

Original article

***Acacia catechu* and *Acacia farnesiana* as antimicrobial agents against periodontal pathogens: An *in vitro* assessment**

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ABSTRACT

OBJECTIVES: To investigate the *in vitro* antimicrobial activity of different concentrations of ethanolic extracts of *Acacia catechu* and *Acacia farnesiana* (L.) against *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg). **MATERIALS & METHODS:** Antimicrobial activity of ethanolic extracts of *Acacia catechu* and *Acacia farnesiana* (L.) in various concentrations were analyzed for *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) by standard agar disc diffusion method. The Minimum Inhibitory Concentration (MIC) of these extracts was measured by Broth dilution method. Chlorhexidine was used as positive control. **RESULTS:** *Acacia catechu* showed profound antibacterial activity against *Aggregatibacter actinomycetemcomitans* (Aa) with the Zone of Inhibition of 25mm and against *Porphyromonas gingivalis* (Pg) with the zone of Inhibition of 18mm. *Acacia catechu* was inhibitory to Aa at all the serial dilutions ranging from 0.2µg/ml to 100µg/ml and to Pg at serial dilutions ranging from 12.5µg/ml to 100µg/ml. *Acacia farnesiana* showed intense antibacterial activity against *Aggregatibacter actinomycetemcomitans* (Aa) with the Zone of Inhibition of 18mm and against *Porphyromonas gingivalis* (Pg) with the zone of Inhibition of 20mm. *Acacia farnesiana* was inhibitory to Aa at all the serial dilutions ranging from 0.2µg/ml to 100µg/ml and to Pg at serial dilutions ranging from 12.5µg/ml to 100µg/ml. **CONCLUSION:** *Acacia catechu* & *Acacia farnesiana* may be used as an adjunct in treatment of periodontal diseases.

Introduction

The paradigm of treatment modalities are subjected to incessant changes with the advancement in the understanding of the etiology and pathogenesis of periodontal disease. Periodontal diseases are now known to be polymicrobial in nature. Although scaling and root planing is considered to be the gold standard, adjuvant antimicrobial therapy has sought our interest in improving the treatment outcomes of periodontal disease. Systemic antimicrobials such as amoxicillin and metronidazole combinations have provided superior treatment outcomes when used as adjunct to

mechanical therapy. Apart from systemic antimicrobials, the literature concerning the utility of chlorhexidine in various formulations for chemical plaque control is enormous providing an undisputable proof of its efficacy. Apart from being used in dental fraternity for therapeutic purposes, chlorhexidine has also been recognized as the positive control to measure the efficacy of newer anti-plaque agents.¹ These synthetic antimicrobials irrespective of their delivery system are associated with clinical concerns such as drug resistance, hypersensitivity reactions and imbalance of normal indigenous microflora.

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Table 1: Minimum Inhibitory Concentration of A. Catechu

Herb/ dilution	100µg/ml	50 µg/ml	25 µg/ml	12.5µg/ml	6.25µg/ml	3.75µg/ml	1.5 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Aa	S	S	S	S	S	S	S	S	S	S
Pg	S	S	S	S	R	R	R	R	R	R

S- Susceptible , R- Resistant

Chlorhexidine is associated with objectionable taste, tooth discoloration, desquamation, and soreness of oral mucosa. Herbal therapy/phytotherapy due to their natural and nonchemical property can be an alternative to overcome these clinically relevant drawbacks.

For centuries, various parts of the plants and plant products have been used for treating various systemic and oral ailments. The major vigor of these natural herbs is that they are time proven to be safe without any adverse reactions. Especially when it comes to their utility as mouthwashes, herbal mouth rinses do not contain alcohol or sugars which are two of the most common ingredients found in most other over-the-counter products. These are the two common substrates upon which microorganisms feed and release byproducts that cause halitosis. Hence, we can take a step towards better oral hygiene and better health by avoiding these harmful ingredients.

Acacia catechu Willd (Family: *Fabaceae* and subfamily: *Mimosoideae*.) is popular for its excellent astringent and antioxidant activities. The extracts of *Acacia catechu* confer several therapeutic benefits like antipyretic, antiinflammatory, antidiarrhoeal, hypoglycaemic, and hepatoprotective activities. From dental perspective, when used externally as a powder, it cures bleeding gums. It is used with great benefit internally in form of gargle to alleviate sore throat, halitosis and dental caries. Literature shows that it is antimicrobial against several oral pathogens namely *streptococcus species* and lactobacillus ² responsible for dental caries, *candida* ³ species causing candidiasis and *enterococcus faecalis* ⁴ commonly found in failed endodontic cases.

