

Anti-bacterial Activity of Methanolic Fruits Extract of Acacia nilotica (L.)

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Abstract

Acacia nilotica (L.) is used to treat different ailments for instance cold, congestion, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhoea, ophthalmic, sclerosis, small pox, intestinal pains and acute diarrhea. Other preparations are used for gargle, toothache, ophthalmic and syphilitic ulcers. The fruits of *A. nilotica* were tested against eight standard bacterial species: two Gram-positive bacteria viz, *Enterococcus faecalis* and *Staphylococcus aureus*, six Gram-negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella Sonnei*, *Salmonella Para A*, *Klebsiella pneumoniae* and *Proteus mirabilis* using the disc diffusion method. Antibacterial activity of fixed oil of *A. nilotica* dissolved in methanol (1:10), showed high activity against the Gram-negative bacteria (*P. aeruginosa* & *E. coli*) (18 & 14 mm). It also showed against Gram positive bacteria (*S. aureus* & *B. subtilis*) (14 & 13 mm) and against (*C. albicans*) (14 mm). This study conducted of *A. nilotica* fruits proved to have potent activities against antibacterial activity in vitro.

Keywords: *Acacia nilotica* (Fruits), Antibacterial activity, Antibiotic

Introduction

Medicinal plants are invaluable, safe, less toxic, cheap, available and reliable natural sources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against the disabling diseases [1,2].

Acacia nilotica Subsp. *nilotica* belongs to the family: Mimosaceae. The tree has yellow mimosa-like flowers and long grey pods constricted between seeds. The bark and branches bear spikes about 2 cm long. The five leaves are densely hairy with 3-6 pairs of pinnate consisting of 10-20 pairs of leaflets that narrow with parallel margins rounded at the

apex and with a central midrib closely crowded. The inflorescence consists of bright yellow flowers in auxiliary head on stalk half way up. The flowering period of the plant is between November and March [3]. The native distribution of *Acacia nilotica* includes much of Africa and the Indian subcontinent [4]. From the Germplasm Resources Information Network: GRIN database [5], the native distribution includes: Africa (Algeria, Angola, Botswana, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Kenya, Libya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe) and Asia (Iran, Iraq, Israel, Oman, Saudi Arabia, Syria, Yemen, India, Nepal, Pakistan) [6]. The bark tinged of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns. The bark is used in the treatment of haemorrhages, cold, diarrhea, tuberculosis, leprosy, colds, bronchitis, diarrhea, bleeding piles and leukoderma [7,8]. Decoction of the bark is largely used as an astringent douche in gonorrhoea, cystitis, vaginitis, leucorrhoea, prolapse of the uterus and piles [9].

Microbial infections are major public health problems in the developed countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistances in human pathogens is increasing [10]. 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. Today infectious diseases account for one-third of all deaths in the world; the World Health Organization 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 [11]. The development and spread of resistance to currently available antibiotics is a worldwide concern, the increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs [11]. This study conducted

of *A. nilotica* fruits proved to have potent activities against antibacterial activity *in vitro*.

Materials and Methods

Plant materials

The *A. nilotica* (Fruits) were collected from central Sudan between January 2016 and February 2017. The plant 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 in a mill until their uses for extracts preparation.

Preparation of crude extracts

Extraction was carried out for the Fruits of *A. nilotica* by using overnight maceration techniques according to the method described by [12]. About 50 g were macerated in 250 ml of methanol for 3 h at room temperature with occasional shaking for 24 h at room temperature, 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 and the yield percentage was calculated then stored at 4°C in tightly sealed glass vial ready for use.

Collection of bacteria strains

The methanolic extract solution of *Acacia nilotica* was tested against bacterial species. Various clinical isolates were obtained from Royal Care International Hospital located at Burri, Khartoum State, Sudan. All Isolates bacteria were identified and characterized using standard microbiology technique [13]. The bacterial cultures were maintained on nutrient agar and inoculated at 37°C for 24 h and then used for tests.

