

Antibacterial and Antifungal Properties of *Acacia nilotica* Leaf Extract

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ABSTRACT

Acacia nilotica is an herbal plant used traditionally for treating several diseases from many decades. The aim of this study is to determine the anti-microbial property of *Acacia nilotica* leaf chloroform and methanol extract. Several bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, and fungal isolates viz. *Candida albicans*, *Aspergillus niger*, *Trichoderma reesei* and *Penicillium chrysogenum* were utilized during the study and were assigned to agar well diffusion assay. Antibacterial activity of *Bacillus subtilis* show highest activity at concentration of 80mg/ml, methanol extract shows maximum zone of inhibition. Antifungal activity of leaves was more effective against *Aspergillus niger* and *Trichoderma reesei* show highest activity at concentration of 80mg/ml. The results of antifungal assay revealed that the extract showed significant inhibitory activity against the tested pathogens compared with standard and significant activity was observed with methanol extract. Crude extracts of *Acacia nilotica* inhibited the growth of various bacteria and fungi thus showed its broad-spectrum antimicrobial potential, which may be employed in the management of microbial infections. From the current study, it can be said that *Acacia nilotica* is a potential antimicrobial agent and can be used at large scale for the synthesis of non-toxic, eco-friendly, green route medicines in the future.

KEYWORDS: *Acacia nilotica*, Anti-bacterial activity, Anti-fungal activity.

INTRODUCTION

India is the largest producer of medicinal plants and is rightly called the "Botanical Garden of the World". The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. Considerable works have been done on medicinal plants to treat cancer, and some plant products have been marketed as anticancer drugs, based on the traditional uses and scientific reports. Medicinal plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. Several reports described that the anticancer activity of medicinal plants is due to the presence of antioxidants in them. In fact, the medicinal plants are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs (Pandey and Madhuri, 2010).

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One such plant is *Acacia nilotica* Linn. commonly known as Babul and Kikar belongs to the family *Fabaceae* (Rather and Mohammad, 2015). It is the second-largest genus of the family *Fabaceae*, with about 1350 species. It was first described by Linnaeus in 1773 (Bashir *et al.*, 2014). It is distributed throughout tropical and warm temperate areas of the world like Asia, Australia, Africa and America (Rajvaidhya *et al.*, 2012; Sharma *et al.*, 2014). It is also known as the Egyptian *Acacia*, gum Arabic tree, thorny *Acacia*, *Acacia gomifera*, and so on (Bashir *et al.*, 2014; Tyagi *et al.*, 2016).

Acacia nilotica has various complex phytoconstituents including alkaloids, volatile essential oils, phenols, phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. This plant is rich in phenolics, consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, (+) -catechin, (-) epi-gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate. The compounds such as kaempferol-3-glucoside, iso-queretin, catechin, kaempferol, galactose, l-arabinose, l-rhamnose etc are also present in this plant. These types of phytoconstituents play a role in the therapeutic actions of *Acacia nilotica*. Earlier traditional description confirmed that *Acacia nilotica* has a rich amount of nutrients and contains a high therapeutic value which is capable of prevention, mitigation, and treatment of various infectious diseases and deleterious conditions (Sadiq *et al.*, 2015). It is considered a safe medicinal plant and modulates the numerous therapeutic actions without any adverse effect. The isolated bioactive constituents of *Acacia nilotica* summarized by Khare, 2005; Parajapati and Kumar, 2005; Prajapati *et al.*, 2009.

Acacia nilotica and its chief phytoconstituents play a pivotal role in several therapeutic strategies. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Prusti *et al.*, 2008). Rahman *et al.*, 2014 reported the high antimicrobial activity in the ethanol extract of *Acacia nilotica* leaf than chloroform extract. *Acacia nilotica* demonstrates highest activity against three bacterial (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) and two fungal strains (*Candida albicans* and *Aspergillus niger*). The stem bark extracts have antimicrobial activity against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method (Umerie, 2016).