In India, *Acacia farnesiana* L is known as sweet acacia. The bark of this plant is used as astringent and demulcent. Woody branches are used in India as tooth brushes. The gummy roots are used for the antispasmodic, astringent, demulcent, diarrhea, febrifuge, rheumatism and stimulant. It is also chewed for sore throat. All the parts extract of this herb are inhibitory to *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Pseudomonas aeruginosa*, and *Escherichia coli*. ⁵

Owing to such proven beneficial properties, the present study was taken up to evaluate the antimicrobial activity of *Acacia catechu* & *Acacia farnesiana* against periopathogens mainly

Table 2: Minimum Inhibitory Concentration of *A. farnesiana*

Herb/ dilution	100µg/ ml	50 µg/ ml	25 µg/ ml	12.5µg/ ml	6.25µg/ ml	3.75µg/ ml	1.5 µg/ ml	0.8 µg/ ml	0.4 µg/ ml	0.2 µg/ ml
Aa	S	S	S	S	S	S	S	S	S	S
Pg	S	S	S	S	R	R	R	R	R	R

S- Susceptible , R- Resistant

Aggregatibacter actinomycetemcomitans (Aa) and *Porphyromonas gingivalis* (Pg).

To the best of our Knowledge, these herbs are not evaluated against periopathogens namely Aa & Pg previously.

MATERIAL AND METHODS:

Plant extracts mainly bark of *A. catechu* & fresh leaves of *A. farnesiana*, free from disease were collected from B.V.V.S Ayurveda College & Hospital, Bagalkot, Karnataka. They were thoroughly rinsed in distilled water and shade dried over a period of 3–4 weeks at room temperature, hand crushed separately to obtain a coarse powder. Later, the fine powder was prepared, stored in airtight containers and preserved in a refrigerator at 4°C until further use. Ethanolic extracts were prepared by Soxhlet apparatus using ethanol as a solvent was utilized for extraction procedure at H. K. E College of Pharmacy, Bagalkot.

This *in-vitro* analysis was carried out against bacterial strains namely *Aggregatibacter actinomycetemcomitans* (ATCC29523) & *Porphyromonas gingivalis* (ATCC33277). Antimicrobial activity of the prepared herbal extracts

were determined against using agar well - diffusion method on brain heart infusion agar at various concentrations i.e. 5µg/ml, 10µg/ml, 25µg/ml, 50µg/ml & 75µg/ml. The extracts were placed on Blood agar plates previously inoculated with the test strains and incubated at 37°C for 48-72 hours. The disc impregnated with chlorhexidine was used as a positive control. The agar plates were observed for zones of inhibition around the disc and the diameter of zone of inhibition was measured using a transparent scale.

Minimum Inhibitory Concentration was determined by broth dilution method wherein serial dilutions ranging from 0.2µg/ml to 100µg/ml were prepared with absolute ethanol. Each extract was added to the sterile tube, to which standard number of strains were added & incubated at 37°C for 48 hrs. Growth of organisms on each concentration was checked to determine the minimum concentration that inhibits growth of test strain organism.

gregatibacter actinomycetemcomitans (*Aa*) and Porphyromonas gingivalis (*Pg*). The ethanolic bark extract of *Acacia catechu* and ethanolic leaf extract *A. farnesiana* exhibited a high degree of antimicrobial activity against the organisms tested. Chlorhexidine was used as positive control.

Acacia catechu showed profound antibacterial activity against *Aggregatibacter actinomycetemcomitans* (*Aa*) with the Zone of Inhibition of 25mm and against *Porphyromonas gingivalis* (*Pg*) with the zone of Inhibition of 18mm. *Acacia catechu* was inhibitory to *Aa* at all the serial dilutions ranging from 0.2µg/ml to 100µg/ml and to *Pg* at serial dilutions ranging from 12.5µg/ml to 100µg/ml (Table 1).

Acacia farnesiana showed intense antibacterial activity against *Aggregatibacter actinomycetemcomitans* (*Aa*) with the Zone of Inhibition of 18mm and against *Porphyromonas gingivalis* (*Pg*) with the zone of Inhibition of 20mm. *Acacia farnesiana* was inhibitory to *Aa* at all the serial dilutions ranging from 0.2µg/ml to 100µg/ml and to *Pg* at serial dilutions ranging from 12.5µg/ml to 100µg/ml (Table 2).

The extracts at different concentrations exhibited antibacterial activity against the bacterial strains tested. *A. catechu* was more effective against *Aa* (zone of inhibition 25 mm at 75 µg/ml) whereas *A. farnesiana* was more effective against *Pg* (zone of inhibition 20 mm at 75 µg/ml).

