

# Medicinal Importance of Leaves Extracts of *Albizia Procera* (Roxb.) Benth., in Sudan against Some Bacterial Pathogens Infected Human and Animal

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## Abstract

The aim of this study is to evaluate antimicrobial effect leaves aqueous extracts against human and animal infectious bacterial strains. The leaves were air-dried, powdered and water extracted at concentration of 0.2 g/ml, 0.4 g/ml and 0.5 g/ml and were tested against different bacterial pathogens (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella dublin*); the antibacterial assay carried using agar diffusion method. Data of antimicrobial effect (inhibition zone in "cm") was subjected to analysis of variance using SAS software where Duncan Multiple Range Test was used for means separation at P=0.05. The results indicated that, leaves of the species showed antimicrobial potential at tested concentrations (0.2, 0.4 and 0.5 g/ml). The results of analysis of variance indicated that, the diameter (cm) of inhibitory zone within the studied bacterial strains increases significantly as concentration increased. The lowest average inhibitory zone (cm) was  $1.4 \pm 0.07$  recorded in *Salmonella dublin* at low concentration (0.2 g/ml), while  $1.9 \pm 0.05$  (cm),  $1.9 \pm 0.06$  (cm) and  $1.9 \pm 0.07$  (cm) were the highest average inhibitory zone obtained by application of 0.5 g/ml leaves aqueous extract against *Escherichia coli*, *Staph aureus* and *Staph epidermidis* respectively.

**Keywords:** *Albizia procera* • Morphology • Antimicrobial effect • Conservation • Economic utilization • Sudan

## Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. In current world, a microbial infection was one of the major threats to human and animal population. Antimicrobial drugs provide the essential basic for treatment of various microbial infections instead of the elevated genetic inconsistency of some microorganisms enhance them to develop quickly as possible antimicrobial resistance. Due to drug resistance nature of microorganisms, there is a need to find out new lead molecules from alternative sources like plants and algae. Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now, but the potential of higher plants as source for new drugs is still largely unexplored. However, systematic screening of them may result in the discovery of novel effective compounds. Moreover, worldwide the pharmaceutical industry has made massive investment in pharmacological, clinical and chemical research in an effort to discover plants-origin more effective drugs. Although, trees remain a

source for some drug ingredients since the use of more potent synthetic ones have become more common.

Sudan is a large tropical sub-Saharan country with unique position reflected in diverse habitats from desert and semi-desert in the north, acacia-wooded grassland in the Sahel zone of the central part of the country and valuable aromatic and medicinal plants species. The species in the country comprise 140 species and 141 taxa, of which few have paid research attention, incorporated in Sudan's economic system and utilized in forestry, horticulture, pasture and agroforestry systems while other species remained unknown since their economic, ethnobotanical as well as medicinal potential until recently not explored. Among these many species within the genus *Albizia* which are important forage, timber and medicinal plants and many are cultivated as ornamentals for their attractive flowers. Within the genus, *Albizia procera* (Roxb.) Benth., in the family Fabaceae is a multipurpose tree species native to India and occurs naturally through East Asia to Papua New Guinea and Northern Australia. The tree is useful for farm and amenity planting, light shade, firebreaks and for the rehabilitation of seasonally dry, eroded and degraded soils, regarded as a soil improver and is used as a nurse tree in tea