The gum, stem bark, leaves and fruits of *Acacia nilotica* are widely used in folk medicine for the treatment of colds, bronchitis, pneumonia, diarrhea, dysentery and for skin ailments. The fruit and stem bark are regarded as tonic and astringent agents and are used for the treatment of sore throats and chest complaints. The water extract of the fruit is used externally to treat syphilitic lesions and other venereal diseases. The vapors of burnt fruit are inhaled to relieve chest congestion and to treat the common cold. The infusion of the fruit in warm water is used as a gargle to relieve sore throats, mouth ulcers and gum infections. A ground fruit poultice is applied to the vagina as a prophylactic agent against postnatal infection (Watt 1962; Pousset 1989).

The present study focuses on leaf part of the *Acacia nilotica* antimicrobial activity against specific

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bacteria and fungi strains which can lead to a new direction in the field of research in the field of medicines and therapeutics.

MATERIALS AND METHODS

1) Collection of plant material

The experimental plant material i.e., *Acacia nilotica* leaf was collected from the campus of the SPNKS Govt. PG. College, Dausa whose plant part leaves were used for the evaluation process.

2) Antimicrobial activity

(i) Microbial Strains, culture medium and inoculum preparation:

Clinical laboratory bacterial isolates of *Staphylococcus aureus* MTCC 3381, *Bacillus subtilis* MTCC 10619, *Pseudomonas aeruginosa* MTCC 425 and *Escherichia coli* MTCC 443, and fungal isolates viz. *Candida albicans* MTCC 183, *Aspergillus niger* MTCC 872, *Trichoderma reesei* MTCC 164 and *Penicillium chrysogenum* MTCC 5108 were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, Rajasthan (India).

(ii) Preparation of Plant Extract

The freshly collected leaves of *Acacia nilotica* have been washed thoroughly with tap water and are subjected to air-drying in the shade at room temperature (32-37°C) for about 2-3 weeks. The dried leaves were grounded into powder form by using a homogenizer. About 50gm of powdered plant leaves (50gm/500ml) were extracted in a Soxhlet extractor for 8 to 10 hours at boiling temperature (>78°C), sequentially with methanol (polar) and chloroform (non-polar). The extracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at 20°C until further tests were carried out.

a) Determination of Antibacterial Assay

In vitro antibacterial activity of the methanolic and chloroform extracts of plant leaves were studied against gram positive and gram-negative bacterial strains by the agar well diffusion method (Perez *et al.*, 1990). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 10 mg/ml. The Mueller Hinton agar was melted and cooled and then poured into sterile petri dishes to give a solid plate. A standardized inoculum (1.5×10^8 CFU/ml, 0.5 McFarland) prepared in sterilized 0.9% saline water was used. Wells were prepared in the seeded agar plates. The test compound (20 μ l, 40 μ l, 60 μ l and 80 μ l) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, Ciprofloxacin (40 μ l). The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the

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mean values are presented.

b) Determination of Antifungal Assay

Anti-fungal activity of the methanolic and chloroform extract of experimental plant leaves were investigated by agar well diffusion method (Bonjar *et al.*, 2005). The fungi were sub-cultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 hours and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile phosphate buffer saline (PBS) and adjusted to a concentration of 10⁶ cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. Wells of 6 mm in diameter were punctured in the culture media using sterile glass tube. 20 µl, 40 µl, 60 µl and 80 µl of fresh extracts were administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 hours bioactivities were determined by measuring the diameter of inhibition zone (in mm). Ketoconazole (40 µl) was used as antifungal positive control. The All experiments were made in triplicate and means were calculated.

STATISTICAL ANALYSIS

Statistical analysis is based on biological studies. Differences between groups were compared by using one way analysis of variance (ANOVA) followed by the student "t" test. All data are presented as mean ± SEM.

RESULTS AND OBSERVATIONS

The antimicrobial activity of the plant extract of *Acacia nilotica* was performed against selected bacterial strains and fungal strains. The antimicrobial effect of the extract was compared with the standard drugs (Ciprofloxacin for bacterial and ketoconazole for fungus). Crude extracts of *Acacia nilotica* inhibited the growth of various bacteria and fungi thus showed its broad-spectrum antimicrobial potential, which may be employed in the management of microbial infections (Kaur *et al.*, 2016). During the study, the antibacterial potential of methanol and chloroform extracts of *Acacia nilotica* leaves was evaluated against the pathogens including gram positive- *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa*, *Escherichia coli* bacteria, were displayed in Table 1-2 and Graph 1-2. The antifungal activity for methanol and chloroform extract of leaves of *Acacia nilotica* is showed in Table 3-4 and Graph 3-4. According to this the methanol extract was found to be effective against all the test fungi *viz.* *Candida albicans*, *Aspergillus niger*, *Trichoderma reesei* and *Penicillium chrysogenum*.

