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Anti-bacterial competency of stem barks of *Azadirachta indica* Juss. and *Acacia nilotica* Linn.

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Abstract
Emergence of antibiotic resistance and toxicity an issue reduce the use of antimicrobial agents and provokes resurgence in research for the development of antimicrobial resistant strains on plants.*Azadirachta indica* Juss. and *Acacia nilotica* Linn. are multipurpose medicinal plants with many health benefits. Different parts of these plants show antibacterial effects against a wide variety of human pathogenic bacteria. Hence, the present study was aimed to explore the antibacterial competency of stem bark of*A. indica* and *A. nilotica* extracted with different solvents against human pathogen. The bark extracts (Acetone, Chloroform, Ethanol, Methanol and Water) were tested against randomly selected common pathogens such as *Staphylococcus aureus* (Gram positive), *Klebsiella pnemoniae*, *Salmonella typhi*, *E.coli* and *Proteus* spp (Gram negative) using disc diffusion method. The results have shown that the bark extracts possessed antibacterial activities against all the tested bacterial strains except *Salmonella typhi*. The highest antibacterial activity exhibited by aqueous extracts of *A. nilotica* against *K. pnemoniae* followed by *A. indica* against *K. pnemoniae* and *E. coli*. On a comparison between the two bark extracts, *A. nilotica* shown maximum activity than *A. indica*.

Keywords: antibacterial activity, disc diffusion method, stems bark extracts, *Azadirachta indica* and *acacia nilotica*

Introduction

Global prevalence of transmittable diseases caused by bacteria is a leading public health issue (Zhang *et al*., 2006; Willamson *et al*., 2008). The bacterial pathogens cause several infectious diseases in human. Development of novel antibiotic resistance from plants could be useful in meeting the demand for new antibacterial agents with improved competency and safety (Srivatsava and Shukla Kumar, 2000). In view of the rich diversity of Indian medicinal plants, it is expected that screening and scientific evaluation of plant extract for their antimicrobial substances may prove beneficial for the mankind (Parihar *et al*., 2003). The organic versatile compounds are mostly the active principles and are responsible for antimicrobial activity of several medicinal plants (Harsh and Nag, 1988).In general, Plants are rich in phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties (Dorman and Deans, 2000; Talib and Mahasneh, 2010). Although the mechanism of action and effectiveness of these herbal extracts in most cases is still needed to be validated scientifically (Cruz *et al*., 2007; Ruberto *et al*., 2000).

In India, medicinal herbs have been the basis of treatment for various traditional methods such as Ayurveda, Siddha and Unani. The medicinal plants selected for the present study are *Azadirachta indica* Juss. And *Acacia nilotica* Linn. *Azadirachta indica* (*A. indica*) belongs to the family of Meliaceae, commonly known as neem, used in traditional medicine as a source of many therapeutic agents. *A. indica* are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against chikungunya, measles (Biswas *et al*., 2002). Different parts of neem (leaf, bark and seed oil) have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial, antimutagenic, anticarcinogenic, anti-inflammatory, anti-hyperglycemic, antiulcer and antidiabetic properties (Talwar *et al*, 1997). *Acacia nilotica* (*A. nilotica*) ,also known as *Mimosa nilotica*, is a member of the family Mimosaceae, used in folk medicine by people in rural areas as a remedy for tuberculosis, leprosy, small pox, dysentery, cough, ophthalmia, toothache, skin ulcers and cancers and as astringents, antispasmodics, aphrodisiac(Duke, 1983; Van *et al*., 2000).

In present days, the use of antibiotics is thought to have spurred evolutionary changes in bacteria that allow them to survive even treatment with the powerful drugs (Anonymous, 2004).

Antibiotic resistance is accelerated by the overuse of antibiotics which has driven selection of mutations in bacteria that bring about drug resistance (Anonymous, 2000).

Hence, the plant extracts with an antimicrobial property, can be of great significance as potential antibiotic agent against various microbial infections (Reddy and Jamilk, 2006; Atefl and ErdoUrul, 2003; Edro, 2002).

The current study was designed to investigate the comparative antibacterial activity of crude extracts from the bark of *Azadirachta indica* and *Acacia nilotica* against human pathogenic bacteria.