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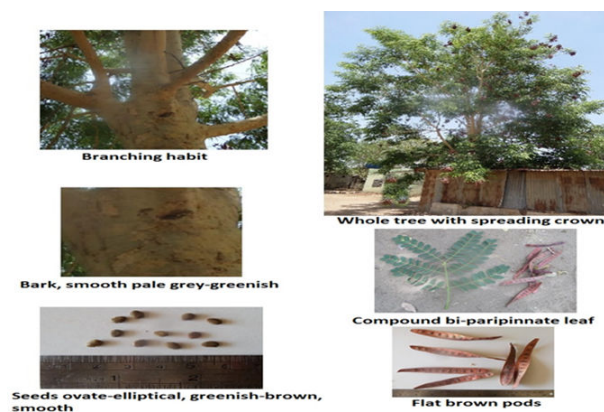
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gardens, coffee, and cocoa plantings. According to the species has good survival and rapid early growth characteristics on both saline and alkaline soils and widely cultivated in afforestation programs as well as in agroforestry systems [1]. This plant is useful in traditional medicine as it act as anticancer, pain, convulsions, delirium and septicemia. However, the decoction of bark is useful against rheumatism, haemorrhage, pregnancy problems, stomachache and sinus. Its leaves extensively used for the treatment of variety of wounds and ulcer and powdered seeds and fruits are used in curing amoebiasis, urinary tract infections as glycosuria, haemorrhoids, fistula and worm infestation as well as suppresses skin diseases. On the hand, Sivakrishnan and Swamivelmanickam, (2017) stated that, the species with high economic potential to be applied in pharmaceuticals, traditional medicines, agricultures, tanning, dyeing, construction and ornamental markets.

In the Sudan, *Albizia procera* (Roxb.) Benth., is commonly known by "Albizia Al Basiga or Al Procera) and introduced from India and grown in public gardens and avenues. From field surveys and observation it clear that, the species can grow and produces high quality fruits and seeds in a wide range of habitats extend from semi-desert to high rainfall savanna region. In the Blue Nile state as in Khartoum state the species found to grown as shade trees in homes, gardens and governmental institutions while healers as well as local people believe on the medicinal activity of its different parts particularly leaves which utilized as decleaves extracts in traditional medicine against some diseases in both for human and animal. This is going in line with the statement that, plant extracts and phytochemical have pharmacological properties give high significance in medicine. However, indicated the antibacterial potential of *Albizia procera* leaves and phytochemical properties of its leaf and bark methanolic extracts investigated by using chromatographic screenin [2]. Even through, traditionally, leaves of *Albizia procera* extensively used for the treatment of variety of wounds stated that, based on medicine, most records of morbidity and mortality occurring as bacterial infections that increases mortality and the length of stay in the hospital due to fact that most of the bacteria pathogens have developed resistance to antibiotics. In addition, the research and the identification of this uncommon tree against some bacterial pathogens for their microbial activity causing disease will be beneficial as sources of antimicrobial substances. Therefore, the present study is aiming to assess the antibacterial activity of *Albizia procera* leaves initially as aqueous extracts in three concentrations (0.2, 0.4 and 0.5 g/ml) against five bacterial strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella dublin* which are known as common in the Sudan to cause serious infections to both human and animal. This with no doubt will add to scientific knowledge about the plant species in the country and provide data for better future management, utilization and conservation.

*Albizia procera* is one of the tree species in the Sudan and showed ability to grow and survive in wide range of habitat with good and healthy habit (Figure 1).



**Figure 1.** Morphological characteristics of *Albizia procera* (Roxb.) Benth., in the Sudan showing whole tree, leaves, pods, seeds, bark and branching habit.

## Materials and Methods

### Study sites selection and description

Due to its limited distribution and few number of individuals observed, Khartoum State and Blue Nile State were selected as study sites and described for location within the agro-ecological zones of the Sudan, general soil condition, topography and rainfalls (Table 1).

Study site and code	Coordinate	Location within agro-ecological zone of the Sudan
Khartoum state (KhaSt)	15°39'N, 32°30'E; 15°35' N, 32°30' E	Semi-desert: <i>Acacia tortilis-crassifolia</i> Desert Scrub, mean annual rainfalls 75-300 mm/year.
Blue Nile state (BNSt)	12°03' N, 34°18' E; 12° 19' N, 34°21' E	Low rainfall woodland savanna on clay soils, mean annual rainfalls vary from isohyets 600-800 mm.

