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Determination of antibacterial activity of *acacia nilotica* against *porphyromonas gingivalis* and *aggregatibacter actinomycetemcomitans* : an *in vitro* study

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ABSTRACT

Periodontal disease is the most common bacterial disease of the mankind affecting more than 10% of the world's population. There are various synthetic drugs used now a days to treat periodontal diseases which causes harmful side effects on prolonged use. Development of Multi Drug Resistance (MDR) by bacteria to antibiotics has also become an alarming threat. Hence it is inevitable to develop an innovative and alternative strategy that ensures safety, efficacy, cost effectiveness and easy availability. One such strategy is to explore the enormous wealth of medicinal plants. To determine the anti bacterial activity of *Acacia nilotica* against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and to impart the significance of herbs in routine life and to identify an alternate natural and safe remedy to periodontal disease. Extract of "*A.nilotica*" [powder form] was obtained and was certified to be free from bacteria, yeast/mould by manufacturer after microbial analysis. *A.actinomycetemcomitans* and *P.gingivalis*, had been sub cultured from maintained frozen stock. Antibacterial activity of these bacteria against extracts of *A.nilotica* was determined using disk diffusion method and Minimum Inhibitory Concentration was recorded. *A. nilotica* showed antibacterial activity for both aqueous and ethanol extracts of which ethanol extracts was more effective than that of aqueous extract. *A.nilotica* exerts antimicrobial activity against *A.actinomycetemcomitans* and *P.gingivalis*. These plant extracts have the potential to be used as antiplaque agents and can be used as an alternate phytotherapeutic for the commercial periodontal drugs available now which are synthetic and causing side effects.

INTRODUCTION

Periodontal disease is the most common bacterial disease of the mankind that affects 10-15% of the world's population. It leads to severe bone and tooth loss if left untreated. It is an inflammatory disease which results in gingival recession, periodontal tissue destruction and loss of alveolar bone. The initiation to control these organisms by mechanical means resulted in minimal long term effects due to their ability to invade gingival epithelial cell in vitro and buccal epithelial cell in vivo. So, adjunct antimicrobial therapy is useful in reduction of bacterias.[1,2]

There are more than 630 different bacterial species found in the oral cavity, out of which 10-15 bacterial species are recognized as potential periodontal pathogens. Among them "*Actinobacillus actinomycetemcomitans"* and "*Porphyromonas*

gingivalis" are major pathogens for initiation and progression in destruction of tooth and supporting structures.[1] Dental plaque is community of microorganisms found on tooth surface as biofilm, which is embedded in a matrix of polymers of host and bacterial origin.[3]*A.actinomycetemcomitans* is a non motile, coccobacillus, capnophilic, Gram-negative, facultative anaerobe. It produces a number of potentially damaging metabolites including leukotoxin, cytolethal distending toxin.[1]Though synthetic drugs such as penicillin, amoxycillin, doxycycline, tetracycline, clindamycin, metronidazole, ciprofloxacin, erythromycin, spiramycin and azithromycin are used nowadays to treat periodontal diseases, it leads to harmful side effects such as nephritis, eosinophilia, hemolytic anemia, allergy, gastrointestinal problems and disturbance in the nervous system on prolonged usage. In addition to that, development of Multi Drug Resistance (MDR) by these bacteria to these antibiotics has also become an alarming threat. Hence it is inevitable to develop an innovative and alternative strategy that ensures safety, efficacy, cost effectiveness and easy availability. One such strategy is to explore the enormous wealth of medical plants.[3]

The traditional medicine has been practiced since the inception of human history which involves the usage of the bioactive compounds occurs indigenously in different plants. *Acacia nilotica* is a shrub or tree belongs to the family Fabaceae. It is widely used in both human and veterinary medicine in resource-poor rural and urban households. The literature review suggested that, *A.nilotica*, exerts antimicrobial, antifungal, anti-plasmodial and antioxidant effects.[4,5]

According to WHO, medicinal plants are best source to obtain variety of drugs. Most of the developed countries used traditional medicines which have compounds derived from medicinal plants. The literature suggests *Acacia nilotica*, exerts an antimicrobial effect.[6]

In our current study, the antibacterial effect of ethanol and aqueous extract of *A.nilotica* was screened against the periodontal pathogens *such as P.gingivalis* and *A.actinomycetemcomitans.*

MATERIAL AND METHODS

Plant materials:

Natural bark of *Acacia nilotica* was collected, identified, and authenticated by a botanist and was stored in the herbarium. It was surface sterilized and shade dried. After complete drying, it was converted into core powder with mechanical or electric blender and stored in cold dry place until use.