(i) Antibacterial activity

In the present study the antibacterial potential of methanol and chloroform extracts of *Acacia nilotica* leaves was evaluated against the pathogens including gram positive- *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa*, *Escherichia coli* bacteria. In antibacterial activity, the methanol extracts of *Acacia nilotica* leaves showed maximum zone of inhibition against *Bacillus subtilis* bacteria (28 mm) at 80 µl concentration while the chloroform extracts showed maximum

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zone of inhibition against *E. coli* (16 mm) at 80 µl concentration. As compared to chloroform extracts, leaves methanol extract gave significant better results against all studied bacteria. Weshaya and Al Sir, 2017 methanol extract of *Acacia nilotica* fruit cover was found effective against all tested Gram negative and Gram-positive bacteria with various inhibition zone (range between 32-16 mm). Aqueous extract of *Acacia nilotica* fruit cover was moderately active against both Gram positive and Gram-negative bacteria inhibition zone (range between 28-14 mm). Singh and Thakur, 2016 data showed that the highest values of the diameter of the zone of inhibition were exhibited by methanol extract against *S. pyogenes* giving a zone diameter of 17.00 ± 0.05 mm, whereas the lowest antibacterial response was observed against *E. faecalis* with 7.33 ± 0.58 mm zone of inhibition. Among the hot solvent extracts, methanol extracts exhibited the highest value of the zone of inhibition against *S. pyogenes* giving zone of diameter of 16.83 ± 0.29 mm. Butanol extract exhibited the lowest value of inhibition zone against *B. subtilis* with 5.00 ± 0.00 mm zone of inhibition. Kavitha *et al.*, 2013 studied the antibacterial effects of *Acacia nilotica* against various clinical bacterial isolates. The leaf extracts at the maximum mean diameter zone of inhibition of 21.11 ± 1.05 mm and 16.83 ± 0.94 mm against *E. coli* and *Salmonella* strains, respectively. The leaves were found more effective in inhibiting bacterial growth as compared to pods and bark extracts. The results demonstrated that all selected pathogens were susceptible to all tested parts of the plant. Likewise, the methanol and chloroform extracts exhibited antimicrobial activities with zones of inhibition ranging from 6 to 22 mm and exhibited appreciable activity against all the clinically important bacterial and fungal species. Overall maximum inhibition zone (22 mm) was observed in extract of methanol and chloroform (75:25) against *Bacillus subtilis* (Kaur *et al.*, 2016). Antimicrobial activity of *Acacia nilotica* and the ethanol extract activity against all tested microorganism and the inhibition zone were *E. coli* 17 ± 3 mm, *Klebsiella pneumoniae* 18.3 ± 4 mm, *Proteus mirabilis* 16.9 ± 4 mm, *Pseudomonas aeruginosa* 17 ± 3 mm, *Enterobacter cloacae* 18.3 ± 0.5 mm, *Citrobacter freundii* 16 ± 5 mm (Alamein *et al.*, 2021). The methanol extract of different parts of *A. nilotica* showed good antibacterial activity against *B. cereus*. The highest zone of inhibition was recorded in leaf extract against *E. coli* (15.30 mm). Cavazos *et al.*, (2021) study aimed to determine presence of antibacterial activity of *Acacia berlandieri* and *Acacia rigidula* leaves. The antibacterial activity was investigated using a disc diffusion assay. *P. alcalifaciens* ($p < 0.001$), *E. faecalis* ($p < 0.01$), *S. aureus* ($p < 0.001$) and *Y. enterocolitica* ($p < 0.001$) were significantly inhibited by *A. rigidula* extracts as compared to *A. berlandieri* extracts.

