Research Article

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Assessment of Antimicrobial Activity of *Acacia nilotica* Extract against Gram-Positive Bacteria Isolated from Clinical Specimens in Shendi Town, Sudan

Leila Mohamed A Abdelgader, Dalia Anwar M Ahmed and Ghanem Mohammed Mahjaf*

Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan

***Corresponding Author**
Ghanem Mohammed Mahjaf, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan.

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Abstract

Background: *Acacia nilotica is one of the products used as an antimicrobial agent since ancient times. With the alarming increase of antibiotic-resistant bacterial strains, Acacia nilotica can be an alternative and safe agent that helps in the treatment of these strains.*

Objectives: *The aim is to evaluate the in vitro antimicrobial activity of different concentrations of Acacia nilotica on gram-positive bacteria isolated from clinical specimens using the agar well diffusion method.*

Methodology: *This is a prospective cross-sectional study in Shendi City, Sudan, during the period from March 2023 to February 2024. A total of 50 samples were collected from urine and wounds, from which four strains of pathogenic gram-positive bacteria were isolated and identified.*

Results: *Out of a total of 50 clinical specimens, Staphylococcus aureus 30 (60%), Staphylococcus epidermidis 13 (26%), Staphylococcus saprophyticus 4 (8%), and Enterococcus fecalis 3 (6%). Acacia nilotica showed remarkable antimicrobial activity against all gram-positive cocci bacteria.*

Conclusion: *The findings of this study showed that Acacia nilotica can be used as an antibacterial agent against gram-positive bacterial strains. The greatest value of the antibacterial effect can be obtained when Acacia nilotica is at a concentration of 100%. As a result, this study established the usefulness of the Acacia nilotica plant as a substitute therapy for bacterial illnesses, one that may be utilized to either totally eradicate or significantly reduce the bacteria's resistance to synthetic antimicrobial drugs.*

Keywords: Antimicrobial, *Acacia nilotica*, Assessment, Sudan

1. Introduction

The primary stay of treatment for microbiological (bacterial and fungal) infections is antibiotics. The medical community had long believed that the development of these antibiotics and their application as chemotherapeutic agents would eventually result in the eradication of infectious diseases. However, the primary cause of the emergence and spread of multi-drug resistance strains of many types of microbes is now the overuse of antibiotics [1]. The global spread of ß-lactamase producers such as *Escherichia coli, Klebsiella pneumoniae, Haemophilus*, and numerous others has emerged as a significant therapeutic challenge. Hospitals are home to several multi-drug resistant strains of *K. pneumoniae* and *E. coli,* which are increasingly being separated from illnesses that are acquired in the community [2, 3]. Natural items have been utilized in traditional medicine for thousands of years, long before antibiotics and other contemporary medications were developed. It has been unbelievable how effective certain plants have been said to be as antimicrobials in treating illnesses. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists [4]. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent [5]. Many secondary metabolites found in plants, including flavonoids, alkaloids, and tannins, have been shown to exhibit antibacterial qualities *in vitro* [6]. *Acacia nilotica* is a thorny tree with yellow flowers. It grows in the dry areas of Africa, Australia, the Arabian Peninsula, and India. This plant has a long history in ancient civilizations, especially among the ancient Egyptians,

Sudanese, and indigenous tribes in Australia. The tree known as *Commiphora myrrha* is used to make myrrh, a resin derived from dried tree sap. The tree is native to the Arabian Peninsula, Oman, Yemen, and Africa (Djibouti, Ethiopia, Somalia, and northeast Kenya). It is called 'Myrrh' in Arabic, meaning bitter [7]. Due to the medical properties of these plants, they have been used to treat many medical conditions, such as respiratory infections, sore throats, and wounds [8]. *Acacia nilotica* is a multipurpose plant that is used for the treatment of various diseases. The plant contains a profile of a variety of bioactive components [9]. The rural population has effectively used *Acacia nilotica* as an astringent, antispasmodic, and aphrodisiac in folk medicine to treat ailments such as tuberculosis, leprosy, smallpox, dysentery, cough, ophthalmia, toothache, and skin cancer [10]. Gram-positive bacteria are a group of heterogenous bacteria that appear purple when stained by gram staining and examined under a microscope. There are two large groups: gram-positive cocci and gram-positive bacilli. These microorganisms have great clinical importance in hospitals because they often require patients to be in intensive care units. Gram-positive cocci are a large family of gram-positive bacteria, and Staphylococci, Streptococci, and Enterococci are large, important genera in this family, which are human pathogens [11]. Therefore, the present work was done to evaluate the antimicrobial activity of *Acacia nilotica* Extract against Gram-positive bacteria Isolated from clinical specimens in Shendi Town, Sudan.

2. Materials and Methods

Study Design

This was a cross-sectional study, conducted in Shendi City that aimed to assess the antimicrobial effect of *Acacia nilotica* on gram-positive bacteria.

