

## ANTIMICROBIAL EFFICACY OF TERMINALIA ARJUNA ON POTENT PERIODONTOPATHOGENS: AN IN VITRO STUDY

Shaswata Karmakar<sup>1\*</sup>, Rashmi Srinath<sup>2</sup>, Shobha Prakash<sup>3</sup> and Mahesh J.<sup>4</sup>

<sup>1</sup>Post Graduate student, Department of Periodontics, College of Dental Sciences, Davangere, Karnataka.

<sup>2</sup>Reader, Department of Periodontics, College of Dental Sciences, Davangere, Karnataka.

<sup>3</sup>Vice-Principal, Professor and HOD, Department of Periodontics, College of Dental Sciences, Davangere, Karnataka.

<sup>4</sup>Post Graduate student, Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere, Karnataka.

\*Corresponding Author: Shaswata Karmakar

Post Graduate student, Department of Periodontics, College of Dental Sciences, Davangere, Karnataka.

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### ABSTRACT

Medicinal plants play an essential role in health care and are the major raw materials for both traditional and conventional medicine preparations. One of the plants which is indigenous to India is Terminalia Arjuna, commonly known as 'Arjuna', which has been used in a wide range of diseases in medical field. On the other hand, Chronic Periodontitis is considered to be one of the most common diseases affecting periodontium, which left untreated, causes loss of teeth. And till date, there are no studies on the efficacy of Arjuna on the potent periodontopathogens. Therefore, the aim of this in vitro study was to analyse the antibacterial efficacy of Arjuna extracts on potent periodontopathogens.

**KEYWORDS:** Terminalia arjuna, Chronic periodontitis, Periodontopathogens, Minimum Inhibitory Concentration.

### INTRODUCTION

The human oral cavity is a complex environment inhabited by diverse microorganisms that mainly live on tooth subgingival and supragingival sites. At these oral sites, bacteria can colonize and accumulate as microbial communities known as dental biofilms or dental plaque. Although planktonic cells can easily be eliminated with antibiotic therapy, biofilms are more resilient and are a cause of many persistent infections and recontamination. Changes in the composition of predominant biofilm species are believed to disturb the balance of the host and initiate oral diseases such as dental caries and periodontal disease.<sup>[1]</sup>

Biofilms associated with susceptibility to periodontal disease, are mainly composed of Gram negative, Anaerobic microorganisms i.e. *Fusobacterium nucleatum*, *Prevotella intermedia*, *P. nigrescens*, *P. gingivalis*, *Tannerella forsythensis* and *Treponemes denticola* and *Aggregatibacter actinomycetemcomitans*. The inhibition of biofilm formation and effective disruption of existing biofilms, on tooth surfaces and periodontal pockets, is a proper way for prevention and treatment of such oral diseases. Several studies have examined the inhibitory effect of antimicrobial agents, such as antibiotics, Fluorides, Povidone-iodine, Triclosan

and Chlorhexidine on the bacterial growth on oral surfaces.<sup>[2]</sup>

However, these agents have some significant side effects including; gastrointestinal irritation, tooth staining and most importantly, risk of developing antimicrobial resistance.<sup>[3]</sup> Therefore, the detection of safe, novel and natural bioactive compounds that interfere with biofilm development and maintenance which simultaneously decrease the risk of bacterial resistance are critical. Recently, a wide range of herbs and medicinal plants are being used for various diseases.

Medicinal plants play an essential role in health care and are the major raw materials for both traditional and conventional medicine preparations; still most of the people choose herbal medicines than conventional medicines.<sup>[4]</sup>

Among the medicinal plants, one of the plants which is indigenous to India is Terminalia Arjuna, commonly known as 'Arjuna', which has been used as a cardiogenic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis and has been used for the treatment of different human diseases like blood diseases, anemia, venereal and viral disease; and to

continue excellent healthiness. It is used in the treatment of fractures, ulcers, hepatic and showed hypocholesterolemic, antibacterial, antimicrobial, antitumoral, antioxidant, antiallergic and antifeedant, antifertility and anti-HIV activities.<sup>[5-7]</sup> However, the evidence on the efficacy of Arjuna on oral cavity is very limited.

Chronic Periodontitis is considered to be one of the most common diseases affecting periodontium, which left untreated, causes loss of teeth. And till date, there are no studies on the efficacy of Arjuna on the potent periodonto pathogens. Therefore, the aim of this in vitro study was to analyse the antibacterial efficacy of Arjuna extracts on potent periodontopathogens.

## MATERIALS AND METHODS

### Reparation of the Original Herbal Extract

Arjuna dry extract was prepared in the Department of Research and Development, Himalaya health care Pharmaceuticals, Bangalore, India and sent for microbiological analysis. The Periodontal microorganism to be tested are mentioned (Table 01).

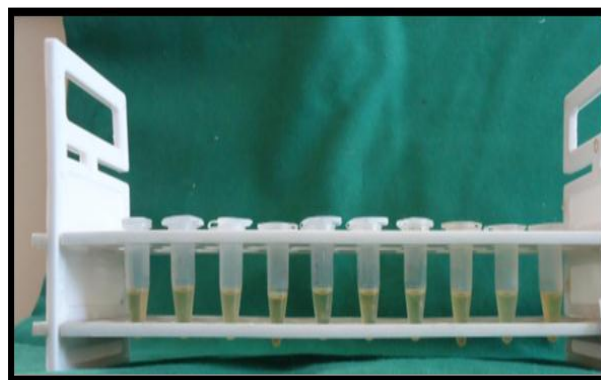
**Table 1: Test organisms for checking MIC.**

Test organism serial no.	Micro organism	Type
1	<i>Actinobacillus actinomycetemcomitans</i>	Gram negative
2	<i>Porphyromonas gingivalis</i>	Gram negative
3	<i>Prevotella intermedia</i>	Gram negative
4	<i>Tanarella forsythus</i>	Gram negative

### Microbiological Procedure

Microbiological procedures were carried out at department of microbiology, Maratha mandal's NGH institute of dental science and research, Belgaum, Karnataka.

