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Antibacterial evaluation of herbal extracts against *Streptococcus mutans:* An *in vitro* study

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ABSTRACT

The objective of our study is to investigate the in vitro antibacterial activity of ethanolic leaf extract of Aerva lanata and root extract of Hemidesmus indicus against acidogenic oral microbe Streptococcus mutans .The inhibitory effect of the extract were tested against S.mutans by using the Macro broth dilution method .The ethanolic leaf extract of Aerva lanata exhibited antibacterial activity against streptococcus mutans with minimum bactericidal concentration of 400mg/ml whereas the MBC for Hemidesmus indicus did not showed any activity even in higher concentrations. The ethanolic leaf extract of Aerva lanata was found to be bactericidal in action against tested bacterial strain and these actions may be due to the presence of phytochemical constituents in it.

Key words: *Aerva lanata, Hemidesmus indicus, Streptococcus mutans,* Anti bacterial activity.

INTRODUCTION

Aerva lanata belongs to the family of *Amaranthaceae,* also known as *polpala* or sunny khur. It is one of the important medicinal plants have ever grown throughout the plains of India.[1-3] It is also found in other countries like Sri Lanka, Arabia, Egypt, tropical Africa, java and Philippines.[4, 5] It is found to be an erect or prostrate herbaceous weed that is common throughout the hotter parts of India.

Aerva lanata had been used in the Indian folk medicine for the treatment of diabetes mellitus, urinary calculi, hematesis, bronchitis, nasal bleeding, cough, scorpion stings, fractures, spermatorrhea, [6-8] to clear uterus after delivery and also to prevent lactation.[9, 10]

Hemidesmus indicus belongs to the family *asclepiadaceae* and commonly named in India as *sarsaparilla* or *anantmool*. Which is most widely applied in folk medicine and as ingredients in ayurvedic and unani preparations.[11] *H.indicus* can be used against the diseases like biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders.[12]

Root portion of *H.indicus* is also used in certain conditions like skin diseases, syphilis, elephantiasis, loss of appetite, blood purification and for kidney and urinary disorders and as well as in some biological activities namely hepatoprotective, antioxidant, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, antidiabetic.[13-16]

Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. [17]acidogenic oral bacteria like *Streptococcus mutans, Streptococcus sobrinus, Streptococcus oralis, Streptococcus intermedius, Streptococcus anginosus, Lactobacillus acidophilus, streptococcus salivarius, Streptococcus mitis, Streptococcus sanguis* is a potent initiator that causes dental caries/plaques.

Streptococcus mutans is a member of endogenous oral micro flora and the principal causative agent of dental caries. Short chain carboxylic acid released from the enzymes of *streptococcus mutans* used as its fermentation by-products demineralize the enamel and lead to cavitations in the tooth. [18] Hence considered to be the most cariogenic of all *streptococci* family. The objective of the study is to check the antibacterial effectiveness of *Aerva lanata* & *Hemidesmus indicus* against dental caries /plaque causing *streptococcus mutans*.

EXPERIMENTAL SECTION

Plant material

Ethanolic leaf extract of *Aerva lanata* and Ethanolic root extract of *Hemidesmus indicus* is obtained from Green chem. herbal extracts & formulations, Bangalore. It was authenticated by NISCAIR, New Delhi by Dr. H.B Singh, Head, Raw materials Herbarium & Museum**.**

Medium used

Trytic soy broth is used as a medium

Strain used

Streptococcus mutans ATCC 25175

Preparation of different concentrations of herbal extract

The herbal extracts each 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.,3.1 mg,6.2 mg,12.54mg,25mg, 50mg,100mg,200mg,400mg/100µl respectively in to wells of micro plates. The tested organism was grown in (TSB)Tryptic soy broth medium [MHA-Hi media ,Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 Mac Farland Standard.[19-21]The different concentrations of extracts were taken in 100µl quantities in a U bottom micro culture plates. 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 triplicate to minimize the test error.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested micro organisms was determined by macro broth dilution method. A series of two- fold dilution of each extract (3.1mg/100µl to 400mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 106 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, was noted as the MBC.

RESULTS AND DISCUSSION

Uses of natural medicinal products have become vital in view of their safety. A novel estimate suggests that, in many developing countries people depends on traditional practitioners and medicinal plants to meet primary health care needs.

Dental plaque plays the primary role in the pathogenesis of the dental caries. Dental plaque is a general term for the diverse microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest threat of disease.

The phytochemical constituents present in *Aerva lanata* plant include alkaloids (ervine, methylervine, ervoside, aervine, methylaervine, aervoside, ervolanine, and aervolanine), flavanoids, methyl grevillate, lupeol, lupeol acetate benzoic acid, β-sitosteryl acetate and tannic acid may be responsible for the pharmacological actions.

The ethanolic leaf extract of *Aerva lanata* and root extract of *Hemidesmus indicus* was tested for the antibacterial activity against *streptococcus mutans* a potent intiator of dental caries and dental plaque.

The extract was tested in various concentrations; the antibacterial activity is seen at a concentration of 400mg/ml for *Aerva lanata* whereas *Hemidesmus indicus* failed to exhibit antibacterial activity at any of these concentrations used in the study.

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, which was represented in Table 1
The least effectiveness of the extract indicates the MIC/MBC

Table 1: Antibacterial evaluation of herbal extracts against *Streptococcus mutans*

Herbal extract against *S.mutans*	Concentration of extracts (mg/ml)									
	3.1	6.2	12.5	25	50	100	200	400	control	MIC/MBC
Aerva lanata	G	G	G	G	G	G	G	NG	G	400mg/ml
Hemidesmus indicus	G	G	G	G	G	G	G	G	G	NO ACTIVITY

NG-NO GROWTH (INDICATES MIC/MBC); G=GROWT4H (NO ACTIVITY)

CONCLUSION

In conclusion, our study provides information about *Aerva lanata* ethanolic leaf extract, based on its antibacterial potential that has never been reported. The anticariogenic activity of the plant extract against oral microbe may be attributed to the various phytochemical constituents present in the refined extract. Hence further research should be conducted *in vivo* for its application in dental field.

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