Testing for antibacterial activity

The cup-plate agar diffusion method [14], was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the isolated bacterial stock suspension (10^8 - 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton Agar which was maintained at 40°C. 20

ml aliquots of the inoculated Mueller Hinton Agar were distributed into sterile Petri-dishes. The agar was left to set and all of these plates 5 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. The cups were filled with 0.1 ml of the extract using automatic μL pipette, and allowed to diffuse a room temperature for two hours. The plates were then incubated 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 the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in mm.

Statistical procedures

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

Results and Discussion

The yield percentage of *Acacia nilotica* methanolic fruits extract was 13.5. The extract was screened for antibacterial activity against six Gram negative bacteria (*Escherichia coli*, *Shigella sonnei*, *Salmonella para A*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabiliss*), and two Gram positive bacteria (*Enterococcus faecalis* and

Staphylococcus aureus) using the cup plate agar diffusion method. The extract obtained from the fruits of *A. nilotica* exerted pronounced activity against several bacteria strains tested as indicated by diameter of growth inhibition zones that varied from (16-38 mm) (**Table 1**). Out of the ten cultures tested, it showed good activity against *Staphylococcus aureus* (38 mm), *Salmonella Para A* (32 mm), *Shigella Sonnei* and *Pseudomonas aeruginosa* (30 mm), *Escherichia coli* (28 mm), *Klebsiella pneumoniae* (22 mm), *Enterococcus faecalis* (18 mm) and *Proteus mirabilis* (16 mm) at the highest concentration checked (100 mg). Methanol extract of fruits of *A. nilotica* was also able to show fairly good activity against Gram positive and negative species. On comparison, only *Proteus mirabiliss* show (16 mm) inhibition zoon in concentration (100 mg). 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 [15]. In our study, the antibacterial activity of methanol extract of *Acacia nilotica* were evaluated and the result indicates that *Acacia nilotica* has activity against of the strains tested. These studies are 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 an antacid it can also treat digestive problems [16].

Table 1: Antibacterial activity of *Acacia nilotica* against isolated bacteria

| No. | Tested bacteria | Zone of Inhibition in mm \pm (SD) | | | | | Antibiotic Gentamicin (10 mgc) |
|-----|-------------------------------|-------------------------------------|---------------|---------------|---------------|---------------|--------------------------------------|
| | | Concentrations (mg/ml) | | | | | |
| | | 100 | 50 | 25 | 12.5 | 6.25 | |
| 1 | <i>Escherichia coli</i> | 28 \pm 0.02 | 27 \pm 0.08 | 22 \pm 0.01 | 18 \pm 0.09 | 12 \pm 0.02 | 30 |
| 2 | <i>Shigella sonnei</i> | 30 \pm 0.05 | 26 \pm 0.03 | 24 \pm 0.06 | 21 \pm 0.02 | 18 \pm 0.08 | 25 |
| 3 | <i>Salmonella Para A</i> | 31 \pm 0.04 | 27 \pm 0.02 | 25 \pm 0.01 | 23 \pm 0.09 | 22 \pm 0.07 | 27 |
| 4 | <i>Proteus mirabilis</i> | 16 \pm 0.03 | 11 \pm 0.01 | - | - | - | 24 |
| 5 | <i>Pseudomonas aeruginosa</i> | 30 \pm 0.04 | 28 \pm 0.02 | 23 \pm 0.06 | 20 \pm 0.01 | 18 \pm 0.02 | 32 |
| 6 | <i>Klebsiella pneumoniae</i> | 22 \pm 0.07 | 16 \pm 0.03 | 14 \pm 0.02 | 11 \pm 0.02 | - | 21 |

| | | | | | | | |
|---|------------------------------|-----------|-----------|-----------|-----------|-----------|----|
| 7 | <i>Enterococcus faecalis</i> | 18± 0.01 | 14 ± 0.06 | - | - | - | 20 |
| 8 | <i>Staphylococcus aureus</i> | 38 ± 0.02 | 30 ± 0.07 | 28 ± 0.09 | 25 ± 0.01 | 22 ± 0.02 | 25 |

Note: 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

Conclusion

Acacia nilotica showed that the various degree of inhibitory activity against the bacteria tested. The obtained results indicated that the *Acacia nilotica* is good antibacterial therapy in traditional medicine in Sudan and the neighbouring 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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Conflict of Interests and Funding Source

None Declared.

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