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Antibacterial activity of *Acacia nilotica* fruits extract against pathogenic bacteria

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Abstract
Microbial infections are major public health problems in the developing countries. Infectious disease consider as the most cause of death for approximately one-half of all deaths in tropical countries. This study was carried out to investigate the *in-vitro* antibacterial activity of *Acacia nilotica* methanolic fruits extract against clinical isolates performed by cup-plate agar diffusion method against 5 Gram negative bacteria *Escherichia coli, Shigella flexneri, Salmonella typhi, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* and 2 Gram positive bacteria, *Listeria monocytogenes* and *Bacillus cereus.* The methanolic extract exhibited inhibitory effects against most of the tested microorganisms with zone of inhibition ranging from (11-39 mm). The largest inhibition zone were obtained from the methanolic extract of *Acacia nilotica* (fruits) against the Gram negative *Salmonella typhi* (39mm) in 100 mg/ml concentration, and Gram positive *Bacillus cereus* (30mm) in 100 mg/ml concentration comparison with Gentamicin (10µg/disc). These studies conducted for *A. nilotica* (fruits) was proved to have potent activities against clinical isolated bacteria *in vitro* and was considered as treatment of several bacterial and viral infections.

Keywords: *In vitro*, antibacterial, *Acacia nilotica*, pathogenic.

1. Introduction
Acacia is the most significant genus of family: Mimosaceae first of all described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, about two-third of them native to Australia and rest of spread around tropical and subtropical regions of the world [1]. The plant is a tree with yellow mimosa-like flowers and long grey pods constricted between seeds. It is known as "garad" among the speaking people in Sudan and it is also known as "Bagaruwa" among the "Hausa" speaking people of northern Nigeria. Due to abuse of commercial antibiotics; the incidence of multiple antibiotic resistances in human pathogens is increasing [2].
The infectious diseases caused by bacteria and fungi affect millions of people worldwide, throughout the history of mankind, infectious diseases have remained a major cause of death and disability. The World Health Organization (WHO) estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind [3]. The development and spread of resistance to currently available antibiotics is a worldwide concern [3].
The use of plants and herbs extract in the treatment of human ailments is a very ancient art, a practice that has been passed through generations and Scientists in Africa and other developing countries are conducting research into local plants abundant in the continent for their possible use in traditional medicine [4]. Herbal drugs are of major importance in Sudanese folk medicine. This was documented during comprehensive ethnobotanical investigations of El Kamali and Khalid [5], El Ghazali *et al*. [6, 7], El Kamali and El Khalifa [8], These authors listed the most common herbs used in Sudan, based mainly on interviews with traditional herbalist, this plant are used for treatment of diarrhea and stomach pain. A number of systematic attempts have been made to verify the claimed antibacterial uses of Sudanese plants to detect or isolate their bioactive agents [9-14].

Acacia nilotica was used by herbalist to treat colds, diarrhea, dysentery, inflammation, itch, measles, sore throat, wounds and Sexual Transmitted Disease. The plant also possessed antiemetic, antibacterial, expectorant and antiseptic activities. Its juice is used in folk remedies for the cancerous condition. Pain and inflammation are common complaints in many patients suffering from acute conditions [15, 16]. Anti-inflammatory agents inhibit the synthesis of prostaglandin which is one of the most important mediators of inflammation. Other mechanism of anti inflammatory activity the stabilization of lysosomal membrane enzymes in leucocytes (lysosomal enzymes destroy cartilage and other tissues and perpetuate inflammation) and antagonism of certain actions of bradykinin [17].

2. Materials and Methods

2.1 Plant materials

The *Acacia nilotica* (fruits) were collected from Sudan (Algaba, Omdurman, Khartoum state) between August 2015 and September 2015. It was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). The fruits were air-dried under the shadow with good ventilation and then ground finely until their uses for extracts preparation.

2.2 Preparation of crude extracts

Extraction was carried out for the fruits of *Acacia nilotica* by using overnight maceration techniques according to the method described by Harbone [18]. About 50 g were macerated in 250 ml of methanol for 3 hrs at room temperature with occasional shaking for 24 h, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration the solvent was then removed under reduced pressure by rotary evaporator at 55 °C. Each residue was weighed, the yield percentage was calculated and then stored at 4 °C in tightly sealed glass vial for future use.

2.3 Standard antibiotic

Gentamicin (10µg/disc) antibiotic discs used in the present study were procured by Hi media Chemicals Ltd, Mumbai, India and stored at 4 °C.

2.4 Collection of bacteria strains

Various clinical isolates were obtained from The Royal Care International Hospital located at Burri, Khartoum State, Sudan. All bacterial Isolates were identified and characterized using standard microbiology technique [19]. The bacterial cultures were maintained on nutrient agar and inoculated at 37 °C for 24 h and then used for tests.

