Comparative Antimicrobial Activity of Acacia nilotica L., Leaves Extracts Against Pathogenic Bacteria and Fungi

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ABSTRACT : The comparative in vitro antimicrobial activity of ethanol and chloroform leaves extracts from Acacia nilotica L., was studied. Leaves extracts exhibited considerable bacteriostatic activity against two Gram-positive and three Gram-negative stains. The antimicrobial action was compared with the effect of Doxycycline antibiotic. The maximum zone of inhibition of 29 mm diameter was observed in Escherichia coli with ethanol extract while a minimum 08 mm zone of inhibition was found in Bacillus subtilis. Klebsiella pneumoniae showed marked resistance towards both ethanol and chloroform extracts. Among the fungal strains tested, Aspergillus flavus exhibited maximum sensitivity action of the extracts. This analysis revealed the high antimicrobial activity in the ethanol extract of Acacia nilotica L., than chloroform extract. It is recommended to isolate, identify and integrate the bioactive principle in these pathogens management.
***Key words*:** Acacia nilotica L., ethanol, Extract, chloroform,pathogenic, MIC.

INTRODUCTION

Medicinal plant researchers pursued with several goals like the development of low cost therapeutic compounds and the discovery of prototypic drugs (Elisabetsky, 1991). Acacia nilotica L., is the member of the family Mimosaceae and is known as babul in Pakistan. Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000m and withstand at extreme temperature (>50^{o} C) and air dryness but sensitive to frost when it is young (Kiran and Bargali, 2009). It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India (Bennison and Paterson, 1994).

Phytochemical analysis of the aerial parts of the plant demonstrated the presence of polyphenolic compounds and flavonoids in the flowers. Tannins, volatile oils, glycosides, coumarins, carbohydrates and organic acids are reported in the fruits (El Shanawany, 1996). Babul has been reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyldjenkolic acid, N-acetyldjenkolic acid, sulphoxides pentosan, saponin, tannin. Seeds contain crude protein 18.6%, ether extract 4.4%, fiber 10.1%, nitrogen-free extract 61.2%, ash 5.7%, silica 0.44%, Phosphorus 0.29% and calcium 0.90% of DM (Pande et al., 1981).

Acacia nilotica L., leaves are very digestible and have high levels of protein. The fruits are higher in aspartic and glutamic acid but lower in most other amino acids. The methionine was absent from the fruit of Australian materials but present in the seeds of African material (Fagg, 2001; Spies and March, 2004)

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Farombi, 2003).

Acacia nilotica L., is specifically and other Acacia species are used in local traditional medicine by people as remedy for various disorders like cancers/ tumors (of ear, eye, or testicles) and indurations of liver and spleen, condylomas and excess flesh. It may also be used for colds, congestion, coughs, diarrhea, dysentery, fever,

gallbladder, hemorrhage, hemorrhoids, leucorrhea, ophthalmia, sclerosis, smallpox and tuberculosis (Hartwell, 1971). Bark decoction drunk for intestinal pains and diarrhea. The resin is mixed with orange-flower infusion for typhoid convalescence.

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).

There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; de Boer, 2005 and Islam et al., 2008). Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Brantner, 1994; Somchit, 2003). Cytotoxic compounds have been isolated from the species of Vismia (Hussein, 2003). According to the WHO, medicinal plants would be the best source for obtaining variety of drugs (Nair, 2006). These evidences contribute to support and quantify the importance of screening natural products.

In Pakistan, huge varieties of medicinal plants are available (Dastur, 1970). Most of these plants are being used for therapeutic purposes without specific knowledge of their active ingredients. In fact, Pakistani medicinal plants, for the purpose of drug development, are one of the least investigated sources of natural compounds (Satyavati et al., 1976). The aim of the present study was to investigate the antibacterial and antifungal activity of the plant leaves ethanol and chloroform extracts of Acacia nilotica L., against both Gram-positive and Gram-negative bacteria and fungal. The anti-microbial activity of the plant leaves extracts was compared with that of standard antibiotic Dox (Doxycycline).