Extraction of Plant:

Soxhlet extractor was used to carry out the extraction protocol. 50 gram of ground powder of plant material was placed in a permeable sack/ thimble - made of strong filter papers and stacked into the main chamber of the Soxhlet extractor. The extractor was accordingly positioned onto refining jars containing the appropriate solvents (Ethanol and water). The Soxhlet extractor was then outfitted with a condenser and the solvents were warmed to reflux. The warm solvents fumes went up through the refining arm and overflowed into the chamber lodging the thimble. It was routinely discharged by a siphon side arm down to the refining cup once the chamber was nearly full. The cycle was permitted to rehash ordinarily with the goal that the ideal compounds get concentrated in the refining cup. The solvent concentrates were separated, concentrated under diminished pressure (30±10mbar) in a rotating evaporator at 30-60^{o}C to a thick consistency and finally dried at room temperature, stored at 4°C and utilized for further antimicrobial studies.[7]

Collection of Bacterial Pathogens:

From the identified and maintained frozen stock cultures of *A. actinomycetemcomitans* and *P. gingivalis,* a small quantity of cells was recovered and sub cultured. Brain Heart Infusion (BHI) broth was used as culture medium to support the growth of these bacteria and utilized for antimicrobial assay and Minimum Inhibitory Concentration (MIC) studies.

Determination of Antimicrobial activity (Disc Diffusion Method):

To affirm antibacterial activity of aqueous and ethanol extract of *A.nilotica* against *A. actinomycetemcomitans* and *P. gingivalis,* antibacterial assay was performed using disc diffusion method. The ethanol and aqueous fractions were mixed with the required quantity of Dimethylsulfoxide (DMSO) to obtain the working concentration of the extract.[3] Stock solution of aqueous and ethanol extracts were prepared by dissolving 100mg of fractions with 10ml of Dimethylsulfoxide (DMSO) in order to produce a 10µg/µl final concentration. 20µl, 40µl, 60µl and 80µl of both aqueous and ethanol extracts were impregnated separately into plain, sterile 6mm discs and completely dried before the application on bacterial yard. Loopful of identified cultures of *A. actinomycetemcomitans* and *P. gingivalis* were sub cultured in Brain Heart Infusion (BHI) broth and incubated at 37°C for 6-12 hrs in anaerobic condition. Bacterial cultures were adjusted to 0.5 McFarland standards using sterile BHI broth which is equal to 1.5×10^{8} colony forming unit/ml. A loopful of each culture was lawned in sterile Muller Hinton Agar plates, after few minutes the discs impregnated with the extracts were placed in appropriate places along with the positive and negative control discs. DMSO was used as negative control and penicillin 30 µg /disc was used as positive control for all bacterial strains. Plates were incubated at 37°C for 24 48 hrs in anaerobic condition. By measuring the zone of inhibition diameter around the discs, the antibacterial activity was evaluated. Antibacterial action was communicated as the mean zone of inhibition diameters (mm) delivered by the plant extract.[8]

Minimum inhibitory concentrations (MIC):

In this study, serial tube dilution technique was followed based on the guidelines of the Clinical and Laboratory Standards Institute to determine MIC values of the extracts. In the initial tube, 100 µl of stock solution was added into 300µl of BHI broth to make a volume of 400 µl, from which nine serial dilutions were prepared in separate test tubes containing 200 µl of BHI broth. To each serially diluted tube, 200 µl of the previously prepared bacterial suspension was added and incubated for 24 h in an anaerobic jar at 37°C and observed for turbidity which indicates the growth of the organisms. The turbidity in each tube was compared with a positive control, which contained the pure culture of the bacterial pathogens alone. The test tube containing least concentration of the plant extract, which does not show any turbidity, was considered as MIC of the plant concentrate for that particular test organism.[1]

RESULTS

The present investigation was carried out on antimicrobial activity of ethanol and aqueous extracts of *A.nilotica* against the oral pathogens such as *A.actinomycetemcomitans* and *P. gingivalis*.

