IJBPAS, August, 2012, 1(7): 957-963

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACT OF *CASSIA AURICULATA*, *CLOHEX*, AND *S- FLO* AGAINST SALIVARY MICROFLORA OF MIXED DENTITION AGE GROUP

DESHPANDE RR[1, 3], MAHAJAN PP[*1], KULKARNI AA[1], JADHAV MV[1], GAIKWAD SA[2] AND DESHPANDE NR[2]

1: Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune-18.

2: Dr. T. R. Ingle Research Laboratory, Department of Chemistry, S.P. College, Pune - 30.

3: Deenanth Mangeshkar Hospital and Research center. Pune - 4.

***Corresponding Author: drpriyankamahajan@gmail.com**

ABSTRACT

The study was designed to evaluate the antimicrobial activity of herbal extract (Cassia articulate), and commercially available antimicrobial mouthrinse against salivary microflora. Children (n=10) attending the department of pedodontics, **Dr. D. Y. Patil Dental College, Pmpri, Pune**. Children in mixed dentition period 6 – 12 years DMFT/def : 4/>4 & having good general health**.** The plant material was collected from the local market of Pune, India. Commercially available mouthrinses, 0.2% *Clohex* and 0.2% *S-flo* were used. The salivary samples were diluted using normal saline with dilution factor of 1:3.The microbial inhibition assay was done using well diffusion method on Muller Hinton agar. ANOVA was used for statistical analysis. The herbal extract of *Cassia Auriculata* inhibited the growth of most of the salivary microflora as compared to*0.2% Clohex* and *0.2% S-flo* The herbal extract (Cassia auriculata) exhibited the maximum inhibitory activity at 3000µg of concentration having maximum zone of inhibition 24mm. The study shows that the antimicrobial activity of cassia auriculata is comparable to that of *0.2%Clohex, 0.2%S-flo*.

Keywords: Cassia Auriculata, Clohex, S-Flo, Salivary Microflora, Herbal Extract, Maximum Inhibitory Concentration

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. However, during this time of transition, the synthesized, purified or extracted active ingredients of pharmaceutical drugs were observed to exhibit significant adverse side effects. The potential risk of using synthetic form of phytochemicals has been reported **[1]**. In the oral cavity, saliva serves as a reservoir for normal commensals as well as pathogenic micro flora causing infectious diseases. Dental decay is a chemico-parasitic process in which the oral microorganisms play a very pivotal role. For prophylactic purposes, it seems reasonable to target processes involved in formation of single or mixed bacterial communities that have the potential to cause or favour initiation of dental caries, without perturbing the balance of the normal flora **[2]**.

Cassia auriculata well known as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as traditional medicine for house-hold remedy against various human ailments, plant is said to contain. The plant has been shown to have antibacterial activity in the laboratory **[3]**.

Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds.

So, in this study acetone extracts of cassia auriculata, were screened for their antimicrobial activity against human salivary microflora.

MATERIALS AND METHODS

Plant Material

Cassia auriculata L were collected from local market, Pune, Maharashtra, India, shade dried authentication was done by comparing with herbarium specimens preserved in Botanical Survey of India, Pune (Maharashtra), its authentication no is BSI/WC/Tech/2009/95.

Preparation of Extracts

Air shade dried powdered stem material (10 g) was extracted using acetone (50 ml) separately by soaking it for 24 hours at room temperature. The solvents were evaporated under reduced pressure to obtain crude extracts.

Criteria for Selection of Patient

Inclusion criteria

Patients in mixed dentition period 6 – 12 years DMFT/dmft 4/>4 & having good general health. (Revised WHO criteria 2003)

Exclusion criteria

Patients with H/o antibiotic therapy use of chemical anti-plaque agents prior to 6 months of study initiation**.**

Method of Saliva Collection and Storage

The informed consent was taken from the subjects. The subjects were told to sit upright and rinse with water, saliva was allowed to accumulate in the floor of the mouth for approximately 2 minutes. The patient were told to spit the saliva in a sterile funnel and was collected in a sterile vial **(Figure 1)**. By following the above mentioned method, 10 samples with 3ml saliva each were collected in the early morning time. These salivary samples were diluted in a sterile vial containing 1 ml of normal saline and were used to inoculate on the Muller-Hinton agar plates. All samples were refrigerated within 30 minutes, and frozen within 4 hours.