**Table 1.** Study sites description, coordinates and location within agro-ecological zone of the Sudan.

### Tree identification and materials collection

Identification of trees made in each site based on written description of the species in local botanical literature and other scientific publications were also consulted. Five mature trees (dbh 25-35 cm) were selected randomly in each site at distance not less than 100 m apart, marked and allocated for position by using handle GPS for further sampling and data collection. Time of vegetative growth, fruiting and seeding took more consideration during field surveys. Fresh leaves (5 Kilograms) harvested by hand from the crown of the selected trees in each site, pressed to dry between clean newspapers in plant press for easy handling and transferred to the laboratory of the Department of Silviculture, Faculty of Forestry, University of Khartoum where it was air-dried and pounded by mortar to fine powder [3].

## Antimicrobial assay

**Preparation of plant samples and extracts:** The previously collected leaves were air-dried at room temperature (37°C) in the laboratory of the Department of Silviculture, Faculty of Forestry, University of Khartoum for 7 days and pounded to fine powder using an electric blender and also mortar. Preparation of aqueous "water" leaves extract was made by dissolving fifty grams' sample into 100 ml distill water using a beaker and the solution was kept in rotary shaker for 3 days. The obtained aqueous (supernatant) was filtrated twice with Whatman filter paper and kept to dry for 2 days at room temperature (37°C). The obtained dried filtrate was weighted (30 g) and transferred into glass bottles (50 ml) and stored at room temperature, then diluted to 0.2, 0.4 and 0.5 mg/ml by dissolving 20 mg, 30 mg, 40 g and 50 mg from extract to 100 ml distill water. The obtained concentrates or dilutes were then stored in sterile glass bottles (100 ml) for further in vitro assay.

**Preparation of culturing and identification of bacteria:** Method adopted in preparation of Enriched media in microbiology laboratory at Faculty of Veterinary Medicine, University of Khartoum. Cultured swabs collected from diseased animals, incubated for 24-48 hrs at 37°C and smears were prepared from different colonies and stained with Grams stain to differentiate between gram positive and gram-negative bacteria. Purified colonies were cultured in nutrient agar and all primary and secondary biochemical testes were made according to Barrow and Feltham (2003) to detect the genera and species [4].

## Antimicrobial assay (sensitivity test)

The agar diffusion method proposed was adopted to assess the antibacterial activity of the prepared leaves water (aqueous) extract of the species at different dilutions (0.2 g/ml, 0.3 g/ml, 0.4 g/ml and 0.5 g/ml). The stocked cultures of isolated bacteria: *Staphylococcus aureus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Salmonella dublin* and *Escheichia coli* were grown in nutrient broth medium at 37°C for 22 hours. A lawn culture of studied bacteria was prepared on the wells (agar discs) prepared initially by removing disc of 6.0 mm in diameter from the Muller-Hinton Agar (MHA, Merck) by using a sterile pasture pipette. Then, from each concentration (0.2, 0.4 and 0.5 g/ml) a sample of equal amounts (0.1 ml) were filled into each well, the tested bacteria were added the using micropipette and the extracts were allowed to diffuse into the agar matrix for 1 hour before incubating in the upright position at 37°C for 24 hours for three times. The antibacterial activity of *Albizia procera* extracts at different concentrates determined by measuring in millimeter diameter of inhibitory zone by using transparent ruler to complete the analysis.

## Data arrangement and statistical analysis

The data (tree morphology, pods and seeds morphology and production, antimicrobial assay) arranged in Excel sheets and mean were subjected to analysis of variance (one-way, two-way ANOVA) by using GLM procedure (Generalized Linear Model: GLM) calculated using SAS Version 9.0 (Statistical Analysis System) (2002) software. The mean separations carried out using Duncan's multiple range tests and significance was determined at  $p < 0.05$ .