Table 1 : Antibacterial activity of aqueous extract of *A.nilotica* against *P. gingivalis* and *A.actinomycetemcomitans.*

S.No	Concentration of the Extract (µg/disc)	Zone of inhibition in mm	
		P.gingivalis	*A.actinomycetemcomitans*
1	Positive Control	18±02	19±02
2	Negative Control	-	-
3	200	9.0±02	8.0±02
4	400	10.5±02	11.5±02
5	600	11.9±02	12.2±02
6	800	12.3±02	13.9±02

Positive Control Penicillin 30 µg/ disc; Negative Control - DMSO

Table 2 : Antibacterial activity of Ethanol extract of *A.nilotica* against *P. gingivalis* and *A.actinomycetemcomitans.*

S.No	Concentration of the Extract (µg/disc)	Zone of inhibition in mm	
		P.gingivalis	*A.actinomycetemcomitans*
1	Positive Control	21±02	19±02
2	Negative Control	-	-
3	200	10.1±02	9.9±02
4	400	11.2±02	11.0±02
5	600	12.1±02	12.2±02
6	800	13.0±02	14.5±02

Positive Control Penicillin 30 µg/ disc; Negative Control - DMSO

Antimicrobial Activity of *A.nilotica* extracts:

In this study, antibacterial activity of ethanolic and aqueous extracts of *A.nilotica* against the periodontal pathogens such as *A.actinomycetemcomitans* and *P.gingivalis* was performed by disc diffusion method. The zone of inhibition was measured by antibiotic zone reader (Table 1 and 2). Both of the two different extracts of *A.nilotica* with different concentrations (200 µg, 400 µg, 600 µg and 800 µg/disc) showed antimicrobial activity against the pathogens. In aqueous extract, *P.gingivalis* exerted 9.0 mm Zone of Inhibition (ZOI) at 200 µg/disc concentration and it has been gradually increased to 12.3 mm at the concentration of 800 µg/disc concentration. Similarly, in *A.actinomycetemcomitans* also the ZOI was 8.0 mm at 200 µg/disc and it was 13.9 mm at 800 µg/disc. *A.actinomycetemcomitans* was more sensitive than that of *P.gingivalis (*Table: 1, Fig: 1a & 1b).

In alcohol extract, *P.gingivalis* exerted 10.1 mm Zone of Inhibition (ZOI) at 200 µg/ disc concentration and it has been gradually increased to 13.0 mm at the concentration of 800 µg/ disc concentration. Similarly, in *A.actinomycetemcomitans* also the ZOI was 9.9 mm at 200 µg/ disc and it was 14.5 mm at 800 µg/ disc. *A.actinomycetemcomitans* was more sensitive than that of *P.gingivalis* (Table: 2, Fig: 1c & 1d). As the concentration increases, the sensitivity of the pathogens to the extracts was also increasing gradually in both extracts. So the concentration and the susceptibility of pathogens are directly proportional to each other.

Minimum Inhibitory Concentration (MIC):

The aqueous and ethanol extracts of *A.nilotica* were tested at various concentrations and evaluated for the value of MIC. In aqueous extract, *P.gingivalis* showed 1.47 optical density value (OD) at 40 and 45 µg/ml concentration of extract and from 50-65 µg/ml concentration of extract the OD value was 1.16. Likewise, as the concentration of extract increases the OD value by *P.gingivalis* was gradually decreased and become 0.00 at 90 µg/ml concentration of extract. Similarly, for

