Research Article

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ANTIBACTERIAL ACTIVITY OF *ABUTILON INDICUM* LEAF EXTRACT AGAINST PERIODONTAL PATHOGENS: AN *IN VITRO* STUDY

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ABSTRACT

Purpose: Periodontitis, the leading cause of tooth loss, is ascribed to periodontopathic bacteria in periodontal tissues. Amongst them, *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Tannerella forsythia* are contemplated to be prime consortium in the succession of periodontitis. As there has been a gradual escalation in antibiotic resistance over the last decade, phyto drugs have presumed an eminent part as a feasible substitute therapy in dentistry. Hence experimentation of new antimicrobial compounds procured from the herbal product is the need of the hour. Functional components of *Abutilon indicum*, i.e., flavonoids, alkaloids, sterols, triterpenoids and glycosides, bring about an anti-inflammatory and antimicrobial effect. So, the present study is conducted to estimate the antimicrobial activity of *Abutilon indicum* extract against *Aggregatibacter actimomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Tannerella forsythia.* Methodology: Bacterial strains, particularly *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Tannerella forsythia,* were cultured on Thioglycollate broth. Minimum inhibitory concentration was detected by serial broth dilution of *Abutilon indicum* extract to 100, 50, 25, 12.5, 6.5, 3.12, 1.6, 0.8, 0.4, and 0.2 mg/ml, respectively. The tubes were subsequently incubated for 48 hours at 37 °C. Results: *Abutilon indicum* impeded the growth of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Tannerella forsythia* in a dose-dependent manner. Conclusion: *Abutilon indicum* has the property of antibacterial activity against periodontopathic bacteria and may be an effective and efficient agent for averting periodontitis.

Keywords: *Abutilon indicum*, Indian mallow, Periodontitis, Antimicrobial activity.

INTRODUCTION

With the discovery of antibiotics, the healthcare community thought the struggle with contagious illnesses was victorious. However, it was soon acknowledged that the microorganisms were proficient in developing resistance against any category of antimicrobial drugs in us.[1] Herbal plants have been acclimated since time immemorial to manage systemic diseases. They are primarily preferred because of their insignificant side effects.[2]. In the last few decades, researchers have turned their contemplation to investigate the potentiality of routine medicinal plants for their alleviative properties.[3]

Abutilon indicum Linn sweet belongs to the family *Malvaceae*, generally called "Country Mallow" is a perennial plant up to 3m in height. It is profusely found as a weed in the sub-Himalayan tract, hotter parts of India, adjacent countries, Malaya, the Philippine islands and China. The plant is used in traditional medicine in India, Pakistan, China and the Philippines to treat various diseases like bronchitis, toothache, body ache, diabetes, jaundice, fever, piles, ulcers, leprosy, gonorrhoea, cystitis and diarrhoea.

Abutilon indicum Linn is reported to have hepatoprotective, hypoglycaemic, antimicrobial, male contraceptive and antidiarrheal activities. Plenty of phytoconstituents have been isolated from various parts of Abutilon indicum, such as carbohydrates, flavonoids, essential oil, sesquiterpenes, amino acids, fatty acids and sterols. Since it has received pharmacological needs.[4]

Periodontal disease is a polymicrobial infection developing from the concomitant interaction of distinct bacterial species. Scaling and root planning is the first advocated step in managing plaque-generated periodontal diseases and are the vital phase of periodontal therapy, but there are elements such as accessibility or presence of plaque in retentive areas that can restrict instrumentation.[5]

Local drug delivery has been recommended to supplement the non-surgical periodontal therapy. Several antimicrobials, such as metronidazole, chlorhexidine, tetracycline, minocycline and doxycycline, are employed to annihilate periodontopathogens. On the contradictory, the considerable drawback is the emanation of antimicrobial-resistant pathogens resulting from the vast utilization of antibiotics in medical science.[6]

Considering the anti-inflammatory and antibacterial effects, cost-effectiveness, availability of *Abutilon indicum* and relatively increased frequency of periodontal diseases, this study was formulated to estimate the antimicrobial effect of *Abutilon indicum* extract against periodontal pathogens.

In the current study, we assessed the antibacterial activity of *Abutilon indicum* extract against periodontopathic bacteria such as *Aggregatibacter actimomycetemcomitans* (ATCC29523), *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), *Fusobacterium nucleatum* and *Tannerella forsythia* (ATCC 43037).