MATERIALS AND METHODS

All the experiments were done in three replicates and average values were used.

Anti-microial Activity of Acacia nilotica L.

All the experimentation was done in aseptic area under laminar air-flow cabinet.

Anti-bacterial Activity

Antibacterial activity of solvent extracts; ethanol and chloroform were determined by Well-diffusion method on nutrient agar medium according to Lino and Degracious (2006) with slight modifications.

Plant Material and Preparation of Extract

Acacia nilotica L. species were collected from Quaid-i-Azam University, Islamabad, Pakistan. A. nilotica was identified and voucher specimens were deposited in the Herbarium, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Leaves of Acacia nilotica were rinsed with distilled water and kept under shade till drying. Leaves of the plants were weighed. Extraction from dried leaves was carried out by simple maceration process. The leaves were taken and grounded to coarse powder. The powder was suspended in 75% ethanol and 75% chloroform for 3-7 days at 60^{o} C in extraction bottle. After two weeks mixture was filtered twice, using Whatman-41 filter paper. Ethanol and chloroform was then completely evaporated by rotary evaporator to obtain the extract. Extracts were stored at 4^{o} C for screening of anti-bacterial activity.

Preparation of samples

The extract (15 mg) was dissolved in 1ml of DMSO. This stock solution 15 mg/ml was again diluted, thus 8 concentrations of the extract were prepared i.e. 15 mg/ml, 12.50 mg/ml, 10 mg/ml, 7.5 mg/ml, 5.00 mg/ml, 3.00 mg/ml, 2.00 mg/ml, 1.00 mg/ml. Along with these solutions of Standard antibiotic (2 mg/ml of the DOX) was also prepared. The solutions of the extracts are used for test control. Standard antibiotics and pure DMSO were used for positive and negative control. Dilutions with DMSO are presented in table 1.

Preparation of media for bacteria

Nutrient broth medium was prepared by dissolving 0.4 g/ 50ml of distilled water for the growth of bacterial inoculum; pH was adjusted at 7.0 and was autoclaved. Nutrient agar medium was prepared by dissolving 2.3 g/ 100ml of distilled water; pH was adjusted at 7.0 and was autoclaved at 121^{o} C.

Mcfarland 0.5 Barium Sulphate Turbidity Standard

The standard was prepared by adding 0.5 ml 0.048 M Barium chloride to 99.5 ml 0.36 N sulphuric acid. Barium Sulphate turbidity standard (4-6 ml) was taken in screw capped test tube and poured to inoculums till the inoculum give the same color as that of turbidity standard (Koneman, 1988).

Bacterial strains used

Five strains of bacteria were used in the study which was collected from Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan. Two were gram positive, Staphylococcus aureus (ATCC 6538) and Bacillus subtilis (ATCC 6633) and three were gram negative; Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 7221) and Klebsiella pneumonia (ATCC 10031). The organisms were maintained on nutrient agar medium at 4°C.

Preparation of Inocula

Centrifuged pallets of bacteria from 24 hours old culture in nutrient broth (SIGMA) of selected bacterial strains were mixed with physiological normal saline solution until a Mcfarland turbidity standard [10^6 colony forming unit (CFU) ml^{-1}] was obtained. Then this inoculum was used for seeding the nutrient agar.

Preparation of seeded agar plates

Nutrient agar medium was prepared by suspending nutrient agar (MERCK) 2.3g in 100ml of distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45° C. Then it was seeded with 10 ml of prepared inocula to have 10^6 CFU per ml. Petri plates were prepared by pouring 75ml of seeded nutrient agar and allowed to solidify. Eight wells per plate were made with sterile cork borer (5mm).

Pouring of Test Solutions; Incubation and Measurement of Zone of Inhibitions

Using micropipette, 100µl of test solutions was poured in respective wells. These plates were incubated at 37°C. After 24 hours of incubation; the diameter of the clear zones of inhibitions were measured by a ruler. Antibacterial activity of 8 dilutions of plant extract was determined against five bacterial strains.