2.4.1 *In vitro* testing of extracts for antibacterial activity

2.4.1.1 Testing for antibacterial activity: The cup-plate agar diffusion method [20] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. 1 ml of the isolated bacterial stock suspension 10^8 - 10^9 CFU/ml were mixed with 100 ml of molten sterile Mueller Hinton Agar which was maintained at 40 °C. 20 ml of the inoculated Mueller Hinton Agar were distributed into sterile Petri-dishes. The agar was left to set were cut by a sterile cork borer (No. 4). The cups (10 mm in diameter) were filled with 0.1 ml of each extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for 2hrs. The plates were then incubated in the upright position at 37 °C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of inhibition zones was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotic Gentamicin (10µg/disc).

2.4.1.2 Statistical procedures

Antimicrobial activity experiments were repeated thrice in each time and the average values with ± standard deviation (SD). Statistical analysis for all the assays results were done using Microsoft Excel program 2010.

3. Results and Discussion

The fruits of *Acacia nilotica* were screened for antimicrobial activity against Five Gram negative bacteria (*Escherichia coli*, *Shigella flexneri, Salmonella typhi, Klebsiella pneumonia and Pseudomonas aeruginosa*) and Two Gram positive bacteria (*Listeria monocytogenes* and *Bacillus cereus*) using the cup plate agar diffusion method. The extract obtained from the fruits of *Acacia nilotica* exerted pronounced activity against several bacteria strains tested as indicated by diameter of growth inhibition zones that varied from (11-39 mm).

Out of the 7 cultures tested, it showed good activity against *Salmonella typhi* (39mm) and *Bacillus cereus* (30 mm) at the highest concentration checked (100 mg/ml). Methanol extract of fruits was also able to show fairly good activity against gram positive more than gram negative species. For each test control positive Gentamicin in concentration (10µg/disc) with zone of inhibition ranging from (32-20 mm) against all bacterial strains tested.

The methanolic (fruits) extract of *A. nilotica* showed significant inhibition against Gram-positive and Gram-negative bacteria [21, 22] including a moderate antimicrobial activity against multi-drug resistant *Salmonella typhi* unlike the result we have in our study by dissolving the *Acacia nilotica* fruits in methanol showed that *salmonella typhi* have a highly sensitive antimicrobial activity multi-drug resistant.

Table 1: The antimicrobial activity of *Acacia nilotica* against the isolated bacteria

No.	Tested bacteria	Zone of Inhibition in mm ± (SD)					
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Gentamicin 10 mgc
1	*Escherichia coli*	20±0.2	15±0.17	12±0.11	15±0.1	_	30
2	*Shigella flexneri*	32±0.19	30±0.13	25±0.13	21±0.09	21±0.08	25
3	*Salmonella typhi*	39±0.7	35±0.3	32±0.1	27±0.05	23±0.15	25
4	*Klepsiella pneumoniae*	31±0.11	27±0.13	25±0.17	24±0.12	23±0.03	20
5	*Listeria monocytogenes*	20±0.20	17±0.4	14±0.21	_	_	28
6	*Bacillus cereus*	30±0.30	27±0.2	25±0.14	22±0.2	20±0.12	27
7	*Pseudomonas aeruginosa*	20±0.22	19±0.12	15±0.17	14±0.12	13±0.9	32

Key: MDIZ (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition.

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics [23]. In our study the anti-bacterial activity of methanol extract of *Acacia nilotica* were evaluated and the result indicates that *Acacia nilotica* has activity against the strains tested. This study is compatible with many of the studies that say: *Acacia nilotica* is commonly used to treat eye conditions, open wounds and dermatological ailments. Acting much as antacid it can also treat digestive problems [24]. *Acacia nilotica* has antibiotic activity and its aqueous extracts are antibacterial [25]. It has soothing, astringent, and antiseptic properties [24]. The methanolic extract exhibited the highest antimicrobial activity; this may be due to stronger extraction capacity of active component responsible for antibacterial activity. There can be several factors which can affect or reduce the efficacy of the medicinal plants in their antimicrobial activity. This is beginning from the time of plant collection (it's recommended that the collection of plant parts in most cases, but not always should be done after the flowering stage of the plant) the state of plant processing and the state of storage of plant [26]. The method of plant extraction is another factor which affects the antimicrobial activity of the medicinal plant. Generally, the results reported from different studies are difficult to compare because of the use of different test methods, bacterial strains and sources of antimicrobial samples used. And the differences in the type and concentrations of the secondary metabolites across different plants, variation in antimicrobial activities are expected.

4. Conclusion

The bark extracts of *Acacia nilotica* showed the various degree of inhibitory activity against the microorganisms tested. The obtained results may justify the use of the Sudanese fruits of *Acacia nilotica* as antimicrobial therapy in traditional medicine in Sudan and the neighboring countries. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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