Anti-Microbial Assay

The microbial inhibition assay was done using the agar well diffusion method. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid Muller Hinton Agar medium in plates **(Figure 2)**. After the media was solidified; a well was made in the plates with the help of a cup-borer (8.0mm). The well was filled with different concentrations of the extract (250µg to 4000µg/ well) and plates were incubated at 37 ± 0.1^{o}C for 24 hours. After incubation, the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. Chlorhexidine and S-flo was used as positive control. The lowest concentration required to attain maximum inhibition of a mixed oral micro flora was recorded.

RESULT

The data was analysed using the ANOVA test showing that there is a significant difference between groups **(Table 2)** as $p<0.005$.

The results of the anti-microbial assay of the acetone extract of Cassia auriculata showing average zones of inhibition (mm) are recorded in **(Table 1)** concentration of 4000 µg of Herbal extract is found to inhibit most of the salivary samples. The 0.2% chlohex showing the maximum Zones of inhibition 20mm **(Figure 4)**. The crude extract

showing the maximum Zones of inhibition 16.2mm and S-flo showing Zones of inhibition 16mm. A dose dependant evaluation of extract on a salivary micro flora is analyzed and is reported in **(Figure 3)**. From the graph **(Figure 5)** it is noted that zone diameter is increased from 250 µg to 3000 µg and remain steady.It indicates that 250 µg is the lowest dose required to attain minimum inhibition of a mixed oral micro flora of fungi and bacteria of the patients saliva.

Table 1: Various Concentrations of Acetone Extract of *Cassia auriculata* Showing the Average ZOI

S. No	Concentrations	Zones of inhibition(mm)
1	250 µg	7.4
2	500 µg	10.8
3	1000 µg	12.4
4	2000 µg	13.1
5	2500 µg	15.1
6	3000 µg	16
7	4000 µg	16.2
8	S-flo	16
Control	2%CHX	20

Table 2: ANOVA Test Showing the Test of Significance

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	4305.691	10	430.569	59.793	.000
Within Groups	712.900	99	7.201		
Total	5018.591	109			

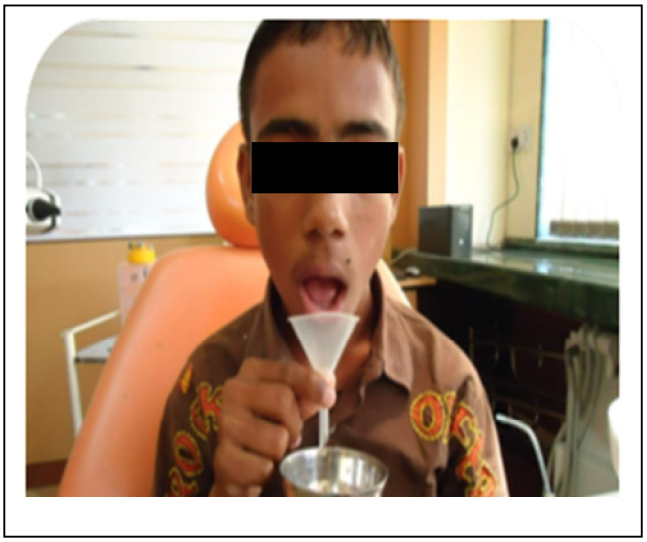

Figure 1: Method of Saliva Sample Collection

Figure 2: Inoculation of the Herbal Extract In the Wells On the Muller Hinton Agar Plates

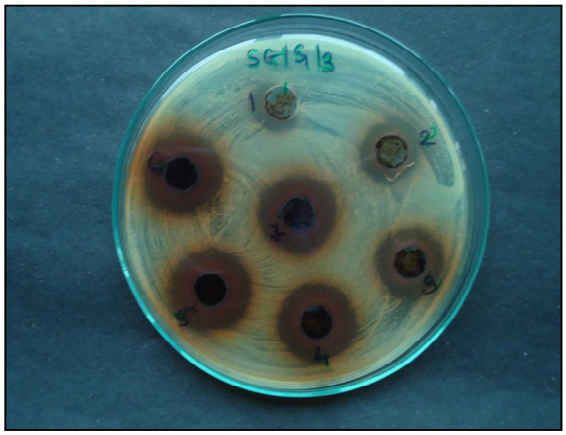

Figure 3: Muller Hilton Agar Plate Showing the ZOI at Various Concentration of *Cassia auriculata*