Antifungal Assay

The agar tube dilution method is used for antifungal activity of extract as reported by Choudhary et al., (1995).

Fungal strains used

Two fungal strains Aspergillus niger and Aspergillus flavus were used in this study. Each fungal strain was maintained on sabouraud dextrose agar medium at 4° C.

Media for antifungal assay

Sabouraud dextrose agar (MERCK) was used to grow fungus for inoculums preparation. Its composition was;

A) Peptone complex	10 gm/l
B) Glucose	40 gm /l
C) Agar	15 gm /l

Preparation of samples

The samples for antifungal assay were prepared from initial stock of 15mg of extract each sample per ml of DMSO. One sample of each extract was prepared, which were used for test. Slants without extract were used for negative control.

Assay Procedure

Media for fungu**s** was prepared by dissolving 6.5 gm/100ml in distilled water pH was adjusted at 5.6. Test tubes were marked to 12 cm mark. The sabouraud dextrose agar (MERCK) dispensed as 4ml volume into screw capped tubes or cotton plugged test tubes and were autoclaved at 121°C for 21 minutes. Only single concentration 24mg/ml was made. Tubes were allowed to cool to 50°C and non-solidified SDA was loaded with 100 µl of 24 mg/ml plant extracts were inserted by compound pipette from the stock solution. Tubes were then allowed to solidify in slanting position at room temperature.

One slant of the extract sample was prepared for each fungus species. The tubes containing solidified media and test compound were inoculated with 4mm diameter piece of inoculum, taken from a seven days old culture of fungus. Negative control test tubes without extract were also inoculated. The test tubes were incubated at 28°C for 7 days. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length of fungus in slant by measuring growth (mm) and growth inhibition was calculated with reference to negative control. Percentage (%) inhibition of fungal growth for each concentration of compound was determined by the following formula.

$$\% \text{ inhibition of fungal growth} = \frac{\text{Linear growth in control - Linear growth in test}}{\text{Linear growth in control}} \times 100$$

RESULTS AND DISCUSSION

Antibacterial study of of Acacia nilotica L.

Extracts of ethanol and chloroform of Acacia nilotica L., were tested against five strains of bacteria. Two strains were Gram-positive i.e. Staphylococcus aureus and Bacillus subtilis and three were Gram-negative i.e. Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. Reading of inhibition zones were taken in millimeter (mm). All dilutions of extracts were made in DMSO (Dimethyl sulfoxide). This solvent has no effect on the growth of bacteria. Eight dilutions of plants extracts sample was made i.e. 15 mg/ml, 12.50 mg/ml,10 mg/ml, 7.5 mg/ml, 5.00 mg/ml, 3.00 mg/ml, 2.00 mg/ml, and 1.00 mg/ml. Only one concentration of 2 mg/ml of Dox (Doxycycline) standard antibiotic was made and 100ul of each plant extracts dilutions were introduced into the wells made by sterile cork.

The antibacterial activity of both ethanol and chloroform extracts from Acacia nilotica L., leaves against all test organisms was reduced with decrease of extracts concentrations. Escherichia coli showed the highest inhibition zone (29 mm) with ethanol extract at the concentration of 15 mg/ml While minimum inhibitory concentration (MIC) value was 1.00 mg/ml, MIC indicated 19 mm inhibitory zone. The inhibition zone reduced gradually with reduction of extract concentration to 25 mm at 12.5 mg/ml, 23 mm at 10 mg/ml, 22 mm at 7.5 mg/ml, 21 mm at 5 mg/ml, 20mm at 3 mg/ml, 18 and 16 mm at concentrations of 2 mg/ml and 1 mg/ml, respectively. The chloroform extract showed smaller inhibition zones, 18 mm at concentration 15 mg/ml and 15 mm at 12.5 mg/ml, 11 mm at 10 mg/ml, 08 mm at 7.5 mg/ml, 06 mm at 5 mg/ml, 05 and 04 mm at concentrations 3 and 2 mg/ml, respectively. The organism showed resistance towards chloroform extract at concentration of 1 mg/ml (Table 2). At 2 mg/ml concentration of standard antibiotic DOX (Doxycycline) showed 38 mm inhibition zone.