Study Area

Different hospitals and clinical centers are located in Shendi locality, River Nile State, Sudan. Shendi is a town in northern Sudan, situated on the east bank of the Nile (150 km) northeast of Khartoum. Shendi is also about 45 kilometers southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is the center of the Ja'aliin tribe and an important historic trading center. Its principal suburb on the west bank is Al-Matamma. A major traditional trade route across the Bayuda desert connects Al-Matamma to Marawi and Napata (250 km) to the northwest.

Study Duration

From March 2023 to February 2024.

Study Populations

This study was performed on patients with symptoms of wound infection and urinary tract infection.

Inclusion Criteria

Patients included in this study were admitted with urinary tract infections and wound infections.

Exclusion Criteria

Patients under antimicrobial treatment were excluded.

Sampling

Different types of samples were collected for this study.

Sample Size

A total of fifty samples (n = 50) were collected.

Data Collection

Data were collected from the patients using a structural questionnaire containing all study variables.

Collection of the Specimens

Under the aseptic condition, wound swabs were collected using sterile cotton swabs moistened with sterile normal saline, and urine was collected in sterile screw-capped universal containers.

Cultivation of the Specimens

Different types of culture media (CLED agar and blood agar) were used for the identification and isolation of clinical isolates.

Interpretation of Cultural Growth

The plates were observed for any bacterial colonies to grow significantly. The bacteria were isolated and then identified by colonial morphology, Gram stain, and biochemical tests.

Identification of the Isolates

The isolated organisms will be fully identified by Gram staining, and the appropriate biochemical tests will follow.

Preparation of *Acacia nilotica* for Testing the Antimicrobial Activity

The extraction of the different plants and preservation of the extracts was done according to the method of Obomanu, et al. [12]. Precisely, about 25 g of each powdered dried seed was extracted for 72 hours using 100 mL of 95% ethanol in a beaker and later transferred to an airtight bottle to avoid loss of the solvent. The sample was filtered using muslin cloth and later filter paper. The solvent was removed by evaporation at room temperature. The extract obtained was kept in an airtight bottle until it was used. Acacia was diluted into different concentrations using D.W. as follows: 100%, 75%, 50%, and 25% to be used against the selected organisms. Clinical isolates were isolated from different samples and subculture. Two ml of normal saline was distributed in test tubes and sterilized in an autoclave at 121°C for 15 minutes. A loopful of purified bacteria was inoculated in sterile normal saline. The inoculum density was compared with the McFarland standard solution.

Preparation of Bacterial Suspension

Clinical isolates were isolated from different samples and subculture. One ml of normal saline was distributed in test tubes and sterilized in an autoclave at 121°C for 15 minutes. A loopful of purified bacteria was inoculated in sterile normal saline. The inoculum density was compared with the McFarland standard solution.

Testing of *Acacia nilotica* for Antimicrobial Activity against Standard Organisms and Clinical Isolates

The agar-well diffusion method is widely used to evaluate the

antimicrobial activity of plants or microbial extracts [13]. Using a sterile wire loop, touch 3-5 well-isolated colonies of similar appearance to the test organism and emulsify in 3-4 ml of sterile physiological saline or nutrient broth. In a good light, match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities, it is easier to compare against a printed card or sheet of paper. Using a sterile swab, inoculate a plate of Mueller-Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate at approximately 60 to ensure even distribution. With the petri dish lid in place, allow 3-5 minutes for the surface of the agar to dry. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer, and acacia is introduced into the well at the desired concentration [13]. Then agar plates are incubated under suitable conditions. By using a ruler on the underside of the plate, measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts [14].

Ethical Approval

Permission was given by the College Ethical Committee of Shendi University and Hospitals. Participants have been noticed no coercion of any sort has been done and any information that may disclose the participator's identity was not considered.

Data Analysis

Data were entered, checked, and analyzed using Microsoft Excel 2007 and SPSS (Statistical Package of Social Science) soft program version 28.0 Proportional data was presented as frequencies and percentages.

3. Results

Table 1: The distribution of clinical specimens according to gender

Gender	Frequency	Percent %
Male	26	52%
Female	24	48%
Total	**50**	**100%**

Table 2: The distribution of clinical specimens according to the sample age

Age group	Frequency	Percent %
15_25 years	13	26%
26_50 years	20	40%
51_75 years	17	34%
Total	**50**	**100%**

Table 3: The distribution of clinical specimens according to the sample Type

Type of sample	Frequency	Percent %
Urine	28	56%
wound swab	22	44%
Total	**50**	**100%**

Table 4: The frequency and percentage of isolated organisms

Pathogen	Frequency	Percent %
S. aureus	30	60%
S. epidermidis	13	26%
S. saprophyticus	4	8%
E. faecalis	3	6%
Total	**50**	**100%**

Table 5: The sensitivity of gram-positive cocci bacteria to Acacia nilotica at different concentration

Pathogen	Concentration of ethanolic A. niltotica extract			
	100%	75%	50%	25%
S. aureus	23	20	18	15
S. epidermidis	22	20	18	14
S. saprophytic	17	14	12	9
E. faecalis	23	18	16	11