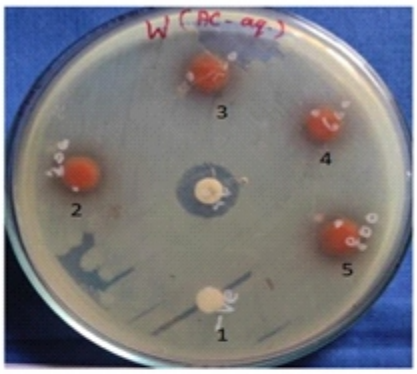

Figure 1a

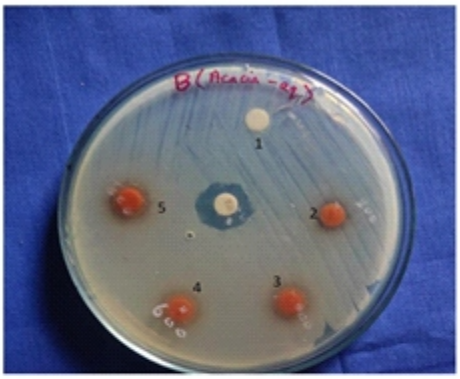

Figure 1b

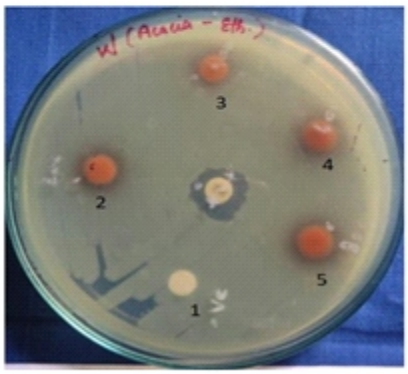

Figure 1c

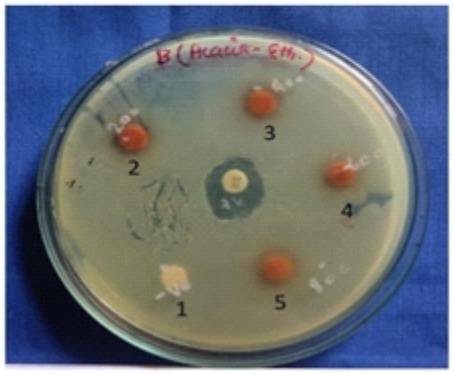

Figure 1d

Figure 1a : Antibacterial activity of aqueous extract of *A.nilotica* against *P. gingivalis* (1 - negative control, 2 - 200 µg/ml, 3-400 µg/ml, 4-600 µg/ml, 5-800 µg/ml) in all the discs.
Figure 1b : Antibacterial activity of aqueous extract of *A.nilotica* against *A.actinomycetemcomitans.* (1 - negative control, 2 - 200 µg/ml, 3-400 µg/ml, 4-600 µg/ml, 5-800 µg/ml) in all the discs.
Figure 1c : Antibacterial activity of ethanol extract of *A.nilotica* against *P. gingivalis* (1 - negative control, 2 - 200 µg/ml, 3-400 µg/ml, 4-600 µg/ml, 5-800 µg/ml) in all the discs.
Figure 1d : Antibacterial activity of ethanol extract of *A.nilotica* against *A.actinomycetemcomitans* (1 - negative control, 2 - 200 µg/ml, 3-400 µg/ml, 4-600 µg/ml, 5-800 µg/ml) in all the discs.

A.actinomycetemcomitans the OD value was 0.42 at 40 µg/ml concentration of extract and drastically reduced as the concentration increased. For both *P.gingivalis* and *A.actinomycetemcomitans* aqueous extract of *A.nilotica* showed complete arrest of bacterial growth at 90 µg/ml.In ethanol extract, *P.gingivalis* showed 0.52 optical density value (OD) at 40 µg/ml concentration of extract. Thereafter, as the concentration of extract increases the OD value by *P.gingivalis* was gradually decreased and become 0.00 at 85 µg/ml concentration of extract. Similarly, for *A.actinomycetemcomitans* the OD value was 0.68 at 40 µg/ml concentration of extract and eventually reduced as the concentration increased. For both *P.gingivalis* and *A.actinomycetemcomitans* ethanol extract of *A.nilotica* showed complete arrest of bacterial growth at 85 µg/ml. (Table: 3, Fig 3&4).

DISCUSSION:

In the present investigation, both aqueous and ethanol extracts of *A.nilotica* exhibited antibacterial activity against *P.gingivalis* and *A.actinomycetemcomitans.* It has been observed that, ethanol extract showed maximum antibacterial activity of 13.0 mm zone of inhibition at 800 µg/disc concentration than that of aqueous extract which showed 12.3 mm at 800µg/disc concentration on *P.gingivalis*. Similarly, ethanol extract showed maximum antibacterial activity of 14.5 mm zone of inhibition at 800µg/disc than that of the aqueous extract which showed only 13.9 mm zone of inhibition at 800µg/disc concentration on *A.actinomycetemcomitans*. It was observed that *A.actinomycetemcomitans* was highly sensitive to both aqueous and ethanolic extract compared to that of *P.gingivalis*. Our results are supported by the findings of Chandra Shekar *et al.,* in 2020 who had also reported that, extract of *A.nilotica* effectively controlled *S. mutans, S. sanguis ,S. salivarius*. [3] In the same way our study showed antibacterial activity of *A.nilotica* against two other different species [*P.gingivalis* and *A.actinomycetemcomitan*] oral pathogen causing plaque. In our studies, ethanol extracts were more

