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COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF ACTIVE COMPONENTS OF *"CASSIA AURICULATA"*; 0.2% CHLORHEXIDINE AND S-FLO IN DIFFERENT CONCENTRATION OF HUMAN SALIVARY MICROFLORA

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ABSTRACT

Medicinal plants are natural sources of compounds that can be used against many diseases today. The medicinal values of these plants lie in bioactive phytochemical constituents that produce definite physiological actions on the human body. The world is now looking towards India due to its rich biodiversity of medicinal plants and abundance of traditional medicine systems. This study compares antimicrobial activity of active compound of '*Cassia auriculata*' with 0.2% chlorhexidine and s-flo, known antimicrobial agent. The antimicrobial activity was assisted by measuring the inhibition zones by well diffusion method. Saliva was collected from children of age group 6-12 years having DMFT value four or above four. Ten salivary samples were tested for antimicrobial property to determine the Minimum Inhibition Concentration in order to increase the reliability and precision of the study. The results confirmed the antimicrobial potential of active compound of '*Cassia auriculata'* plant at different concentrations are comparable with chlorhexidine and s-flo and can be used as preventive and therapeutic measure in dentistry.

Keywords: ***Cassia Auriculata*, Chlorhexidine, S-FLO, Antimicrobial Property**

INTRODUCTION

India, a developing country faces many challenges in rendering oral health needs. The majority of Indian population resides in rural areas, of which more than 40% constitute children. These children cannot avail dental facilities due to inaccessibility, financial constraints and stagnation of public dental healthcare services. This entails the health professional to adopt a more practical approach to achieve prevention of oral diseases **[1]**. The burden of oral disease in children is significant. Most established oral diseases are irreversible, will last for a lifetime and have an impact on quality of life and general health.

Dental caries is the most commonly occurring oral disease and is multifactorial in origin. The oral cavity consist of complex and highly diverse microflora containing colonies of bacteria like streptococcus, actinomycetes, staphylococcus etc. which varies in different individuals **[2]**.

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care needs **[3]**.

Cassia auriculata, Linn comes from Caesalpiniaceae family, is a shrub with large bright yellow flowers, growing wild in Central Provinces and Western peninsula and cultivated in other parts of India. In this study we are investigating the antimicrobial properties of active compound of *'Cassia Auriculata'* in acetone extract with chlorhexidine and s-flo at increasing concentration in order to contribute to the bigger picture of – 'Ayurveda - an alternative to synthetic drugs.

MATERIALS AND METHOD

Plant Material

'Cassia auriculata L' were collected from Western 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 gm) was extracted using acetone and ethanol (50 ml) separately by soaking it for 24 hours at room temperature. The solvents were evaporated to dryness under reduced pressure to obtain crude extracts.

Criteria for Selection of Patients

In the present study, patients of 6-12 years of age, in mixed dentition period

with DMFT four or above four were included. These patients had no history of antibiotic therapy or use of chemical anti-plaque agents prior to six months of study initiation.

Method of Saliva Collection and Storage

The subjects were told to rinse with water; saliva was allowed to accumulate in the floor of the mouth for approximately two minutes and by asking the subject to spit in funnel, saliva (3ml) was collected in vial. By following the above mention method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1) in a sterile vile containing 1ml of normal saline and were used to inoculate on the agar plates. All samples were refrigerated within 30 minutes and frozen within 4 hours. (If collection is being carried out in the field, it may not be practical to freeze the samples until the end of the day, but samples should be kept cold until they are returned to the lab).

Antimicrobial Assay

The microbial inhibition assay was prepared using the agar well diffusion method. Sterile 8.0 mm diameter of well were impregnated with the extract of different concentrations ranging from 62.5µg to 4000µg per well. 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. 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 (62.5µg 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 was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded.

Emodin Isolation from *Cassia auriculata* L.

In Ayurveda different species of cassia (Family-Cesalpinieacea) are used as an medicine.