A number of factors such as, thickness and uniformity of the medium, size of the disc, temperature and pH of the medium which affect the accuracy and reproducibility of the disc diffusion method were also taken into consideration to obtain reliable results.

Materials and Methods

Collection of raw materials and preparation of extracts

Azadirachta indica barks were collected from college campus itself and the barks of *Acacia nilotica* were collected from Salem district. The collected samples were cleaned; shades dried for 12-15 days and are pulverized into fine powder in a mechanical grinder. Fifteen gram of fine powder was mixed with 100 ml of Acetone, Chloroform, Ethanol, Methanol and water separately in closed dark container and soaked for 3 days at room temperature with intermittent mixing. The extracts were filtered using standard Whatmann No.1 filter paper and stored in air tight containers at 4ºC for further analysis.

Chemicals

All chemicals and drugs used were of analytical grade and are commercially obtained from Hi-Media Laboratories Ltd, India. Muller Hinton Broth and Muller Hinton Agar procured from HI-Media and Mumbai were used in the study and prepared as per manufacturer's instructions.

Nutrient Medium Preparation

A known quantity nutrient agar medium was taken in a concial flask and dissolved in 1000ml of distilled water. The contents were mixed thoroughly then, the conical flask with the medium was tightly plugged with cotton and subjected for sterilization. The steam sterilization process was carried out in an autoclave at 15 pounds pressure for 15 minutes. Along with the nutrient medium, necessary glass wares such as petri dishes, forceps and inoculation needle were also sterilized.

Bacterial inoculum preparation

Bacterial inoculum was prepared by subculturing the commercially available strains procured from clinics. A loop full of test organisms were taken and inoculated into 5ml of nutrient broth and incubated at 37˚C for 3 to 5 hours till a moderate turbidity was developed. This was used as a source of bacterial inoculum.

Test Microorganisms

The clinical isolates used in the present study include Gram positive *Staphylococcus aureus,* Gram negative *Klebsiella pnemoniae*, *Salmonella typhi*, *Escherichia coli* and *Proteus* spp. All the cultures were purchased from department of Clinical Microbiology, K.A.P.V. Government Medical College, Tiruchirappalli. The bacterial strains were maintained in Muller Hinton Agar (MHA, pH 7.2) at 37±1ºC. The stock culture slants were maintained at 4ºC.

Staphylococcus aureus: Staphylococcus aureus is one of the most important human pathogens associated with hospital and community-acquired infections (Lee *et al*., 2011). It is a facultative gram-positive cocci and a non-motile anaerobe. It typically forms colonies with golden yellow pigment and grows luxuriantly under aerobic than anaerobic conditions. *S. aureus* is the causative agent of cutaneous abscess, acute staphylococcal enterocolitis, food poisoning, dermatitis, scarlantia, bullous impeting and staphylococcal scalded skin syndrome etc.

Klebsiella pnemoniae: *Klebsiella pneumoniae* is a signifcant Enterobacteriaceae considered as one of the opportunistic pathogens causing broad spectra of diseases and showing increasingly frequent acquisition of resistance to antibiotics. It is a gram-negative, non-motile, capsulated rods and it is the most important pathogen of *Klebsiella* group. This organism accounts for about one-third of all Gram-negative infections such as urinary tract infections, cystitis, pneumonia, surgical wound infections, endocarditis and septicaemia (Navon-Venezia *et al*., 2017).

Salmonella typhi: *Salmonella typhi* is a Gram-negative, obligate anaerobe that belongs to the serogroup D. *Salmonella typhi* causes enteric fever (typhoid and paratyphoid fever) characterized by high fever, abdominal pains, headache, vomiting, and diarrhea followed by constipation and rashes lasting for several weeks or months. Humans are the only reservoir for these organisms and can be both cases and vehicles. Sources of infection can be contaminated food and water, or contact with stools of infected people. These bacteria are in many foods, including prepared foods, dairy products, meat products, shellfish, vegetables, and salads (Jaroni, 2014) [21].