Table 3 : MIC of Aqueous & Ethanol extracts of *A.nilotica* against *A.actinomycetemcomitans* and *P. gingivalis*

S.No	Concentration of Extracts (µg/ml)	OD Value Aqueous extract		OD Value Ethanol extract	
		P.gingivalis	*A.actinomycetemcomitans*	*P.gingivalis*	*A.actinomycetemcomitans*
1	Blank	0.00	0.00	0.00	0.00
2	control	1.53	1.54	1.53	1.54
3	100	0.00	0.0	0.00	0.00
4	95	0.00	0.00	0.00	0.00
5	90	0.00	0.00	0.00	0.00
6	85	0.02	0.02	0.00	0.00
7	80	0.05	0.08	0.05	0.06
8	75	0.10	0.11	0.07	0.11
9	70	0.52	0.18	0.09	0.13
10	65	1.16	0.23	0.12	0.26
11	60	1.16	0.28	0.19	0.31
12	55	1.16	0.34	0.24	0.40
13	50	1.16	0.38	0.29	0.41
14	45	1.47	0.38	0.32	0.46
15	40	1.47	0.42	0.52	0.68

effective than that of aqueous extract. This may be due to stronger extraction capacity of active components in ethanol which might be responsible for antibacterial activity. Deshpande and Kadam *et al.,* in 2013 found that, the ethanolic extracts of *A. nilotica* was effective in inhibiting the growth of *S. mutans*. In the same way our current study also showed ethanolic extract of *A.nilotica* was effective in inhibiting the growth of *P.gingivalis* and *A.actinomycetemcomitans*.[5]

Our results are corroborating with the findings of Rwarinda U Angelo in 2015 who found that the ethanolic extract of *Acacia nilotica* significantly suppressed the growth of *Bacillus subtilis* and *Escherichia coli* (26mm).When compared to the ethanolic, methanolic and acetonic extracts, ethanolic extract showed the highest zone of inhibition among the organisms. Our results are supported by the findings of Rwarinda U Angelo in 2015, who also reported that ethanolic extract of *Acacia nilotica* showed 13.0 mm zone of inhibition against *Aspergillus niger,* were as in our study same way ethanolic extract of *A.nilotica* showed maximum zone of inhibition for both A.*actinomycetemcomitans* and P. gingivalis at 800 µg/disc.[4]

During extraction with the solvents, active phytocomponents otherwise could be called as phytotherapeutics are effectively extracted in the solvents such as ethanol, chloroform, water etc.,. Tenguria *et al.,* and Deshpande and Kadam, reported that the phytochemical analysis of *A.nilotica* plant parts revealed the presence of anthraquinones, terpenoids, saponins, flavonoids, tannins, and cardiac glycosides.[5, 9]

In our study the antimicrobial activity of *A.nilotica* against *P.gingivalis* and *A. actinomycetemcomitance* might be because of phytoconstituents present in the bark of *A.nilotica* as *Dabur et al.,* said in his study.[10]

In our study we found that the zone of inhibition for aqueous extract of *A.nilotica against P. gingivalis* and *A.actinomycetemcomitans* as 12.3±02 and 13.9±02 respectively, and zone of inhibition for ethanoilc extract of *A.nilotica against P. gingivalis* and *A.actinomycetemcomitans* as 13±02 and 14.5±02 respectively, which is found to have a good antibacterial activity. In our study we have also observed MIC for both *P.gingivalis* and A.actinomycetemcomitans for aqueous and ethanolic extract of *A.nilotica, which* was found to be 90µg/ml and 85 µg/ml respectively. When compared to aqueous extract ethanol was effective. Similarly, Rwarinda U Angelo in 2015 reported that the MIC of ethanol extract was low (2.5mg/ml) as compared to other extracts (10 mg/ml). The lower MIC is an indication of high effectiveness of extract.[4]

CONCLUSIONS:

Acacia nilotica exerts antimicrobial activity against Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. These plant extracts have the potential to be used as

antiplaque agents and can be used as an alternate phytotherapeutic for the commercial periodontal drugs available now which are synthetic and causing side effects. The results of present study support the valuable use of *Acacia nilotica* in traditional medicines for the treatment of infections caused by above tested bacteria and pave the way to explore the enormous wealth of medicinal plants.

CONFLICT OF INTEREST:

Nil.

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