(ii) Antifungal activity

In the present study, methanol extract was found to be effective against all the test fungi. It gave maximum zone of inhibition against *Candida albicans* (23 mm) at 80 µl concentration. While observing the results for antifungal activity of chloroform extract of *Acacia nilotica*, it was found less effective than methanol extract. The maximum zone of inhibition was found against *Candida albicans* (14 mm) at 80 µl concentration. Prabhakar *et al.*, 2012 tested the ethanol extract of *Acacia nilotica* (300 mg/ml) recorded maximum zone of inhibition against *Aspergillus flavus* (25 mm) followed by *Candida albicans* (15 mm), *Aspergillus fumigatus* (13 mm), *Penicillium chrysogenum* (11 mm) and

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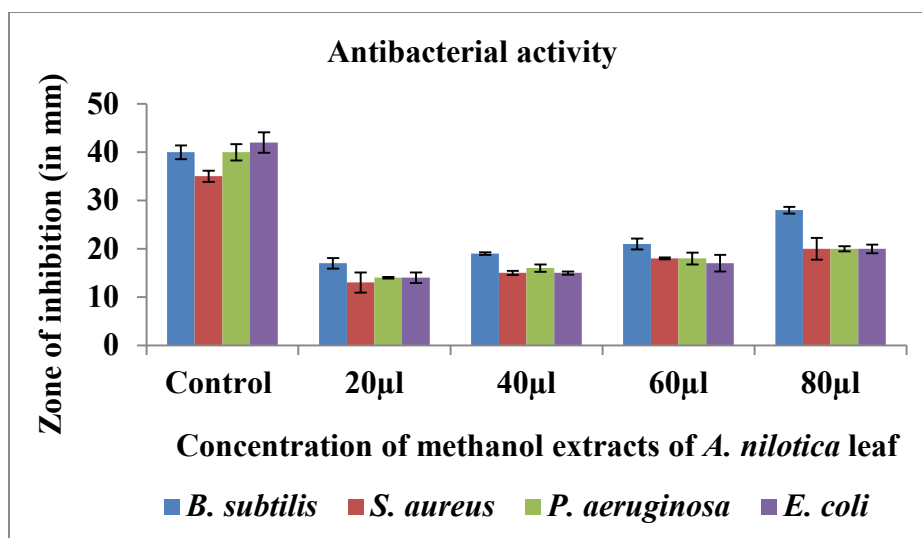
Candida glabrata (8 mm). For ethyl acetate extract, maximum zone of inhibition was recorded against *Aspergillus flavus* (15 mm), *Aspergillus fumigatus* (12 mm), *Candida albicans* (10 mm) and *Penicillium chrysogenum* (7 mm). No zone of inhibition was seen against *Candida glabrata*, *Aspergillus niger* and DMSO control. The ethanol extract of *Acacia nilotica* showed more fungal inhibitory activity when compared to ethyl acetate extract. *Acacia catechu* seed extract was highly effective in showing the antifungal activity against fungal pathogens *Aspergillus niger* and *Candida albicans* (2.0 mg/ml concentration showed 13.5 mm of zone of inhibition in *Candida albicans* and 7.5 mm of zone of inhibition in *Aspergillus niger* in an average) (Ahmed and Thangavelu, 2020). Antifungal activity of leaf extract showed significant activity when compared with the bark/root extract. *Acacia nilotica* bark and leaf extract showed antifungal activity against *Aspergillus flavus* (12 mm). Methanol extracts and aqueous extract of *Acacia nilotica* zone of inhibition ranging from 34.27±1.45 mm to 93.35±1.99 mm (Mahesh and Satish, 2008).

Table 1: Antibacterial activity of methanol extract of *Acacia nilotica* leaf against pathogenic bacterial strains

Pathogenic bacterial strains	Zone of Inhibition (in mm)				
	Control	20 µl	40 µl	60 µl	80 µl
B. subtilis	40±1.43	17±1.09	19±0.28	21±1.14	28±0.69
S. aureus	40±1.17	13±2.1	15±0.41	18±0.21	20±2.27
P. aeruginosa	40±1.69	14±0.18	16±0.75	18±1.21	20±0.54
E. coli	40±2.13	14±1.11	15±0.33	17±1.73	20±0.90