Klebsiella pneumoniae showed marked resistance towards both ethanol and chloroform extracts at concentrations of 3, 2 and 1 mg/ml extracts. It was only affected by 15, 12.5, 10 and 7.5 mg/ml of the Acacia nilotica L., ethanol and chloroform extracts. Where as minimum inhibitory concentration (MIC) value was 1.00 mg/ml. MIC indicated 14 mm inhibition zone. Standard antibiotic DOX (Doxycycline) exhibited 25 mm inhibition zone at the concentration of 2 mg/ml. Only One concentration of antibiotic was made against test bacteria. On the other hand, Staphylococcus aureus had a marked sensitivity towards both ethanol and chloroform extracts except with 2 and 1 mg/ml chloroform extracts. This sensitivity was markedly reduced with decrease in extract concentration.

Bacillus subtilis tended to show the smallest inhibition zones at all concentrations of both ethanol and chloroform extracts when compared with other organisms. It also had a marked clear resistance against chloroform extracts at 3, 2 and 1 mg/ml concentrations. Here also minimum inhibitory concentration (MIC) value was 1.00 mg/ml, and MIC exhibited 08 mm zone of inhibition. Standard antibiotic DOX (Doxycycline) showed 47 mm inhibition zone.

The antimicrobial activity of ethanol and chloroform extracts from the A. nilotica fruits against Pseudomonas aeruginosa was more effective than Bacillus subtilis. All concentrations of ethanol and chloroform extracts showed inhibition zones except chloroform extract at concentrations of 3, 2 and 1 mg/ml. Where as MIC value was 2.00 mg/ml. MIC (minimum inhibitory concentration) showed 09 mm inhibition zone, where as at 1.00 mg/ml extract did not show any antibacterial activity. Standard antibiotic DOX (Doxycycline) showed 14 mm inhibition zone. The ethanol and chloroform extracts from the Acacia nilotica gave smaller inhibition zones when compared with Dox antibiotic except fewer concentrations.

Antifungal study of Acacia nilotica plant

This study was done to check antifungal activity of Acacia nilotica L., plant. Only one concentration of plant extracts, were prepared by dissolving 24 mg/ml in solvent DMSO (Dimethylsulfoxide). The fungi used in this study

were Aspergillus niger and Aspergillus flavus. After inoculation and incubation of the samples for about one week, antifungal assay gave the following results.

Acacia nilotica L. ethanolic extracts showed 4.91 % and 116 mm growth inhibition while 7.0% and 93 mm growth inhibition against Aspergillus niger. Acacia nilotica L. ethanolic extracts showed 4.61 % and 124 mm growth inhibition while 10.9% and 98 mm growth inhibition against Aspergillus flavus.

These plant extracts were considered as test and control i.e. with and with out extract growth of fungus on media in the test tubes. The species Aspergillus niger gave 122, 100 mm growth in ethanolic and chloroform extracts and it was considered as control, where as Aspergillus flavus gave 130, 110 mm growth in test tube and it was also taken as control. All antifungal results were compared with control in test growth in control was taken as standard to compare the growth of inhibition in test.

DISCUSSION

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are incurable in other system of medicine. The results of the present study provide a scientific validation for the popular use of the medicinal plants studied and serve as a guide who may help in selection of plants with antimicrobial activities for further phyto-chemical work on the isolation and the identification of the active compounds.

Pakistan is rich in diversity of plants. People living in rural areas are interested in the use of plant-based drugs, because plant based drugs have no side effects and they are inexpensive. Acacia nilotica L., appear to have potential for testing as a plant of high medicinal values for various antimicrobial activities as well other biological activities. This plant is abundantly found in Pakistan and easily accessible.