***Escherichia coli*:** *Escherichia coli* is a member of the family Enterobacteriaceae, which are Gram-negative facultatively anaerobic rods (possessing both a fermentative and respiratory metabolism) and do not produce the enzyme oxidase. *Escherichia coli* cells are typically 1.1–1.5 μm wide by 2–6 μm long and occur as single straight rods and are either motile or non-motile. *E. coli* behaves as commensal in the intestine and it causes lesions outside of the body. It causes respiratory infection and diarrhea. E. coli is sensitive to Tetracycline, Ampicillin and Cephalosporins (Desmarchelier and Fegan, 2002) [22].

***Proteus* spp:** *Proteus* spp. is Gram-negative bacteria belonging the *Enterobacteriaceae* family and are potentially pathogenic residents of the human gastrointestinal tract. They are clinically recognized as a cause of urinary tract infections and gastrointestinal disease (Penner JL 2005) [23].

Determination of antibacterial activity

The bark extracts of *A. indica* and *A. nilotica* were screened for antibacterial activity by disc diffusion method. Nutrient agar plates were prepared for each species of bacterium under study in sterilized condition. Twenty ml of the sterile Muller-Hinton agar medium was poured into the petriplates aseptically. The nutrient agar plates were allowed to solidify. Each petriplates were labeled according to the bacterial strain to be used for streaking. A loop full of inoculums was streaked on the surface of Muller Hinton Agar plate in aseptic condition. The uniform growth of bacteria was ensuring by repeating the streaking for second and third time by turning the plate through 60° angle. Standard disc of Streptomycin (10μg/disc), 6 mm in diameter were used as positive control and DMSO was used as negative control. The plates were incubated overnight at 37° C for 18-24 hours. Antibacterial activity was evaluated by measuring zone of inhibition. The diameter of the inhibition zone included the diameter of the disc.

Data analysis

All the experiments were independently repeated as triplicates, and average zone of inhibition of test extracts relative to negative control was calculated using Microsoft Excel 2010.

Results

In the present study, antibacterial activity of crude extracts from stem barks of *Azadirachta indica* and *Acacia nilotica*

were analysed against the human pathogenic bacteria *Staphylococcus aureus*, *Klebsiella pnemoniae*, *Salmonella typhi*, *E. coli* and *Proteus* spp.

All the test strains of bacteria were found to be sensitive to Positive control Streptomycin. DMSO was used as the negative control which did not show any zone of inhibition against the tested bacterial strains. Among the extracts tested, the aqueous bark extracts of both *A. indica* and *A. nilotica* showed a highest activity against *K. pnemoniae*, with 18 mm and 25mm zone of inhibition respectively. Followed the aqueous extract, acetone bark extracts of *A. indica* exhibits 12mm zone of inhibition against *K. pnemoniae* and *A. nilotica* showed10mm zone of inhibition against*S. aureus* and *K. Pnemoniae* . The results were presented in table -1 (Figure1 and 2).

The methanolic bark extracts of *A. indica* showed moderate activity against *K. Pnemoniae* (10mm) and *S. aureus* (7mm) and *A. nilotica* exhibits positive activity against *K. pnemoniae* and *Proteus* spp with 9mm zone of inhibition and *S. aureus* with 7mm zone of inhibition respectively.

The chloroform extracts of *A. indica* exhibits activity only against *E. coli*, whereas, *A. nilotica* have activity against three microbes such as *S. aureus*, *K. pnemoniae* and *Proteus* spp. The ethanlolic bark extracts of *A. indica* showed activity against two pathogens (*S. aureus*, *K. pnemoniae*) and *A. nilotica* ethanlolic bark extracts showed maximum positive response to four pathogens (*S. aureus, K. pnemoniae, E. coli* and *Proteus* spp). Among the five test strains, *Salmonella typhi* showed more virulence, no extracts exhibit activity against *S. typhi.*