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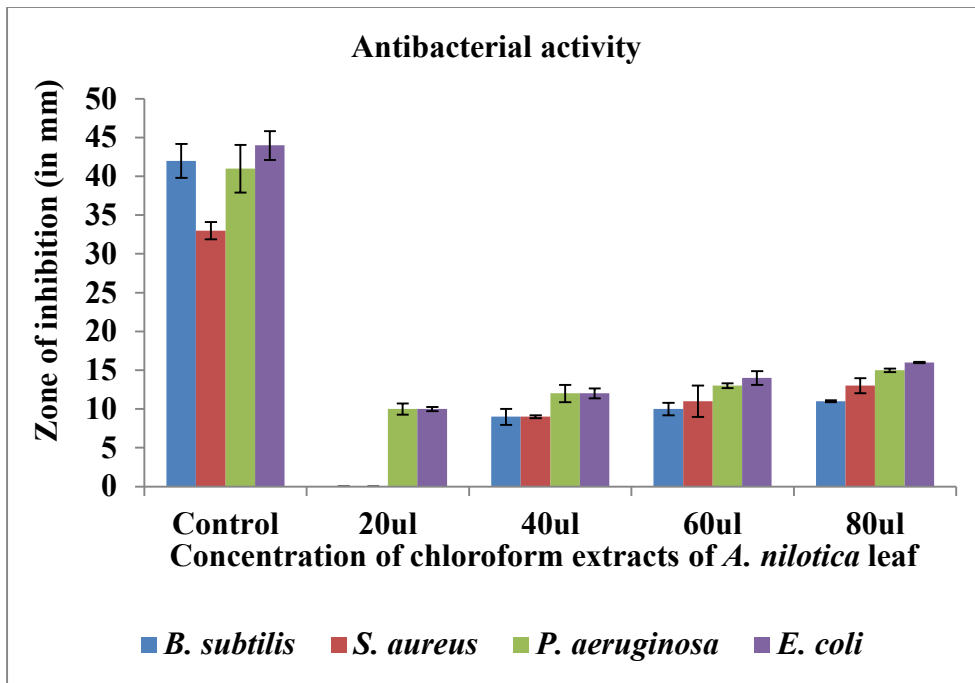
Graph 1: Antibacterial activity of methanol extract of *Acacia nilotica* leaf against pathogenic bacterial strains

Table 2: Antibacterial activity of chloroform extract of *Acacia nilotica* leaf against pathogenic bacterial strains

Pathogenic bacterial strains	Zone of Inhibition (in mm)				
	Control	20 µl	40 µl	60 µl	80 µl
<i>B. subtilis</i>	40±2.19	Nil	9±1.03	10±0.81	11±0.11
<i>S. aureus</i>	40±1.12	Nil	9±0.19	11±2.01	13±0.98
<i>P. aeruginosa</i>	40±3.08	10±0.71	12±1.13	13±0.31	15±0.23
<i>E. coli</i>	40±1.87	10±0.26	12±0.64	14±0.88	16±0.1

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Graph 2: Antibacterial activity of chloroform extract of *Acacia nilotica* leaf against pathogenic bacterial strains

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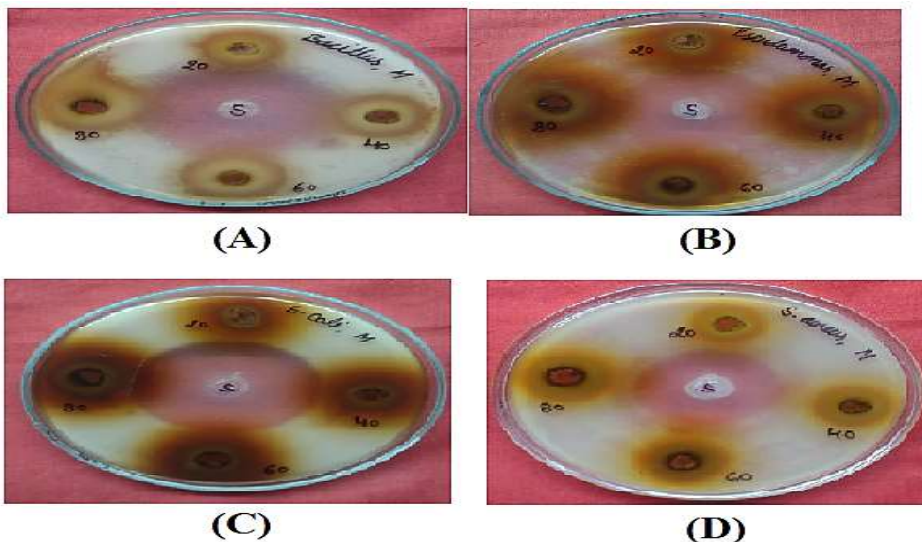