MATERIALS AND METHODS

The current study was framed to determine Minimum Inhibitory Concentration (MIC), which was carried out as per the guidelines of the Clinical and Laboratory Standards Institute. The bacterial cultures of periodontopathogens utilized in this study were procured from Maratha Mandal's Nathajirao G. Halgekar's Institute of Dental Science and Research Centre, Belagavi and were germinated at 37 °C to a stationary phase for 24 hours.

The standardized ethanolic extract of *Abutilon indicum* leaf (known as Indian mallow) is readily available in the market. The stock solution of *Abutilon indicum* leaf extract (test agent) was concocted in dimethyl sulfoxide to ensure absolute solubilization. For attaining MIC, nine dilutions of each drug [*Abutilon indicum* extract and 0.2% chlorhexidine] were done with Thioglycollate broth.

In the first tube, 20 microlitres (µl) of the drug was added into 380 µl of Thioglycollate broth. In order to procure the dilutions, 200 µl of Thioglycollate broth was added into the succeeding 9 tubes separately. From the initial tube containing the drug, 200 µl was transmitted to the first of 9 tubes accommodating 200 µl of Thioglycollate broth. The resultant dilution was considered as a 10^{-1} dilution. 200 µl from the previously mentioned tube was transferred to the second tube to make a 10^{-2} dilution. This method was duplicated until 10^{-9} dilution was procured for each drug. A culture suspension was formulated by adding 5 µl from the maintained stock cultures of desired organisms (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Tannerella forsythia*) into 2 ml of Thioglycollate broth. 200 µl of formulated culture suspension was added into each serially diluted tube. The tubes were incubated for 48-72 hours in an anaerobic jar at 37 °C and discerned for turbidity. The merest concentration of the extract showing no turbidity was noted as MIC.

RESULTS

The Minimum Inhibitory Concentration of *Abutilon indicum* leaf extract against the tested micro-organisms is shown in Table 1.

The tests were triplicated to attain and ensure the most incredible accuracy. *Abutilon indicum* leaf extract suppressed the growth of *Tannerella forsythia* at a low concentration of 25 µl/ml, whereas *Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans* were suppressed at a concentration of 50 µl/ml. *Fusobacterium nucleatum* was suppressed at a higher concentration of 100 µl/ml. The present test agent was sensitive for all 5 strains at 100 µl/ml.

***Abutilon indicum* leaf extract**	**100 µl/ml**	**50 µl/ml**	**25 µl/ml**	**12.5 µl/ml**	**6.25 µl/ml**	**3.12 µl/ml**	**1.6 µl/ml**	**0.8 µl/ml**	**0.4 µl/ml**	**0.2 µl/ml**
Porphyromonas gingivalis	S	S	R	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	S
Prevotella intermedia	S	S	R	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	S
Tannerella forsythia	S	S	S	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	S
Fusobacterium nucleatum	S	R	R	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	S
Aggregatibacter actinomycetemcomitans	S	S	R	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	S

S: Sensitive, R: Resistant.

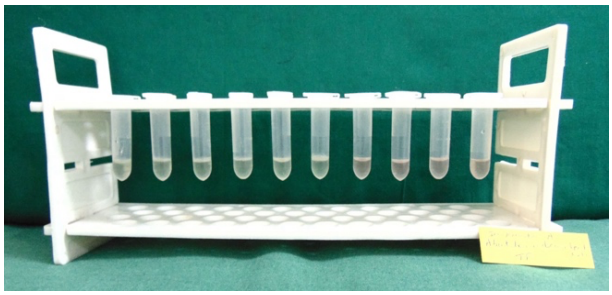

Figure 1: Serial dilution [*Tannerella forsythia*]

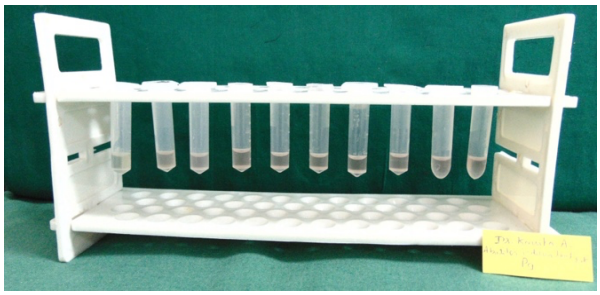

Figure 2: Serial dilution [*Porphyromonas gingivalis*]