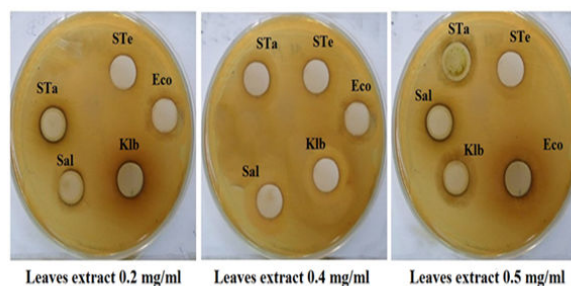
## Results

In this study, we only focused on accessing the antimicrobial activity of leaves aqueous extracts against some bacterial strains based on the information that, the species is recently used on local medicine and not studied in scientific manner. Results of analysis of variance indicated, significant variation ( $P < 0.0001$ ) in the diameter of inhibition zone (cm) obtained by the different concentrations (0.2 g/ml, 0.4 g/ml and 0.5 g/ml) of *Albizia procera* leaves extracts against the studied bacterial strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Salmonella dublin* and *Escheichia coli* (Table 2; Figure 2 and 3). The aqueous leaves extract at the studied concentrations (0.2, 0.4 and 0.5 g/ml) were active against the studied bacterial strains with different variation in inhibitory zones (cm) that increase as concentration increased (Figure 2).

Name of pathogens	Concentrations of <i>Albizia procera</i> (Code: ALP) leaves extraction and diameter of inhibitory zone (cm)		
	0.2 g/ml water extract (Code: 0.2mg/mlW)	0.4 g/ml water extract (Code: 0.4mg/mlW)	0.5 g/ml water extract (Code: 0.5mg/mlW)
<i>Staphylococcus aureus</i> (STa)	1.6c (± 0.07) +ve	1.72 <sup>b</sup> (± 0.05) +ve	1.9 <sup>a</sup> (± 0.06) +ve
<i>Staphylococcus epidermidis</i> (STep)	1.7b (± 0.07) +ve	1.7 <sup>b</sup> (± 0.05) +ve	1.9 <sup>a</sup> (± 0.07) +ve
<i>Klebsiella pneumonia</i> (Klb)	1.5c (± 0.07)	1.7 <sup>b</sup> (± 0.06)	1.8 <sup>a</sup> (± 0.05)
<i>Escherichia coli</i> (Eco)	1.5b (± 0.07) +ve	1.6 <sup>b</sup> (± 0.06) +ve	1.9 <sup>a</sup> (± 0.05) +ve
<i>Salmonella dublin</i> (Sal)	1.4c (± 0.07) +ve	1.6 <sup>b</sup> (± 0.06) +ve	1.7 <sup>a</sup> (± 0.07) +ve
Pr>F	<0.0001	<0.0001	<0.0001
F-value	47.24	68.78	77.86
R-square	0.7857	0.8899	0.9984

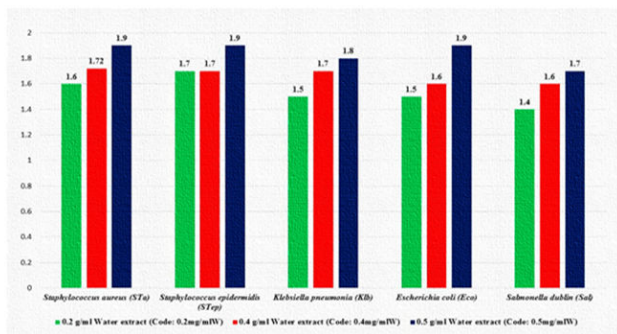
Means (± Standard deviation) with the same letter along the same columns do not differ significantly at  $P=0.5$  according to Duncans Multiple Test; +ve: sensitive to plants extracts; -ve: not sensitive to plants extracts.

**Table 2.** Showing type of human and animal infectious bacterial pathogens and diameter of inhibitory zone (cm) obtained by different concentrations of *Albizia procera* leaves water "aqueous" leaves extracts.