Figure 1: Antibacterial activity of methanol extract of *Acacia nilotica* leaf against (A) *Bacillus subtilis* (B) *Pseudomonas aeruginosa* (C) *Escherichia coli* (D) *Staphylococcus aureus*

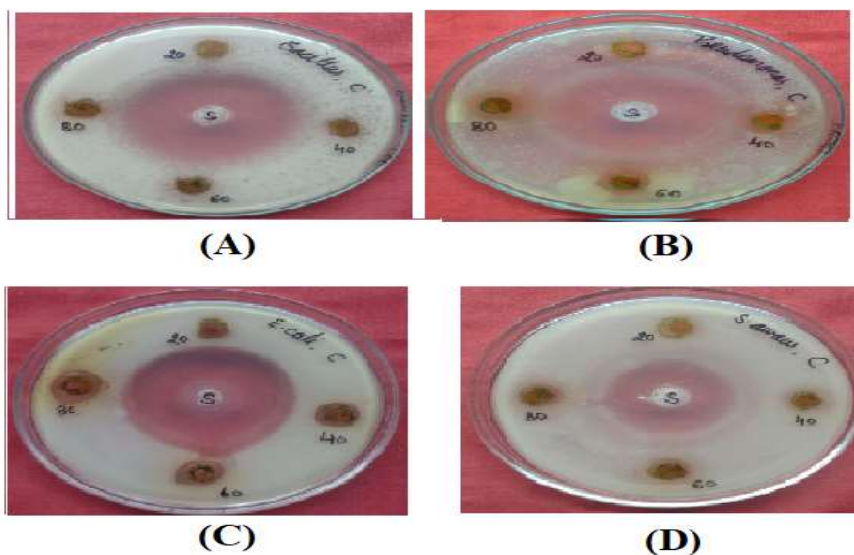


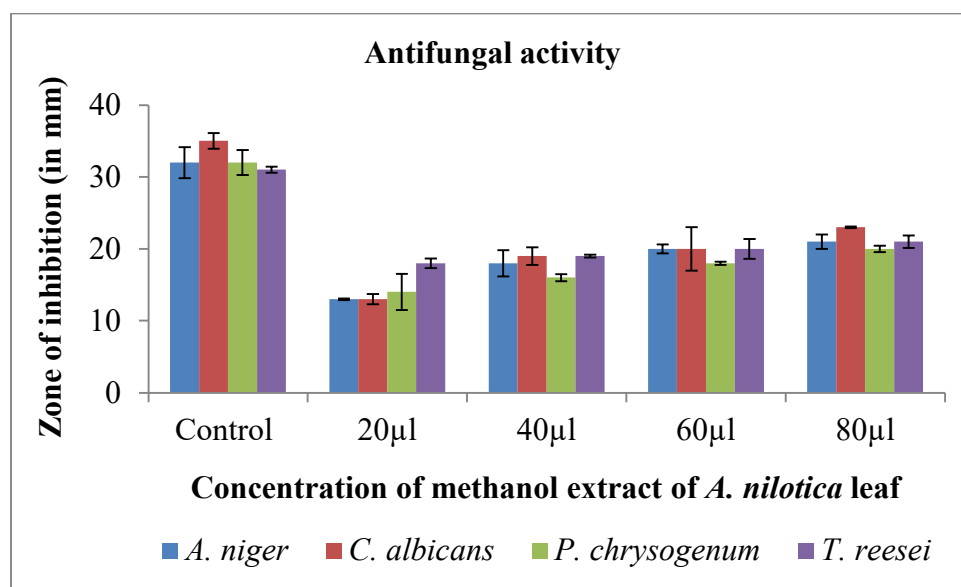
Figure 2: Antibacterial activity of chloroform extract of *Acacia nilotica* leaf against (A) *Bacillus subtilis* (B) *Pseudomonas aeruginosa* (C) *Escherichia coli* (D) *Staphylococcus aureus*