DISCUSSION:

The complex etiopathogenesis of periodontal diseases has a polymicrobial etiology which necessitates the use of antimicrobials as adjunct to scaling and root planing. Considering the current scenario of

RESULTS:

Both the plants extracts inhibited the growth of Ag developing drug resistance and adverse reactions to systemic therapies, there is a need for effective alternatives. Development of a treatment strategy in a cost effective manner has now become a topic of major concern. Administration of antimicrobials in different formulations as an adjunct to augment the efficiency of routine mechanical plaque control procedures is considerable. *Acacia* herbs namely *Acacia catechu* and *Acacia farnesiana* (*L.*) possess antimicrobial and antioxidant properties which may provide a better alternative in the perspective of periodontal treatment outcomes.

The present study aimed at evaluating the antimicrobial activity of *Acacia* herbs against periopathogens thereby verifying its scope in treatment of periodontal diseases. *Acacia* herbal extracts were analyzed in different concentrations for their antimicrobial activity against *Aa* and *Pg* by standard agar disk diffusion method which demonstrated that both the periopathogens are susceptible to these herbal extracts. Besides, minimum inhibitory concentration of each herbal extract was determined by broth dilution method which showed that *Acacia* herbs are inhibitory to the tested strains of periodontal pathogen at various concentrations.

Although chlorhexidine is the gold standard ¹, many products have been tried to substitute or replace CHX in order to get rid of the set of adverse effects suffered by the patients using CHX in their daily routine. Also recently, a case has been reported regarding urticaria due to chlorhexidine use. ⁶ However, none of the tested products have been proven superior to chlorhexidine. Therefore, it is considered to be a positive control in any study, *in vitro* or *in vivo*. So, we

also took CHX as a positive control wherein *A. catechu* showed greater zone of inhibition against Aa than CHX.

Preliminary phytochemical analysis of *Acacia catechu* showed the presence of tannins, phenol, saponins, carbohydrates, flavonoids, alkaloids and glycosides.² The extract lacks the presence of phlobatanins, proteins and amino acids. Similarly phytochemical analysis of *Acacia farnesiana* showed the presence of hydrolysable tannins, terpenes, flavinoids such as quercetin, salicylic acid, carbohydrates and/or glycosides, alkaloids and nitrogenous bases, unsaturated sterols and coumarins.^{5,7,8} These are few of the identified components which confers them with beneficial properties.

Several studies have shown effective antimicrobial activity of *A. Catechu* against various gram positive & gram negative organisms. *A. catechu* extract was found to be equally effective against gram positive and gram negative bacteria.³ Acetone bark extract of *Acacia catechu* was found to be bactericidal against *E. Faecalis*.⁴ Similarly, ethanolic bark extract of *Acacia catechu* is antibacterial against *streptococcus sanguis* and *Lactobacillus acidophilus*.² *A. catechu* extract has also shown significant antimicrobial activity against the poultry pathogens.⁹ Our present study consolidates the findings of the available literature on antimicrobial properties of this herbal extract.

A study on *Acacia Farnesiana* concluded that *Acacia farnesiana* flowers don't show significant antimicrobial potency against microorganisms.¹⁰ Contradictory to this study, we tested *Acacia farnesiana* leaves which contain certain above mentioned compounds conferring them with the antimicrobial properties. As of now, current literature lacks the data regarding the potency of this particular

herb which invites further research in this zone. A noteworthy feature of these herbs is that it exhibited certain degree of inhibition against the tested gram-negative organisms in spite of certain permeability differences. Generally, the cell wall of gram negative organisms will be more complex than that of gram-positive thereby acting as a diffusion barrier for other synthetic antimicrobials. This usually makes them less susceptible to the antibacterial agents than the gram positive bacteria.

To summarize, *Acacia* herbs evaluated in the present study exhibited considerable degree of antimicrobial activity against major periodontal pathogens. The limitation of the present study would be that these herbal extracts are tested against only a particular strain of periodontal pathogens. Considering the diversity of the bacterial flora and numerous strains causative of periodontal disease, the present data might be insufficient. The findings of this study shall open a new arena for future studies being conducted on various periodontal pathogens and its strains. Also, it must be able to widen the zone of applications of these herbs in periodontal therapeutics in multiple formulations as deemed rational.

CONCLUSION:

The present study findings conclude that the herbal extracts, *A. catechu* and *A. farnesiana* exerts antimicrobial actions against major periodontal pathogens. Further investigations, *in-vitro* and *in-vivo* as well are warranted to confirm the findings of the present study and explore the therapeutic implications of these herbal extracts in management of periodontal patients.

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