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INVITRO ANTIBACTERIAL SCREENING OF *ARCTOSTAPHYLOS UVA URSI* LEAF EXTRACT ON SELECTED ORAL PLAQUE FORMING MICROORGANISMS

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ABSTRACT

Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. dental caries/plaque is an extremely prevalent infectious disease that has been shown to be associated with serious health problems. The objective of our study is to evaluate the antibacterial activity of ethanolic leaf extract of *Arctostaphylos Uva ursi* against selected cariogenic oral bacteria that causes dental plaque in fixed orthodontic appliances patients. *Arctostaphylos Uva ursi* is used medicinally since second century. the ethanolic extract of *uva ursi* leaf were used to find out the antibacterial potency against *streptococcus mutans, streptococcus mitis streptococcus sanguis ,streptococcus salivarius, streptococcus sobrinus and Lactobacillus acidophilus*. the extract exhibited significant antibacterial activity against *streptococcus mitis, streptococcus mutans ,streptococcus sanguis & streptococcus salivarius, streptococcus sobrinus* with minimum bactericidal concentration of 1mg/ml and 5mg/ml respectively. whereas, the extract does not show antibacterial activity against *Lactobacillus acidophilus.*

Keywords: *Arctostaphylos Uva ursi,* anti bacterial evaluation, MBC, oral bacteria, dental plaque.

INTRODUCTION

Orthodontic treatments may induce oral ecologic changes, leading to increase of *Streptococcus mutans* in saliva and plaque.[1,2 3] Orthodontic brackets play a significant role in gathering microbial plaque .[4, 5]

Orthodontic appliances, both fixed and removable, impede the maintenance of proper oral hygiene and result in plaque accumulation. Many studies report that changes in the dental flora occurs after starting the orthodontic treatment. Human dental plaque was one of the ecosystems in which microorganism was first observed. dental plaque refers to the aggregates of bacterial cell embedded in a polysaccharide and protein matrix which adheres to the teeth by a characteristic bacterium, *Streptococcus mutans.* This organism metabolizes sucrose in a peculiar way, producing an extracellular adhesive polysaccharide , a sticky insoluble glucan which promotes the firm adherence of the organisms to the tooth surface contribute the formation of dental plaque, subsequently leads to localized decalcification of the enamel surface .[6,7]

Arctostaphylos uva ursi which is also known as bearberry, grows in the northern United States and Europe. In fact, until the discovery of sulfa drugs and anti-biotics,

Uva Ursi is the treatment of choice for bladder and related infections through modern day scientific research in test tubes and animals, researchers have discovered that *Arctostaphylos uva ursi's* antibacterial properties, which can fight infection, are due to several chemicals, including arbutin and hydroquinone.[8]

The herb also contains tannins that have astringent effects, helping to shrink and tighten mucous membranes in the body. That, in turn, helps reduce inflammation and fight infection.*uva ursi* is used to treat cystitis. It increases renal circulation and stimulates tubular function. [9] The leaves, long used by practitioners of herbal medicine, have antiseptic effects. The leaf extract is also a diuretic, helping to remove excess liquids from the body. As a popular home remedy, *Arctostaphylos uva ursi* was traditionally used for treatment of renal infections, and renal stones. It was also recommended for treatment of bronchitis.

The herb has also been used as a general tonic for weakened kidneys, liver or pancreas. Native Americans used it as a remedy for headaches, to prevent and cure scurvy and to treat urinary tract infections. [10]

This herb helps prevent postpartum infection. *Arctostaphylos Uva Ursi* is also helpful for chronic diarrhea. as a nutritional supplement and muscle relaxant, it soothes, strengthens, and tightens irritated and inflamed tissues. The herb neutralizes acidity in the urine, increasing urine flow, therefore reducing bloating and water retention, making it beneficial for weight loss. *Arctostaphylos Uva Ursi's* astringent properties may also assist in the treatment of some bed wetting problems.[11]

Japanese researchers conducted a study on comparing arbutin extracted from *Arctostaphylos Uva ursi* with indomethacin and proved it as good anti inflammatory drug, the results was published in Journal of pharmacological society of Japan.

Research shows *uvaursi* possess potent anti bacterial,anti fungal ,anti plaque, [12] anti viral activity.

Several anti-plaque agents are being available in the market. However, due to several unwanted side effects associated with these agents stimulated the search for alternate agents .[13] In recent years, there has been focus on plants or plant products used in folk dental practice for curing oro dental infections .[14]

Hence an attempt was made to evaluate the antibacterial potency of *Arctostaphylos Uva ursi* leaf extract against selected oral microbes causing dental plaque in fixed orthodontic appliance patients.

MATERIALS AND METHODS

Plant material

Arctostaphylos Uva ursi ethanolic leaf extract were obtained from Green Chem Herbal Extracts & Formulations. Bangalore, India

Test microorganisms

Bacterial strains used were *streptococcus mutans* (ATCC 25175) ,*streptococcus salivarius* (ATCC 25975),*streptococcus mitis* (ATCC 9811),*streptococcus sanguis*(ATCC 10556) *streptococcus sobrinus (*ATCC 27607)*Lactobacillus acidophillus* (ATCC 4356).The organisms were obtained from Department of Microbiology , Saveetha Dental College & Hospitals, Chennai .