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Table 3: Antifungal activity of methanol extract of *Acacia nilotica* leaf against pathogenic fungal strains

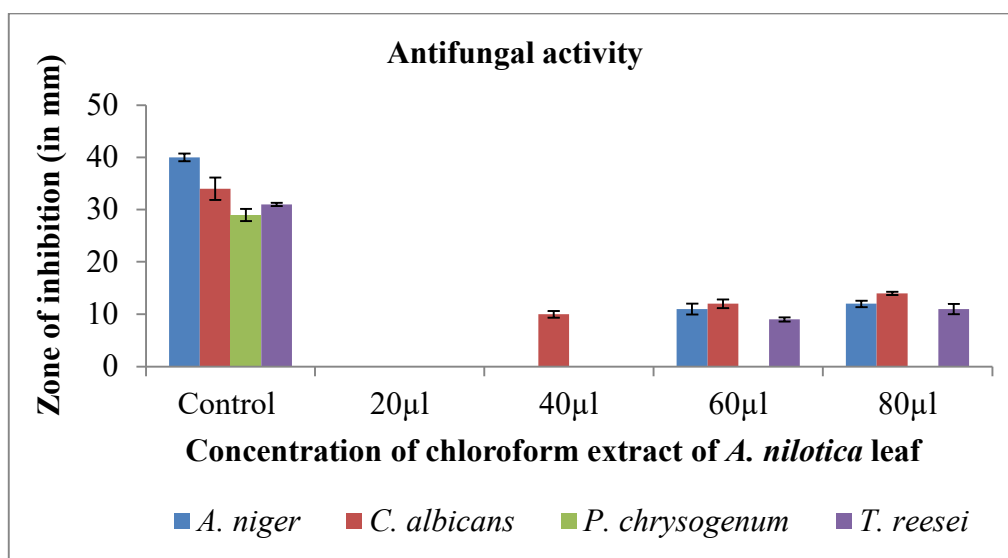
Pathogenic fungal strains	Zone of Inhibition (in mm)				
	Control	20 μ l	40 μ l	60 μ l	80 μ l
<i>A. niger</i>	34 \pm 2.17	13 \pm 0.12	18 \pm 1.81	20 \pm 0.61	21 \pm 0.99
<i>C. albicans</i>	34 \pm 1.09	13 \pm 0.71	19 \pm 1.23	20 \pm 3.01	23 \pm 0.11
<i>P. chrysogenum</i>	34 \pm 1.74	14 \pm 2.51	16 \pm 0.49	18 \pm 0.21	20 \pm 0.43
<i>T. reesei</i>	34 \pm 0.43	18 \pm 0.66	19 \pm 0.19	20 \pm 1.36	21 \pm 0.87

Graph 3: Antifungal activity of methanol extract of *Acacia nilotica* leaf against pathogenic fungal strains**Antibacterial and Antifungal Properties of *Acacia nilotica* Leaf Extract**

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Table 4: Antifungal activity of chloroform extract of *Acacia nilotica* leaf against pathogenic fungal strains

Pathogenic fungal strains	Zone of Inhibition (in mm)				
	Control	20 μ l	40 μ l	60 μ l	80 μ l
<i>A. niger</i>	34 \pm 0.71	Nil	Nil	11 \pm 1.03	12 \pm 0.62
<i>C. albicans</i>	34 \pm 2.13	Nil	10 \pm 0.63	12 \pm 0.82	14 \pm 0.32
<i>P. chrysogenum</i>	34 \pm 1.15	Nil	Nil	Nil	Nil
<i>T. reesei</i>	34 \pm 0.31	Nil	Nil	9 \pm 0.4	11 \pm 0.97



Graph 4: Antifungal activity of chloroform extract of *Acacia nilotica* leaf against pathogenic fungal strains