The present study showed that Acacia nilotica L., leaves extracts were effective inhibitors of bacterial and fungal growth. The extracts of the plant showed varying degrees of activity against Gram-positive and Gram-negative bacteria as well as fungi. It showed inhibitory zones ranging from 04 to 29 mm against five strains of bacteria; whereas, at 3, 2 and 1.00 mg/ml against Klebsiella pneumoniae it did not show any antibacterial activity. Satish et al., (2008) found that that aqueous extract of the Acacia nioltica L., exhibited significant study against the test bacteria. Acacia nilotica L., showed zones of inhibition from 9-35 mm, whereas in this study zones of inhibitions were from 04 to 29 mm, and the extracts was of ethanol and chloroform. The results revealed that ethanol extract was more effective against all test organisms than chloroform extract. This may be due to the ability of the ethanol to extract a wide range of chemical compounds of the plant leaves while the chloroform might have extracted less number of the constituents.

The leaves extracts showed higher activities against Escherichia coli compared with other test bacteria (Gram negative). As Acacia nilotica L., leaves contains, flavonoids, polyphenolic compounds, tannins, glycosides, organic acids and coumains (El-Shanawany, 1996) the anti-microbial activity of the plant leaves might be due to the polyphenolic compounds. It was found that the polyphenolic compounds are responsible for the anti-bacterial activity of plants.

The studies of Cheesbrough (1984) also indicated that polyphenolic compounds and/ or volatile oils cause inhibition of a wide range of microorganisms. Phenol is well known as a chemical antiseptic. The presence of tannins may have accelerated wound healing probably due to their astringent effect. Al-Yahya et al., (1990) found that both ethanol and chloroform extracts from Acacia nilotica L., fruit were equally effective against both Bacillus subtilis and Staphylococcus aureus and that the ethanolic extracts was also active against Proteus vulgaris.

Saini et al., (2008) found that the mathanolic extracts of Acacia nilotica (pods) and Acacia catechu (bark) were reported to be most active against different bacterial and fungal strains. The methanolic extract of Acacia nilotica (pods) showed highest activity against Escherichia coli, Staphylococcus aureus and Aspergillus niger. In this study Acacia nilotica L., showed highest activity against Escherichia coli followed by Klebsiella pneumoniae, Bacillus subtilis and Pseudomonas aeruginosa. Plant was active against all test micro organism.

Banso (2009) using agar diffusion method found that the ethanolic stem bark extract of Acacia nilotica L. produced antimicrobial activity against Streptococcus viridans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Shigella sonnei. The extract contained the active principles– terpenoids, tannins, alkaloids, saponins and glycosides. MIC value against Bacillus subtilis and Escherichia coli was 35 mg/ml and 45 mg/ml. In this study it was found that ethanolic extract of Acacia nilotica L., exhibited significant activity against all test pathogens. MIC value against Escherichia coli, Bacillus subtilis was 1 mg/ml. This is contrast MIC value to the work of Banso (2009).

Al-fatimi et al., (2007) found that the methanolic extract of Acacia nilotica L., did not show any activity against Escherichia coli and Pseudomonas aeruginosa, where as it exhibited antimicrobial activity against Staphylococcus aureus, Bacillus subtilis and Micrococcus flavus. They found that it gave antifungal activity against Candida krusei, Aspergillus fumigatus, Absidia corymbifera and Trichophyton mentagrophytes. In this study it was found that ethanolic and chloroform extracts of Acacia nilotica L. was equally efficient against gram negative Escherichia coli and Pseudomonas aeruginosa as well as against fungi.

Dabur et al., (2007) analyzed that water extracts of Acacia nilotica, Justicia zylanica, Lantana camera and Saraca asoca were found to be the most active against bacteria as well as fungal pathogens. The wells containing a concentration of 9.375-150.0 µg/ml extracts of water and methanol inhibited the visible growth of all the bacterial species. Methanol extracts of Acacia nilotica L. and Justicia zylanica exhibited good activity in the range of 18.75-75.0 µg/ml. Where as in this research ethanolic and chloroform extracts of Acacia nilotica L. was prepared and it gave remarkable activity against all pathogens.