Discussion

In order to reveal the medicinal properties of stem bark of *A. indica* and *A. nilotica*, a comparative antibacterial activity study was carried out. From the present investigation, it was observed that, both the plants showed varied activities such as high, moderate to low anti-microbial activities. The variation in the antibacterial activity is due to the distribution of various phytochemical compounds present in plants. The plants which are rich in alkaloids, glycosides and tannins have been shown to possess antimicrobial activity against a number of microorganisms. Specifically, tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973) [24]. In our findings, *A. nilotica* bark showed significant antibacterial activity against the test strains used when compared to *A. indica*. The aqueous extracts of *A. nilotica* and *A. indica* showed the maximum zone (25mm and 18mm) of inhibition against *K. pnemoniae* on comparing the other extracts and strains used. Similar report was recorded in *Cassia alata* (Somchit *et al*., 2003) [26]. They inferred that, among the different extracts tested, the water extract of *C. alata* exhibited higher antibacterial activity with the zone of inhibition of 11 – 14mm. In the present investigation, the ethanolic bark extracts of *A. nilotica* showed maximum positive antibacterial activity, against four pathogens (*S. aureus*, *K. pnemoniae*, *E. coli* and *Proteus* spp), our results are in accordance with the report of Ali and Yagoub (2007) [27] in *A. nilotica*.

Conclusion

The stem bark of *A. indica* and *A. nilotica*, have a major role in inhibiting the test organisms such as the human pathogenic bacteria *Staphylococcus aureus*, *Klebsiella pnemoniae*, *Salmonella typhi*, *E. coli* and *Proteus* spp. Among the solvent extracts used for the study, ethanol extract showed effective antibacterial activity against maximum number of bacterial culture. Therefore, the present investigation forms the basis as preliminary study of antibacterial competency of stem bark extracts of *A. indica* and *A. nilotica* against human bacterial pathogens. The best extract was scrutinized and further analysis will be carried out to examine the role of phytocompounds against the test organisms as future work. Hence, it can be concluded that, the phytochemical compounds present in stem bark of *A. indica* and *A. nilotica*, may have a promising role in the antibacterial activity against the tested microbes and may serve as selective agents for the maintenance of human health against the bacterial pathogens.

Table 1: Comparative antibacterial activity of *A. indica* and *A. nilotica* bark extracts against human pathogenic bacteria.

Solvent extract	Diameter of inhibition zone (in mm)									
	Azadirachta indica					*Acacia nilotica*				
	S. aureus	*K. pnemoniae*	*S. typhi*	*E. coli*	*Proteus spp.*	*S. aureus*	*K. pnemoniae*	*S.typhi*	*E. coli*	*Proteus spp.*
Water	---	18	---	15	---	6	25	---	---	---
Methanol	7	10	---	---	---	7	9	---	---	9
Ethanol	6	7	---	---	---	12	10	---	9	13
Chloroform	---	---	---	9	---	7	10	---	---	8
Acetone	---	12	---	---	---	10	10	---	---	7
Streptomycin (PC)	11	12	23	17	11	12	27	7	17	17
DMSO (NC)	---	---	---	---	---	---	---	---	---	---

PC: Positive control, NC: Negative control, mm: millimeter, --- indicates no zone of inhibition.

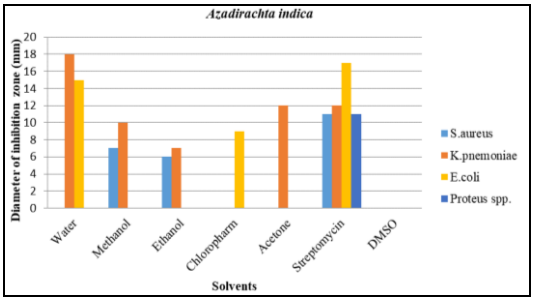

Fig 1: Antibacterial activity of *Azadirachta indica* bark extracts against the selected human pathogenic bacteria.

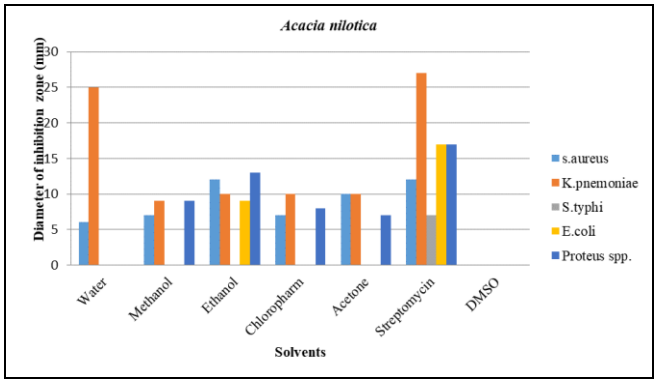

Fig 2: Antibacterial activity of *Acacia nilotica* bark extracts against the selected human pathogenic bacteria.

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