Nine dilutions of the drug were done with brain heart infusion (BHI) broth for MIC (Figure 01). In the initial tube 20 microliter of drug was then added to 380 microliter of BHI broth. For dilutions, 200 microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 microlitre was transferred to the first tube containing 200 microliter of BHI broth, this was considered as 10-1 dilution. From 10-1 diluted tube 200 microliter was transferred to second tube to make 10-2 dilution. The serial dilution was repeated upto 10-9 dilution for the drug. From the maintained stock cultures of the required organism, 5 microliter was taken and added to 2ml of BHI broth. Finally, in each serially diluted tube 200 microliter of the above culture specimen was added and the tubes were incubated for 24 hours in anaerobic jar at 37°C and observed for turbidity.



**Figure 01: Brain Heart infusion broth for determination of MIC.**

## RESULTS

**Table 02: Sensitivity of the microorganisms against T.arjuna in various concentrations.**

Sl. No.	Sample	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
	Arjuna Extract										
1	Aa	S	R	R	R	R	R	R	R	R	R
2	Pg	S	R	R	R	R	R	R	R	R	R
3	Tf	S	S	S	S	S	S	S	R	R	R
4	Td	R	R	R	R	R	R	R	R	R	R

S- Sensitive, R- Resistant

Concentration values are in the unit of Micrograms per milliliter ( $\mu\text{g/ml}$ )

A.a, and P.g, showed sensitivity at the concentration of 100 $\mu\text{g/ml}$  whereas T.f showed sensitivity at the concentration of 1.6 $\mu\text{g/ml}$ . T.d was resistant to T.arjuna gel upto the concentration of 100 $\mu\text{g/ml}$ . (Table 02).

## DISCUSSION

Terminalia Arjuna has been used as a cardi tonic in heart failure, ischemic, cardiomyopathy, atherosclerosis, myocardium necrosis and has been used for the treatment of different human diseases like blood diseases, anemia, venereal and viral disease; and to continue excellent healthiness. It is also used in the treatment of fractures, ulcers, hepatic and showed hypocholesterolemic, antibacterial, antimicrobial, antitumoral, antioxidant, antiallergic and antifeedant, antifertility and anti- HIV activities.<sup>[5-7]</sup>

The major constituents of Arjuna in stem bark, root bark, fruits, leaves and seeds are well characterized (Table 02). The preliminary phytochemical analysis of existing compounds in Arjuna was carried out according to various standard protocols as mentioned by Harbone.<sup>[8]</sup>

**Table 03: Major constituents present in Arjuna.**

Phytoconstituents	Test
Alkaloides	Dragendroff's test
Carbohydrates	Molisch's test
Flavonoids	Lead acetate test
Glycosides	KellereKilliant test
Lactones	Legal's test
Phenolic compounds and tannins	5% FeCl <sub>3</sub> test
Proteins	Ninhydrin test
Phytosterols	Salkowski's test
Saponins	Foam test
Triterpenoids	LiebermanneBurchard's test

Aqueous extract of *T. arjuna* is reported to have 23% calcium salts and 16% tannins. Polyphenols, flavonoids, tannins, triterpenoids, saponins, sterols and minerals are the major constituents of *T. arjuna*. Such amino acids like tryptophan, tyrosine, histidine and cysteine are also the ingredients in *T. arjuna*.<sup>[9-12]</sup>

Kapoor et al.<sup>[13]</sup> investigated the therapeutic potential of *T. arjuna* on the inflammatory markers in subjects with stable coronary artery disease (CAD). In a placebo-controlled, randomized double-blind study, 116 patients with stable CAD who were on standard cardiac medications for more than three months were enrolled and received either placebo or 500 mg of *T. arjuna* from Himalaya Herbal Healthcare, Bangalore, India twice a day in addition to receiving the conventional treatment. A significant decrease in serum triglycerides as well as in various inflammatory cytokines such as hsCRP, IL-18 ( $P < 0.001$ ), IL-6 and TNF- $\alpha$  ( $P < 0.05$ ) was observed at 3 months in patients who were on drug treatment as compared to the placebo. The effects were maintained till 6 months follow-up and showed a further reduction in hyperlipidemia and inflammatory markers with time.

Mandal et al.<sup>[14]</sup> investigated antioxidative and antimicrobial properties of methanolic extract of *T. arjuna* bark. The antimicrobial activity showed that higher inhibition against Gram negative bacteria than gram positive bacteria and showed a promising antioxidant activity, as absorption of DPPH radicals decreased in DPPH free radical scavenging assay. Methanol extract from bark of *T. arjuna* exhibited medicinal as well as physiological activities. Extracts from the leaves and bark of *T. arjuna* were tested for their antimicrobial activity against *Staphylococcus aureus*, *Acinetobacter* sp., *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. In the present study *Arjuna* extract showed the antimicrobial efficacy for *Prevotella intermedia*, *Porphyromans gingivalis* and *Actinobacillus actinomycetemcomitans*, with the concentration of 100 $\mu$ l/ml.

## CONCLUSION

*Arjuna* extract showed significant antimicrobial efficacy on single and mixed cultures. As the limits of this *in vitro* study, it can be concluded that with consideration of its significant, antimicrobial properties on key oral pathogens, *Arjuna* extract could be beneficial as an alternative therapeutic strategy against biofilm-related oral diseases such as periodontitis. The results of the present study imply that the action of *Arjuna* extract should be investigated clinically for its beneficial effects on oral health.

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