Table 6: The result of sensitivity of gram-positive cocci bacteria to antibiotics

Drug	Concentration Mcg	*S. aureus*	*S.epidermidis*	*S.saprophyticus*	*E.faecalis*
Vancomycin	30	13	12	0	21
Gentamicin	10	15	25	0	19
Ceftriaxone	30	18	25	8	24
Meropenem	10	26	22	23	33

Table 7: The sensitivity and resistance of gram-positive cocci bacteria to antibiotic

Pathogen	Vancomycin		Gentamicin		Ceftriaxone		Meropenem	
	S	R	S	R	S	R	S	R
S. aureus	20	10	24	6	25	5	28	2
S. epidermidis	8	5	12	1	11	2	13	0
S.saprophyticus	2	2	2	2	3	1	4	0
E. faecalis	3	0	2	1	3	0	3	0
Percentage	**66%**	**34%**	**80%**	**20%**	**84%**	**16%**	**96%**	**4%**

Table 8: The comparison of sensitivity between gram-positive cocci bacteria to antibiotics and different concentrations of *A. nilotica* extract

Pathogen	Antibiotics				Different concentrations of A. nilotica			
	VA	CN	CRO	MEM	100%	75%	50%	25%
S. aureus	20 (67%)	24 (80%)	25 (83%)	28 (93%)	30 (100%	30 (100%	30 (100%)	30 (100%)
S. epidermidis	8 (61%)	12 (92%)	11 (84%)	13 (100%	13 (100%	13 (100%	13 (100%)	13 (100%)
S.saprophyticus	2 (50%)	2 (50%)	3 (75%)	4 (100%	4 (100%	4 (100%	4 (100%)	4 (100%)
E. faecalis	3 (100%)	2 (67%)	3 (100%	3 (100%	3 (100%	3 (100%	3 (100%)	3 (100%)

4. Discussion

Antimicrobial resistance is the greatest problem in the world that results from misused antimicrobial agents. The emergence of resistant strains of pathogenic bacteria to the most effective antibiotic made us shift to the use of herbal medicine, which can contribute to resolving this problem. The use of *developing antimicrobial resistance quickly A. nilotica* as a traditional remedy for microbial infections dates back to ancient times. Antimicrobial drugs provide the essential basis for the treatment of various microbial infections instead of the elevated genetic inconsistency of some microorganisms enhancing them to develop antimicrobial resistance quickly; therefore, there has been a continuing study for new potential antimicrobial drugs [15]. The present study shows that *A. nilotica* fruit extracts have an inhibitory effect on bacterial growth. The plant extracts show varying degrees of action adjacent to gram-positive bacteria. Extract of Acacia nilotica was investigated (100%, 75%, 50%, and 25%) mg/ml of plant extract was prepared from the stock and examined as anti-bacteria against gram-positive cocci bacteria isolated from the different clinical specimens using the agar well diffusion method. *Acacia nilotica* showed the highest activity against all selected bacteria inhibited by different concentrations. Almost for all bacterial types used in this study, results show that the concentration of 100 mg/ml was the most effective concentration on bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, and Enterococcus fecalis), which agrees with the study by Hameed and his colleagues [16]. Our study agrees with a

study done by Mustafa and his colleagues which shows an extract of *Acacia nilotica* with high activity against S. aureus [17]. Al-Yahya and his colleagues found that both ethanol and chloroform extracts from the *A. nilotica* fruit were equally effective against both Bacillus subtilis and S. aureus and that the ethanolic extract was also active against Proteus vulgaris. Also, Sotohy and his colleagues reported the effect of ethanol extracts against Clostridium perfringens [18]. I also agree with a study done by Jafer Salah Eldeen that shows the ethanolic extract of *Acacia nilotica* has high activity against Staphylococcus aureus. However, Jafer disagreed with our study on Staphylococcus epidermidis. Jafer showed that ethanolic extract had moderate activity against S. epidermidis, but in our study, the ethanolic extract of the plant had high activity against S. epidermidis [19]. The plant has very promising antimicrobial activities and thus can be used traditionally to cure various infectious diseases caused by these resistant bacteria and could serve as a useful source of new antibacterial agents. It is recommended that further studies should be carried out for the isolation and characterization of bioactive compounds. Investigations on the toxicity of the plant resulting from overdosage cannot be over-emphasized [20].

5. Conclusion

Antimicrobial resistance is reported to be on the increase due to gene mutations in disease pathogens. *Acacia nilotica* was chosen for this study because of its reputation in folklore medicine as an antimicrobial agent and because it is used in many diseases. In this study, it was concluded that Acacia nilotica possesses antimicrobial activity, but with varying degrees of effectiveness. *Acacia nilotica* is the most potent antibacterial agent against selected bacteria. Traditional medicinal practice could provide a source for new drugs, and therefore efforts should be directed towards evaluating traditional medicinal practice based on the scientific methodologies available. Resort to a new source of antimicrobial agents to treat antibiotic-resistant microbes to avoid the high cost and the side effects of medications. These results justify the use of *Acacia nilotica* in folk medicine.

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