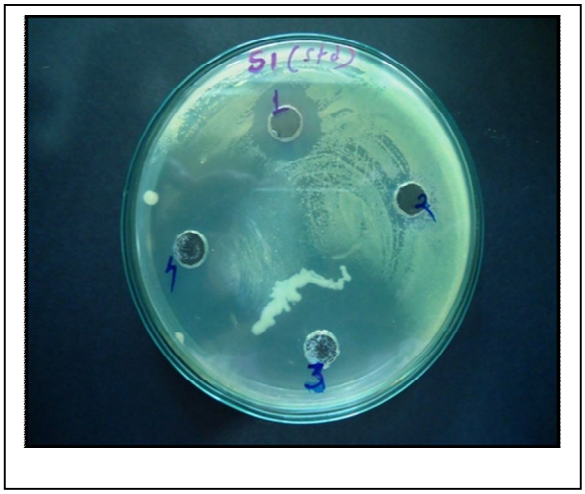

Figure 4: Muller Hilton plate showing the zones of inhibition of (1) 0.2 % Chlox and (2) 0.2% S-Flo

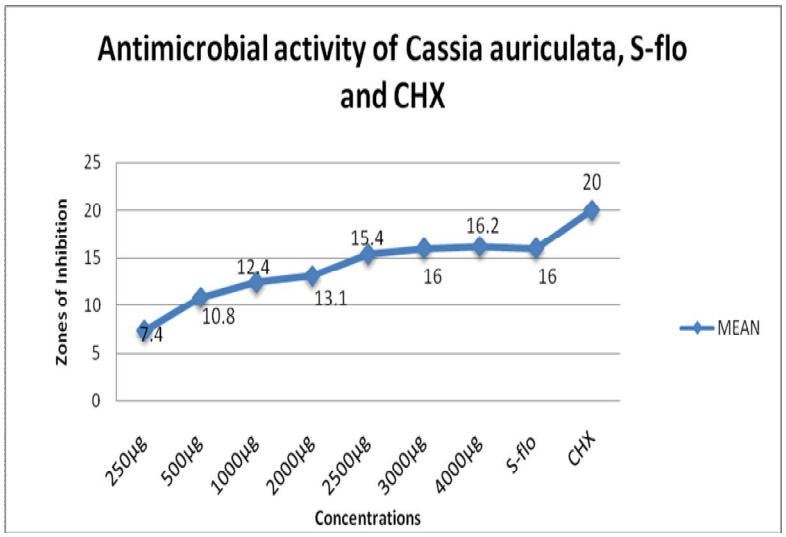

Figure 5: It shows the acetone extract of Cassia Auriculata with the Average zones of inhibition

- **3000µg and 40000µg of acetone extract shows the zones of inhibition of 16 and 16.2mm which is comparable to the S-flo having zones of inhibition of 16mm and chlorhexidine showing the maximium inhibition at 20mm**

DISCUSSION/ CONCLUSION

Caries is a more gradual disease process, with demineralization and remineralization occurring over time. Thus, efforts need to be made for the primary prevention of dental caries initiation, rather than its treatment, throughout the life. Natural products of higher plants may provide a new source of antimicrobial agents with possibly primordial prevention type of mechanism of action. **[4-6]** Also alternatives to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. Awareness for misuse of antibiotics and also the potential risk of using synthetic form of phytochemicals have been reported. This has necessitated the requirement of second and third line drugs.

In the present study, the results confirmed the antibacterial potential of the Cassia auriculata which is comparable with the S-flo. Fluoride which is considered as a pivot in preventive dentistry and continues to be cornerstone in caries preventive programme exerts its antibacterial action through enzyme inhibition, suppressing the flora but as the present study reveals that the antimicrobial action of fluoride is comparable to that of Cassia auriculata showing the zones of inhibition at 16 and 16.2mm indicated that the herbal extracts can be used as a primary preventive and therapeutic agent against dental caries.

The scientific approach has confirmed the antimicrobial potential of the plant extract thus adding weight to its use as a preventive remedy for various microbial diseases of hard tissues in the oral cavity in traditional medicine. The study provides a lead molecule which can be further developed against dental caries. But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter.

ACKNOWLEGEDEMENT

Deshpandes clinic of oral healthcar

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