Received on 15 April, 2014; received in revised form, 02 July, 2014; accepted, 31 July, 2014; published 01 November, 2014

ANTIMICROBIAL POTENTIAL OF ACACIA NILOTICA EXTRACTS ON FEW DENTAL PATHOGENS

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Keywords:

Dental pathogen, *Acacia nilotica*,
Pod extract, Bioactivity

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ABSTRACT: The acute dental abscess is frequently underestimated in terms of its morbidity and mortality. The risk of potential serious consequences arising from the spread of a dental abscess is still relevant today with many hospital admissions for dental sepsis. *Acacia nilotica* commonly known in India as Babul has wide range of medicinal uses. In the present study antibacterial activity of different parts of *Acacia nilotica* was studied to evaluate their potential for being used as natural antibacterial agent against various dental infections. The bioactivity *Acacia nilotica* was analyzed crude extracts of bark, leaves and Pod of *Acacia nilotica* against five dental pathogens (*Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans*) using agar diffusion technique and determine the minimum inhibitory concentration of each extract against dental pathogen. Present study conducted to evaluate the highest anti dental infection property of different extract of *Acacia nilotica*. In this study the pod extract shows highest antibacterial potential followed by the bark extract and leaves extract.

INTRODUCTION: Microbial populations colonizing the teeth are a major source of pathogens responsible for oral and dental infections, including periodontal diseases, gingivitis, pericoronitis, endodontitis, peri-implantitis, and postextraction infections. Each entity has distinct clinical and microbial features. Bacterial species associated with oral infections include *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Campylobacter rectus*, *Eubacterium* species, *Fusobacterium nucleatum*, *Eikenella corrodens*, and *Peptostreptococcus micros*.

Treponema pallidum-related spirochetes have been associated with acute necrotizing ulcerative gingivitis. *Porphyromonas endodontalis* appears to be specifically related to endodontic infections¹⁻³. Other Oral bacteria include streptococci, lactobacilli, staphylococci, corynebacteria, and various anaerobes in particular bacteroides.

The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguinis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species⁴. Bacteroides and spirochetes colonize the mouth around puberty⁵. Periodontal disease develops usually because of two events in the oral

<p>QUICK RESPONSE CODE</p>	<p>DOI: 10.13040/IJPSR.0975-8232.5(11).4756-59</p> <hr/> <p>Article can be accessed online on: www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).1000-09</p>
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cavity: an increase in bacterial quantity of anaerobic Gram negative bacteria and a change in the balance of bacterial types from harmless to disease causing bacteria. Sease-causing bacteria. Among the bacteria most implicated in periodontal disease and bone loss are *Actinobacillus actinomycetemcomitans* and *P. gingivalis*. Other bacteria associated with periodontal disease are *B. forsythus*, *T. denticola*, *T. sokranskii* and *P.intermedia*^{6, 7}. The use of natural products, such as medicinal plants as therapy against infectious diseases, is an age-long practice, especially in developing countries⁸.

Acacia is an important plant genera that is commonly used in a variety of infections¹⁷. It is widely distributed in Asia, Australia and America and its efficacy has been demonstrated in the treatment of gonorrhoea, leucorrhoea, diarrhoea, dysentery and wounds^{9, 10}. Present study taken to evaluate the antimicrobial potential of pods extract of acacia nilotica and compared with the other parts extracts of acacia nilotica against the dental pathogen- *Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans*

MATERIAL AND METHODS:

Collection of Plant material

Bark, leaves and pod samples of *Acacia nilotica* Lam (Mimosaceae) was collected from the forest near Ambajogai, District Beed, Maharashtra and plant species authenticated in the Department of Botany. Yogeshwari College of Art, Science and Commerce Ambajogai.

Preparation of plant extract

For the extraction plant samples were dried at room temperature and powdered via. mortar and pestle. Further, the plant samples were successively extracted with 80% ethanol (100 ml/ gm dry weight) on a water bath for 24 hrs ethanol using soxhlet apparatus. The solvents were evaporated using a rotary vacuum-evaporator at 50°C. The extracts used for the detection of anti-bacterial activity.¹¹

Test Microorganisms:

Total five Clinical isolates of Dental pathogen used in this study were obtained from Department of Microbiology, Government Medical College Latur.

Loopful of test microorganism was transferred to 10-ml Nutrient Broth (NB) and incubated for 24 h at 37⁰ C for bacteria. Optical density of the 24-h culture was measured at 625 nm (OD₆₂₅) using a spectrophotometer (Perkin Elmer lambda 35 UV-Visible spectrophotometer). OD₆₂₅ was then adjusted to 0.1 by adding NB^{12, 17}. The prepared culture was appropriately diluted to achieve an inoculum size of approximately 10⁶ cfu/ml.

FIG 1: ACACIA NILOTICA

FIG 2: POD OF ACACIA NILOTICA

Antimicrobial assay by well diffusion method

The antimicrobial assay was performed using the agar diffusion method of Collins et al. (1995) with slight modifications. The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of sterilized forceps.