**Figure 2.** Sensitivity tests by used the leaves aqueous "water" extracts of *Albizia procera* at concentration of 0.2 g/m, 0.4 g/ml and 0.5 g/ml against *Staphylococcus aureus* (STa), *Staphylococcus*

*epidermidis* (STep), *Klebsiella pneumonia* (Klb), *Escherichia coli* (Eco) and *Salmonella dublin* (Sal) bacterial strains.



**Figure 3.** Histogram showing the variation in diameter of inhibitory zone in "cm" (Y axis) obtained by used the leaves aqueous "water" extracts of *Albizia procera* at concentration of 0.2 g/ml, 0.4 g/ml and 0.5 g/ml against *Staphylococcus aureus* (STa), *Staphylococcus epidermidis* (STep), *Klebsiella pneumonia* (Klb), *Escherichia coli* (Eco) and *Salmonella dublin* (Sal) bacterial strains.

However, the leaves aqueous extract of *Albizia procera* was inhibited *Staphylococcus epidermidis* with inhibitory zone diameter of  $1.7.2 \pm 0.07$  cm,  $1.7 \pm 0.05$  and  $1.9 \pm 0.07$  cm in the concentration of 0.2, 0.4 and 0.5 g/ml respectively followed by inhibitory zone of  $1.6.2 \pm 0.07$  cm,  $1.7 \pm 0.05$  and  $1.9 \pm 0.06$  obtained with the same concentrations against *Staphylococcus aureus*. Furthermore, the inhibitory zone diameter obtained by different concentrations (0.2, 0.4 and 0.5 g/ml) of the leaves aqueous extract were  $1.5 \pm 0.07$  cm,  $1.7 \pm 0.06$  and  $1.8 \pm 0.05$  cm against *Klebsiella pneumonia*,  $1.5 \pm 0.07$  cm,  $1.6 \pm 0.06$  and  $1.9 \pm 0.05$  against *Escheichia coli* and  $1.4 \pm 0.07$  cm,  $1.6 \pm 0.06$  and  $1.7 \pm 0.07$  against *Salmonella dublin* bacterial strains.

## Discussion

The present study shows that leaves aqueous extracts have inhibitory effect on bacterial growth, which increases as concentration increased. The plant extracts show varying degrees of action adjacent to gram-negative bacteria (*Salmonella dublin*, *Klebsiella pneumonia* and *E. coli*), and gram positive bacteria (*Staph aureus* and *Staph epidermidis*). Phytochemical investigation on *Albizia procera* has revealed that, the ethanolic extract of its aerial parts of have high phytochemical contents like triterpenoids, carbohydrates, glycosides, phytosterols, phenolic compounds, saponins, tannins and flavonoids. In addition, phytochemical analysis of leaf, root and stem bark ethanolic extracts of *Albizia anthelmintica* revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins and diterpenes.

The detected compounds have been vastly reported for their antimicrobial and antioxidant activities. Similarly, phytochemical components were extracted from *Ziziphus* leaves to have and inhibition activity against bacteria strains namely alkaloids, saponins, tannins, glycosides, flavonoids and terpinoids. Thus, the presence of these bioactive components in *Albizia procera* leaf, root and stem bark ethanolic extracts may account for the antibacterial and antioxidant activity demonstrated by the extracts. Furthermore, in phytochemical screening of *Albizia chelvalieri* leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenes [5].

## Conclusion

In this study, water (aqueous) extract extracts of the leaves of *Albizia procera* were used at three concentrations (0.2 g/ml, 0.4 g/ml and 0.5 g/ml) to evaluation of the antimicrobial activity. The extract at different concentrations used (0.2 g/ml, 0.4 g/ml and 0.5 g/ml) was effective in all bacterial strains with different diameter of inhibitory zone (cm). The highest effectiveness was demonstrated by the aqueous extract of *Albizia procera* leaves at the highest concentration (0.5 mg/ml) among all the studied Gram negative and positive bacteria.

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