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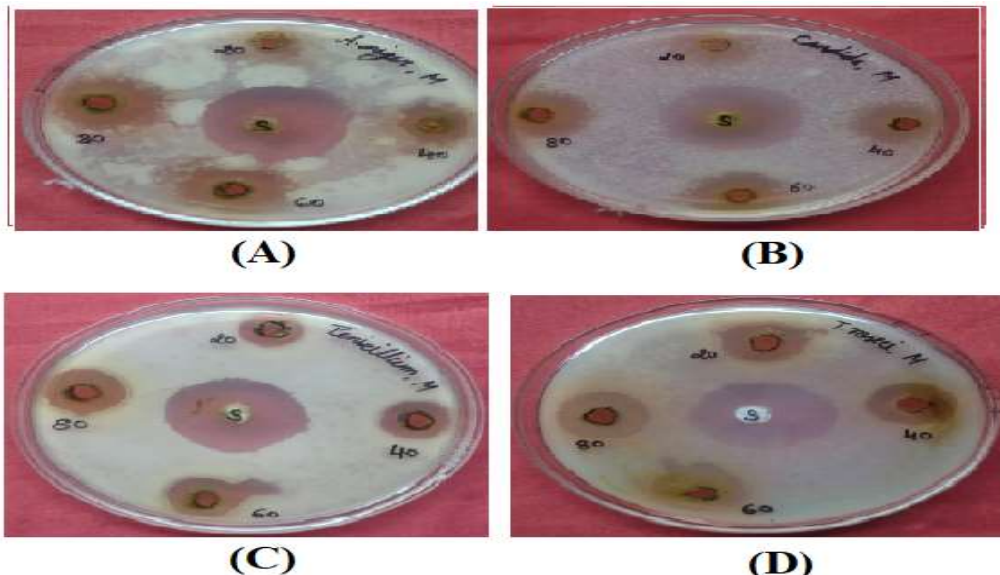


Figure 3: Antifungal activity of methanol extract of *Acacia nilotica* leaf against (A) *Aspergillus niger* (B) *Candida albicans* (C) *Penicillium chrysogenum* (D) *Trichoderma reesei*

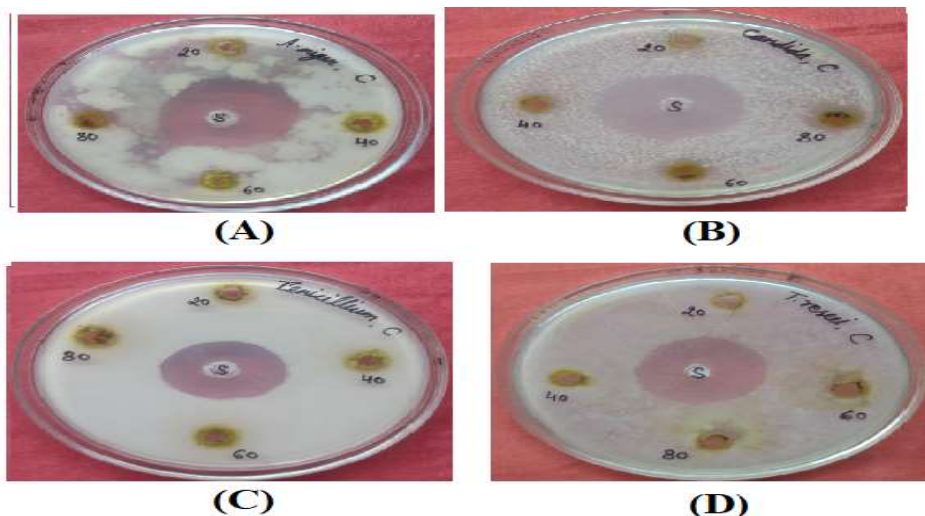


Figure 4: Antifungal activity of chloroform extract of *Acacia nilotica* leaf against (A) *Aspergillus niger* (B) *Candida albicans* (C) *Penicillium chrysogenum* (D) *Trichoderma reesei*

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CONCLUSION

Medicinal plants are rich sources of herbal properties contributing in the discovery of new drugs towards various disorders, diseases including cancer without any toxic effects on the individuals treated. *Acacia nilotica* is widely used as tradition medicine such as for the treatment of venereal diseases, burns, wounds, stomachache etc. This study concluded that the *Acacia nilotica* has antibacterial and antifungal activity in their leaves part. The antimicrobial property of *Acacia nilotica* can be due to the presence of several phytochemical compounds in the leaf that inhibits the growth and development of bacterial and fungal species. Thus, *Acacia nilotica* plays an important role in the prevention and management of various diseases. It can be used in future as herbal drugs with ecofriendly, non-expensive and without any side effects.

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