To each well was introduced various concentrations (5, 10, 15, 20, 25 and 30 mg/ml) of the extracts. Control experiments comprising inoculum without plant extract were set up. The plates were allowed to stand for one hour at room temperature ($25 \pm 2^\circ\text{C}$) for diffusion of the substances to proceed before the growth of organisms commenced. The plates were incubated at 37°C for 24 h. The zones of inhibition were then recorded.

Determination of Minimum Inhibitory Concentration (MIC)

Various concentrations of the plant extract ranging between 5 and 30 mg/ml were introduced into different test tubes, each tube was inoculated with an overweight culture of *Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans* diluted to give a final concentration of 10^6 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the MIC in each case as per the slandered method of Collins et al.¹³

RESULT AND DISCUSSION:

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay¹⁴. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have

Enormous therapeutic potential as they can serve the purpose with lesser side effects that is often associated with synthetic antimicrobials¹⁵⁻¹⁶. Continued further exploration of plant-derived Antimicrobials are needed today. A total of 3 extracts of *acacia nilotica* from three different parts of same plant (leaves, bark, and pod) were investigated. Extracts of the different parts of *acacia nilotica* used in this study were shown in **Table 1**. The Antibacterial susceptibility by means of disk diffusion method showed that the 3 plant extracts tested exhibited an antimicrobial effect against *Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans*.

Out of three different extract of *acacia nilotica*, Barks extracts of *acacia nilotica* possess a lower zone of inhibitory activity as compared to the leaf extracts while pod extract of *acacia nilotica* showed highest zone of inhibition against all test microorganism as compare to leaf extract and bark extract of *acacia nilotica*. All the three extracts of *acacia nilotica* shows highest antimicrobial potential against the *Streptococcus sanguinis* followed by *Streptococcus salivarius* and lowest antimicrobial potential against *Lactobacillus acidophilus*. In the present study highest antimicrobial potential of *acacia nilotica* observed in the pod extract followed by leaf extract and bark extract against all test microorganisms which is shown in **Table 1**. The MIC values showed that pod extract was more effective than leaf extract and bark extract against all test microorganism (**Table 2**).

TABLE 1. THE ANTIBACTERIAL SUSCEPTIBILITY BY MEANS OF WELL DIFFUSION METHOD

Sr. no.	Conc. of Extract (mg/ml)	Mean diameter of Zone of inhibition(mm)											
		<i>Lactobacillus acidophilus</i>			<i>Streptococcus sanguinis</i>			<i>Streptococcus salivarius.</i>			<i>Aggregatibacter actinomycetemcomitans</i>		
		BE	PE	LE	BE	PE	LE	BE	PE	LE	BE	PE	LE
1	5	00	00	00	00	00	00	00	00	00	00	00	00
2	10	00	00	00	00	00	00	00	00	00	00	00	00
3	15	00	6.5	00	7.5	9.5	8.5	7.5	9.0	8.5	00	00	00
4	20	7.0	8.5	8.0	9.5	12.5	10.5	7.5	10.	9.5	6.5	7.5	7.0
5	25	8.5	10	9.5	12	15.5	13.5	9.5	11.5	11	7.5	9	8.5
6	30	9.5	12.5	10.5	14	18.5	16.5	11.5	14.5	13	9	11	10.5

BE- Bark extract, PE- Pod extract, LE – Leaves extract.

TABLE 2 MINIMUM INHIBITORY CONCENTRATION OF VARIOUS EXTRACTS OF ACACIA NILOTICA.

S. No.	Test organism	MIC of BE	MIC of PE	MIC of LE
1	<i>Lactobacillus acidophilus</i>	40	35	40
2	<i>Streptococcus sanguinis</i>	35	25	30
3	<i>Streptococcus salivarius</i> .	35	30	35
4	<i>Aggregatibacter actinomycetemcomitans</i>	45	40	45

BE- Bark extract, PE- Pod extract, LE – Leaves extract.

CONCLUSION: A number of bacteria have now become antibiotic-resistant. This increases the importance of ayurvedic drugs. We report, here, the activity of different extracts (pod extract, bark extract, leaf extract) of acacia nilotica against dental pathogens *Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans*. This study help to various pharmaceuticals companies to developed the more potential natural herbal based drug and tooth pest against the dental pathogen for the treatment of various dental infections. This study help to replaces the chemically synthesized antibiotics with this natural antimicrobial agent and this agent serve as a selective agents for the maintainance of human health and biochemical tools against dental infection.

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How to cite this article:

Pote M and Hirapure P: Antimicrobial Potential of *Acacia Nilotica* Extracts on Few Dental Pathogens. Int J Pharm Sci Res 2014; 5(11): 4756-59.doi: 10.13040/IJPSR.0975-8232.5 (11).4756-59.

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