Various biological properties are mentioned in Ayurveda. Quath *of Cassia auriculata* L leaves is prepared as per standard protocol of Ayurveda. Acetone fraction of the quath is used for the further investigation. The compound was isolated by repeated

chromatographic techniques. It was purified by repeated crystallization using mixed solvent system (Chloroform :Methanol) It was characterized by modern spectral techniques which confirms compound 1.3.8 trihydroxy 6- methyl anthraquinone- Emodin. **(This is an procedure for the extract as well as standard Emodin Isolation as discussed with Hon. Deshpande Madam)**.

RESULTS AND DISCUSSION

This stud compares antimicrobial activity of active compound of 'Cassia Auriculata' with 0.2% chlorhexidine and S-flo. The zone of inhibition are measured by excluding the diameter of well. The mean value of average zone of inhibition of active compound of 'Cassia Auriculata' with 0.2% chlorhexidine and S-flo in ten salivary samples has taken for comparison. These zones of inhibition are directly proportional to the concentration.

Table 1 represents the mean value of average zone of inhibition of the active compound of cassia auriculata at seven different concentrations. **Table 2** represents the mean value of average zone of inhibition of chlorhexidine and S-flo at seven different concentrations. Results were obtained after 24 hours of incubation. Number 1 and 2 in Fig 2 represents zone of inhibition of chlorhexidine and S-flo respectively. **Figure 1** represents average zones of inhibition (mm) of active compound of 'cassia auriculata'.

This study proves that the antimicrobial activity of cassia auriculata at higher concentration is comparable with 0.2% chlorhexidine and S-flo. Statistically, **Kruskal-Wallis** test followed by post-hoc test proved that all results are comparable as the p value is 0.0001 which is significant ($p< 0.5$).

Emodin is a biologically active, naturally occurring anthraquinone derivative **[4]**. From ancient times, herbal extracts of 'cassia auriculata' have been used in medical treatment **[5]**. Several scientific studies of its biological activity have been performed. But to prove antimicrobial activity of active compound of cassia auriculata with chlorhexidine and S-flo we need to take further higher concentration.

Table 1: Mean Value of Zones of Inhibition (in mm) of Active Compound (Emodine) of '*Cassia auriculata*'

CONCENTRATIONS	MEAN VALUE OF AVERAGE ZONE OF INHIBITION (mm)
5 mg	0.0000
10 mg	1.4000
15 mg	3.7000
25 mg	4.4000
30 mg	5.2000
50 mg	7.0000
80 mg	10.2000

Table 2: Mean Value of Zones of Inhibition (in mm) of Standard Antimicrobial Agent

ANTIMICROBIAL AGENT	MEAN VALUE OF AVERAGE ZONE OF INHIBITION
0.2% Chlorhexidine	20.0000
Sflo	16.0000

Figure 1: Average Zones of Inhibition (mm) of Active Compound of '*Cassia auriculata*'

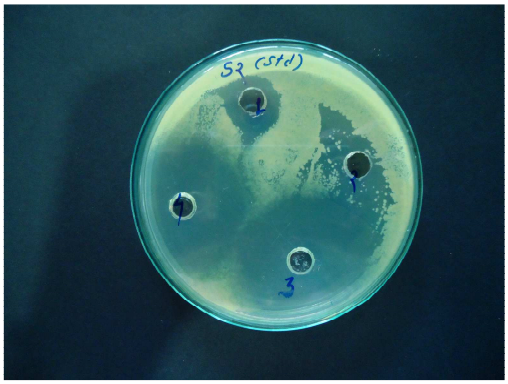

Figure 2: Average Zones of Inhibition (mm) of Antimicrobial Agents [number1 and 2 Represents Chlorhexidine and S-Flo Respectively]

CONCLUSION

The antimicrobial activity of active compound of cassia auriculata at higher concentration is comparable with 0.2% chlorhexidine and s-flo. This study has confirmed the antimicrobial potentials of the plant, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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