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery (Gandhiraja et al., 2009).

CONCLUSIONS

Results indicate the potential of this plant for further work on isolation and characterization of the active principle responsible for antimicrobial activity and its exploitation as therapeutic agent. All Pakistani medicinally important floras should be tested against all pathogens in order to develop new and cheaper drugs using modern techniques like TLC, HPLC, and spectrophotometery. There is need of phytochemical as well as biological activities of plants in Pakistan. There is need of developing drugs from plants as microorganisms are becoming resistant to antibiotics and creating health problems.

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Table 1. Different dilutions with DMSO (Dimetthyl sulphoxide)

S. No.	Conc.(mg/ml)	Stock Sol (ml)	DMSO (ml)	Final Vol.(ml)
1.	15	1.00	0.00	1
2.	12.50	0.833	0.167	1
3.	10.00	0.66	0.334	1
4.	7.50	0.500	0.500	1
5.	5.00	0.334	0.666	1
6.	3.00	0.200	0.800	1
7.	2.00	0.133	0.867	1
8.	1.00	0.100	0.900	1

Table 2: Anti-bacterial activity of ethanol and chloroform extract from Acacia nilotica L., leaves

	Inhibition zone (mm) Ethanol extract (conc.)								Inhibition zone (mm) Chloroform extract (conc.)							
Bacteria	15	12.5	10	7.5	5	3	2	1	15	12.5	10	7.5	5	3	2	1
Escherichia coli	29	25	23	22	21	20	18	16	18	15	10	08	06	05	04	0®
Staphylococcus aureus	24	22	21	19	17	15	12	11	14	10	08	06	05	04	0®	0®
Pseudomonas aeruginosa	16	14	13	12	11	10	10	09	11	10	08	06	04	0®	0®	0®
Klebsiella Pneumonia	21	20	19	17	14	0®	0®	0®	16	13	09	05	0®	0®	0®	0®
Bacillus subtilis.	15	14	13	12	11	10	09	08	11	10	08	05	04	0®	0®	0®

® = Resistant.

Table 3: Anti-microbial activity of standard antibiotic DOX (Doxycycline)

	Inhibition zone (mm) Dox (conc.)
Bacteria	2 mg/ml
Escherichia coli	38
Staphylococcus aureus	39
Pseudomonas aeruginosa	14
Klebsiella Pneumonia	25
Bacillus subtilis	47

Table 4: Anti-fungal activity of ethanol and chloroform extracts from Acacia nilotica L., leaves

	Linear growth in control (LGC) (mm)		Linear growth in test (LGT) (mm)		% inhibition	
Fungi	Ethanol	Chloroform	Ethanol	Chloroform	Ethanol	Chloroform
Aspergillus niger	122	100	116	93	4.91%	7.0%
Aspergillus flavus	130	110	124	98	4.61%	10.9%

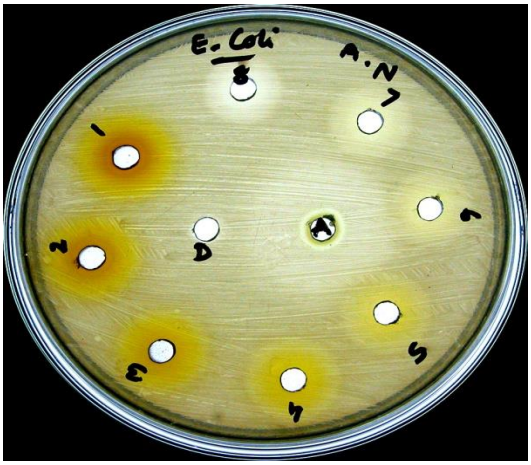

Plate (a):