DISCUSSION

Periodontal diseases are chronic inflammatory diseases of the investing and supporting tissues of the teeth. Plaque biofilm is accountable for the gingival inflammation advancing to increased alveolar bone loss, which brings about tooth loss in due course of time.[7] Around 700 different species of bacteria exist in harmony in the oral cavity. *Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Tannerella forsythia* and *Fusobacterium nucleatum* are the chief gram-negative bacteria which reside in the anaerobic environment of the deep periodontal pockets and are accountable for progressive attachment loss.[8, 9]

In the current study, we assessed the minimum inhibitory concentration of *Abutilon indicum* extract against these bacteria to use as a local drug delivery agent shortly.

P. gingivalis (ATCC 33277) is a gram-negative bacterium, non-motile obligatory anaerobic rod, asaccharolytic, and colonies are black pigmented formed on blood agar plates and need iron for its growth.
P. intermedia (ATCC 25611) is gram-negative, non-motile, obligatory anaerobes; single cells grow in an anaerobic growth environment.

T. forsythia (ATCC 43037) is an anaerobic, gram-negative bacterial species in a red complex in the Cytophaga-bacteroidetes family, which has been implied in periodontal diseases.

A. actinomycetemcomitans (ATCC 29523) is an immobile, microaerophilic, facultatively anaerobic, gram-negative coccoid rod, significantly linked with the pathogenesis of periodontal diseases.[10]

F. nucleatum is included in the Bacteroidaceae family is a gram-negative anaerobe, an intermediate colonizer linking the association between gram-positive and gram-negative organisms having co-aggregation properties permitting it to transport periopathogens.[11]

In the present study, *Abutilon indicum* leaf extract has exhibited antibacterial activity, which is in accordance with the study done by Gowtham *et al.* [2019][12] where it has shown antibacterial activity against numerous bacteria such as *Bacillus cereus, E. coli,* and *K.pnemoniae, Pseudomonas aeuroginosa* and also in accordance with the study done by Mishra D.N *et al.* [2017][13] where methanolic extracts of Root, Stem, Leaf and Whole plant of *Abutilon indicum* showed antibacterial activity against *Escherichia coli.*

In the study by Vig M *et al.* [2017][14], this plant's leaf extract has shown maximum inhibition zones against *Bacillus subtilis, Escherichia coli* and *Lactobacillus species* compared to stem and root extract in both *In vitro* and *In vivo* cultures. In the present study, the leaf extract has also shown antibacterial activity against periopathogens.

In the short communication by Tripathi P *et al.* [2012][15], the ethanolic extract of *Abutilon indicum* was found to be practically non-toxic when administered orally to rats. The phytochemical screening revealed the presence of flavonoids, which could be responsible for their anti-inflammatory activity. The present study had shown its antibacterial activity on periopathogens, and the short communication by Tripathi P *et al.* [2012][15] said that it has anti-inflammatory activity. Hence *Abutilon indicum* can be used in treating periodontal disease.

This study has shown antibacterial activity against various gram-negative pathogens, which is in accordance with the study done by Razia M *et al.* [2013][16], where the *Abutilon indicum* leaf extract has shown antibacterial activity against *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae and Shigella flexneri* and also following the study by Wakale S *et al.* [2021][17] where it has shown antibacterial activity against gram-negative bacteria *Escherichia coli.*

In the study by M. Poonkothai *et al.* [2006][18], Chloroform, ethanol and aqueous extracts of the leaves of *Abutilon indicum* were investigated for antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi.* Among the various extracts, maximum antibacterial activity was exhibited by ethanol extract, followed by chloroform extract, while aqueous extract showed no activity. It is pursuing the present study, in which also alcoholic extract was used.

CONCLUSION

Our study upholds the usage of *Abutilon indicum* as a potential adjuvant with periodontal therapy as a mouthwash, topical gel and local drug delivery agent owing to its low cost, availability, low toxicity, antibacterial, anti-plaque, anti-biofilm and anti-inflammatory properties. Although *In vitro* and *In vivo* studies have already suggested *Abutilon indicum* as a potential therapeutic anti-inflammatory agent. Further clinical studies are needed to realize its potential against various periodontal diseases.

Therefore, we conclude that *Abutilon indicum* can be used as an adjuvant to standard periodontal therapy to overcome the side effects of anti-inflammatory agents.

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