Methodology

The herbal extract 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB).From the stock solution various concentrations were prepared,viz.,62µg,125 µg, 250 µg,500 µg/100µl ,1mg,5mg,10mg/100µl respectively in to wells of micro plates.100µl of these concentration were taken and the plates were incubated at 37°C for 24hrs.

Screening of Antibactericial activity

The tested organisms was grown in (TSB) Tryptic soy broth medium [Hi media ,Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 McFarland standard.[15-17]The above concentration of extracts were taken in 100µl quantities in a U bottom micro culture plates. 100µl of the bacterial suspension was added to each well. control well received plain broth without plant extract. the plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in duplicate to minimize the test error.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganism was determined by broth culture method .[18]

A series of two- fold dilution of each extract (62 µg/100µl to 10mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 10^6 organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990)[22]The control well received plain broth without herbal extract .The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by visual methods. hence subcultures from all the wells were made and growth/no growth is detected. then the MBC were obtained.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by selecting wells that showed no growth . The least concentration, at which no growth was observed, were noted as the MBC

RESULT & DISCUSSION

The human mouth is a unique infrastructure. Our teeth is capable of holding a great deal of germs.In fact, dental plaque itself contains 1,000 bacteria. For this reason, dental plaque is considered the human body's most diverse form of biofilms.[19]

Dental plaque , a colorless biofilm that forms around the tooth surfaces. It can attach on every tooth surface especially on intedental surface, occlusal surface, irregular surface of the tooth enamel, and close with the gingival tissue. The biofilm forms just 1-2 minutes after brushing your teeth. The teeth are covered by a film of salivary mucoproteins which is colonized by 350 different kind of bacteria. The most important are *Streptococcus mutans, salivaris, mitis, Lactobacillus, acidophillus* etc.They use carbohydrates stuck on the tooth surface for their metabolism and multiply making dental plaque thicker and more complex.

Dental plaque can also give rise to dental caries (tooth decay) or periodontal disease such as gingivitis and periodontitis.[20]

Fixed orthodontics may be associated with accumulation of *Mutans Streptococci* (MS), enamel demineralization, and an increased number of carious lesions, predominantly in sites adjacent to brackets.[.21]

Multi bracket orthodontic appliances increase dental plaque retention and make teethbrushing more difficult for patients. As a result, advice from the orthodontist on oral hygiene along with patient motivation regarding teeth brushing are particularly important One change that alters the nature of dental plaque is the placement of orthodontic bands and arch wires.

In a study Bloom and Brown [22] found an average increase of 90,000 *lactobacilli*/ml of saliva in orthodontic patients after the placement of bands and arch wires. Since *lactobacilli* require a special plaque

environment, this 3,500% increase represents a striking change in the plaque after banding procedures.

In our study table 1 indicates that *Arctostaphylos Uva ursi* ethanolic leaf extract shows No growth (MBC) at a concentration of 5mg/ml against *streptococcus mutans and streptococcus mitis*.and *streptococcus sobrinus*. The extract also shows No growth (MBC) at a concentration of 1mg/ml against *streptococcus sanguis & streptococcus salivarius*. In conclusion , *Arctostaphylos Uva ursi* leaf extract is highly effective against *streptococcus sanguis* , *streptococcus salivarius* and less effective against *streptococcus sobrinus streptococcus mutans* and *streptococcus mitis* comparatively.however the extract showed no activity against *lactobacillus acidophillus.*

Our finding suggest the presence of No growth is an indication of high effectiveness of the extract whereas presence of Growth indicates the less effectiveness of the extract , that is documented in table 1

CONCLUSION

Orthodontics help both children and adults not only with achieving a beautiful smile but with dental health as well. For instance, overcrowded teeth can cause problems with brushing and flossing creating the perfect breeding ground for cavity-causing bacteria and plaque.

The occurrence of *mutans streptocooci* and *streptococcus sobrinus* together makes the oral environment more conductive to caries/plaque. acid production by both *S. mutans* and *S. sobrinus* plays an important role in the pathology of dental caries/plaques..the results of our study clearly indicates that ethanolic extract of *Arctostaphylos Uvaursi* possess good anti bacterial activity against oral plaque forming microbes , hence we conclude that *Arctostaphylos uvaursi* as an potent antibacterial herb to treat dental plaque associated with fixed appliances patients undergoing orthodontic treatment. further studies are also required to isolate and elucidate the bioactive principle responsible for anti bacterial activity of the herbal extract.

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Conflict of Interest

Nil

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Table 1 – Antibacterial activity of *Uva ursi* ethanolic leaf extract on selected oral plaque microbes

Uvaursi leaf extract	62µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1mg/ml	5mg/ml	10mg/ml	Control	MBC
Streptococcus mutans	++	++	++	++	++	--	--	++	5mg/ml
Streptococcus mitis	++	++	++	++	++	--	--	++	5mg/ml
Streptococcus salivarius	++	++	++	++	_	++	--	++	1mg/ml
Streptococcus sanguis	++	++	++	++	_	_	--	++	1mg/ml
Streptococcus sobrinus	++	++	++	++	++	--	--	++	5mg/ml
Lactobacillus acidophillus	++	++	++	++	++	++	++	++	No activity

++ =Growth
-- = No Growth