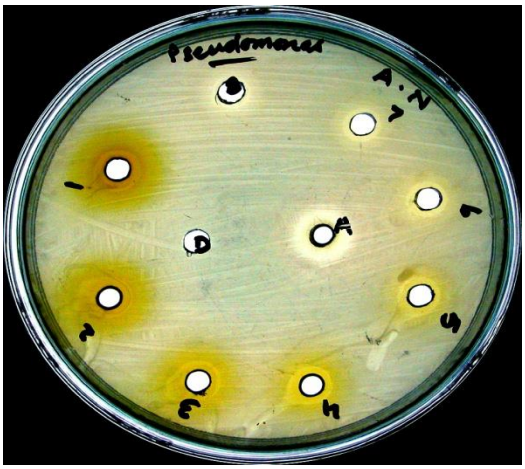

Plate (b):

Plate (c):

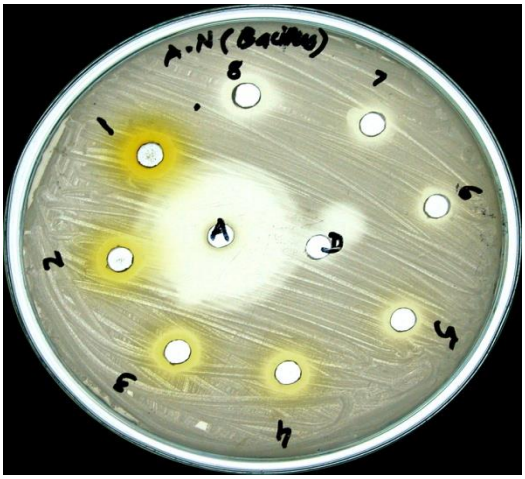

Plate (d):

Antibacterial activity of Acacia nilotica L. against Escherichia coli (a), Pseudomonas aeruginosa (b), Klebsiella pneumonia (c) and Bacillus subtilis (d).

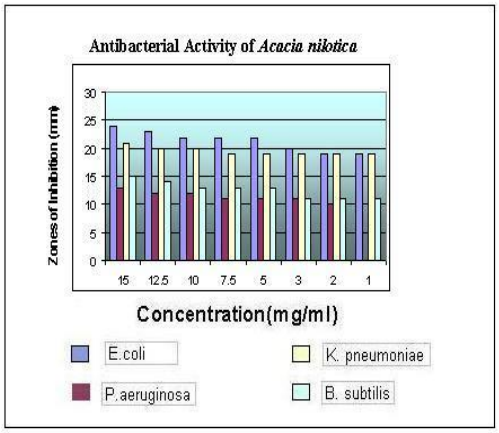

Plate (e):

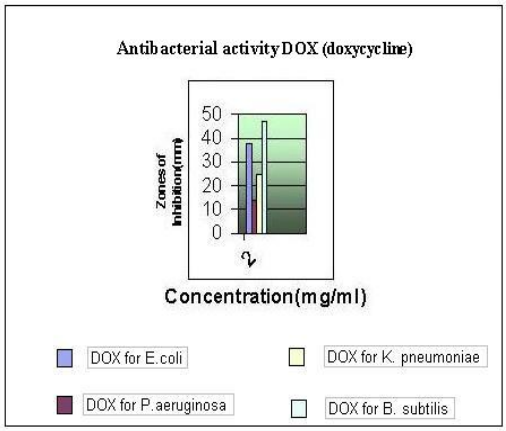

Plate (f):

Zones of inhibition (mm) showing Antibacterial activity of Acacia nilotica L., against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Bacillus subtilis (e) and Antibacterial activity of standard antibiotic DOX (doxycycline) using 2 mg/ml concentration (f).

REFERENCES

Activity of Mulberry Leaves: Inhibition of Streptococcus mutans Biofilm by 1 Deoxynojirimycin Isolated from Morus alba J. Antimicrob. Chemother. (in press).

