneumonia*. Medicinal plants can offer valuable phytochemicals constituents which can play a significant role in fighting infection. Also, natural products have become the current idea of pharmacological research and drug discovery. Investigated the antibacterial activity of three medicinal plants using cup-plate agar diffusion method. Plants tested counter to Gram-negative bacteria (*E. Coli* and *K. pneumonia*). There are slightly different activities between them, and this could be attributed to itschemical constituents. Generally, the biological activities of Sudanese herbal medicines connected with their ability to treat or prevent ailments, the most bioactive compounds recognized in these plants are phenolics, alkaloids, tannins, flavonoids, saponins, and steroids (12).

In this study, *A. nilotica* and *S. aromaticum* showed the best antibacterial result for Gram-negative bacteria (*E. Coli* and *K. pneumonia*). Several studies have reported the antibactrial activity of *A. nilotica* against a wide range of bacteria, and our finding ultimately agreed with their finding (29-31). The result of this study is in accordance with previous reports suggesting potent antibacterial activity of *A. nilotica* (32-35). These antibacterial activities may be due to presence of flavonoids, tannins, saponins, glycosides, steroids, saponin glycosides, terpenes and phenols which were previously isolated from A. *nilotica* and showed significant activity towards bacteria (36). For *S. aromaticum* our result likely agree with the previous study stated that this herb has antimicrobial properties after investigation of antimicrobial activity crude extract against Gram-positive, Gram-negative and antifungal (37, 38). (39) found similar to our result that the volatile oil of *S. aromaticum* showed a broad spectrum of activity against *E. Coli* and *S. auerus*. Previous study by (40) demonstrated the antibacterial activity of the essential oil exhibited activity from *S. aromaticum* dried flower buds against the Gram-positive *S. aureus* and the two Gram negative organisms (*E. Coli* and *P. aeruginosa*). Another study by (41) found similar to our result that the essential oil of *S. aromaticum* exhibited pronounced and varying degree of growth inhibition against *B. subtilus*, *S. aureus* and *E. Coli*. Therefore, the essential oil of *S. aromaticum* showed high activity against all organisms tested and this may be attributed to the contents of active ingredients such as mono –and sesquiterpene hydrocarbons in the plant detected by many co-workers (42-45). The leaves extract of *Z. spina-christi* has no antibacterial activity against the Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*), as shown in Table (1). (46) found similar to our result that the *Z. spina-christi* showed no antibacterial activity against the Gram-negative bacteria against *Escherichia coli* and *Klebsiella pneumonia*. Another study showed no effect against standard *Escherichia coli* (46).

The antibacterial efficacy of this extract was also evaluated by MIC assay, as shown in Table (1). The results revealed that the pod extract of *A. nilotica* represented effective MIC activity against all tested bacteria, even at low concentration (12.5 mg/ml) against the gram-negative bacteria, inparticular. This is in agreement to some extend with (46) who stated that the MIC of pods of *A. nilotica* aqueous extract were 25 mg/ml for *Bacillus subtilis* and *Klebsiella pneumoniae*, and 200 mg/ ml for *Staphylococcus aureus*, respectively. Finally, the pods of this plant are definitely considered promising source of effective antibacterial drug.

The antibacterial efficacy of this extract was evaluated by MIC assay, as shown in Table (1). The results revealed that the pod extract of *S. aromaticum* represented effective MIC activity against all tested

The interesting outcomes of this investigation providing encourage the antibiotics researchers to give more attention to the natural plant products particularly those mentioned or applied in traditional or folk medicine.

Conclusion

The present study indicated that the pods of *A. nilotica* and *S. aromaticum* have antibacterial activity against the tested organisms, while *Z. spina-christi* has no antibacterial activity. The study also revealed that the highest inhibitory effects of *A. nilotica* and *S. aromaticum* extracts were found against the tested organisms. Further studies should be conducted to isolate and identify the active compounds responsible for the antibacterial effects, as well as to perform toxicological and other necessary pharmacological studies for use in modern drug development.

Conflict of Interest

The authors declare no conflict of interest.

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