Bennison JJ, Paterson RT (1994). The use of Trees by Livestock Acacia Production Programme. **1**: 160-164.

Bonjar S (2004). Evaluation of Antibacterial Properties of Some Medicinal Plants Used in Iran. J. Ethnopharmacol., 94: 301-305.

Brantner A, Grein E (1994). Antibacterial Activity of Plant Extracts Used Externally in Traditional Medicine. J. Ethnopharmacol., 44: 35-40.

Choudhary MI, Dure-e-Shawar, Perveen Z, Jabbar A, Ali I, Rehman A (1995). Antifungal steroidal lactones from Withiana coagulance. Phytochem., 440 (4):1243-1246.

Dastur JF (1952). Medicinal plants of India and Pakistan. D. C. Taraporevala Sons and Co. Private Ltd., Bombay, India: 212.

Dastur JF (1970). Medicinal plants of India and Pakistan. D. C. Taraporevala Sons and Co. Private Ltd., Bombay, India: 212

de Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, Levenfors JJ (2005). Antifungal and Antibacterial Activity of Some Herbal Remedies from Tanzania. J. Ethnopharmacol., 96; 461-469.

Elisabetsky E (1991). Sociopolitical, economical and ethical issues in medicinal plant research. J. Ethnopharm., 32(1-3): 235-239.

El-Shanawany MA (1996). Medicinal Plants Used in Saudi Traditional Medicine, King Abdel Aziz City for Science and Technology, Riyadh.144.

Fagg C (2001). Acacia nilotica: Pioneer for Dry lands. In: Agroforestry species and Technologies. Roshetko, J.M. (Ed.), (Winrock International, Arizona, USA.:23-24

Farombi EO (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African J Biotech. 2: 662-671.

Hartwell JL (1967-1971). Plants used against cancer; A survey. Lioydia 30-34.

Hussein AA, Bozzi B, Correa M, Capson TL, Kursar TA, Coley PD, Solis PN, Gupta MP (2003). Bioactive Constituents from Three Vismia Species. J. Nat. Prod., 66: 858-860.

Islam B, Khan SN, Haque I, Alam M, Mushfiq M, Khan AU (2008). Novel Anti-adherence

Kiran B, Bargali SS (2009). Acacia nilotica: a multipurpose leguminous plant. Nat and Sci., 7(4):11-19.

Koneman EW (1995). Color Atlas and text book of Diagnostic Microbiology.2nd Ed. Roma. A. Delfino:550-605

Lino A, Deogracios O (2006). The in-vitro antibacterial activity of Anona senegalensis, Securidacca longipendiculata and Steanotaenia araliaceae- Ugandan Medicinal Plants. Afr. Health Sci., 6(1): 31-35.

Nair R, Chanda S (2006). Activity of Some Medicinal Plants Against Certain Pathogenic Bacterial Strains. Indian J. Pharmacol. 38: 142-144.

Pande MB, Talpada PM, Patel JS, Shukla PC (1981). Note on the nutritive value of babul (Acacia nilotica L.) seeds (extracted). In: Indian J Animal Sci. 51(1):107–108.

Prusti A, Mishra SR, Sahoo S, Mishra SK (2008). Ethanobotanical leaflets 12: 227-230.

Satish S, Raghavendra MP, Raveesha KA (2008). Evaluation of antibacterial potential of some plants against human pathogenic bacteria. Adv in Bio Res 2 (3-4):44-48

Satyavati GV, Raina KK, Sharma M (1976). Medicinal Plants of India, Vol. I, A-G. Council of Medicinal Research, New Delhi, India.,: 68-78.

Somchit MN, Reezal I, Nur IE, Mutalib AR (2003). In vitro Antimicrobial Activity of Ethanol and Water Extracts of Cassia alata. J. Ethnopharmacol., 84: 1-4.

Spies P, March N (2004). Prickly acacia- Approaches to the management of prickly Acacia (Acacia nilotica) in Australia. Department of Natural Resources, Mines and Energy, Queensland, Australia.