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Research Article

COMPARATIVE EVALUATION OF DIFFERENT CONCENTRATIONS OF CASSIA AURICULATA EXTRACT AS AN ANTIMICROBIAL AGENT AGAINST HUMAN SALIVARY MICROFLORA

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ABSTRACT

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practioners of traditional medicine. In this study the acetone extract of Cassia Auriculata is investigated for its antimicrobial activity against salivary microflora. The salivary samples were collected from children of mixed dentition age group having DMFT 4 and above 4. The microbial inhibition assay was done by 'well diffusion method' on the Muller-Hinton agar . The results were compared with 2%chlorhexidine a known commercial available antimicrobial agent. The results confirmed the antimicrobial potential of this extract and indicated that the acetone extract of Cassia Auriculata can be used as the preventive tool for dental caries.

Key words : Cassia Auriculata, chlorhexidine, salivary microflora, acetone extract.

INTRODUCTION

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds[1]. Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem[2]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action[3,4]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[5]. 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[6].

In this study, acetone extracts of cassia auriculata, which had been described in herbal books and folklore medicine of India, were screened for their antimicrobial activity against 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 solvent were evaporated under reduced pressure to obtain crude extracts.

Criteria for selection of patient

In the present study, patients of 6-12 years of age, in mixed dentition period with four or more decayed teeth were included. These patients had no history of antibiotic therapy or 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 patients were told to spit the saliva in a sterile funnel and were collected in a sterile vial. By following the above mentioned method, 10 samples 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. 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 (50µg to 800µg/ well) and plates were incubated at 37 ± 0.1°C for 24 hours. After incubation, the plates were observed for zones of inhibition and the diameters of these zones were measured in millimeters by using bacterial inhibition zone reading scale.(figure:2) All the tests were performed under sterile conditions. Chlorhexidine was used as positive control. The lowest concentration required to attain maximum inhibition of a mixed oral micro flora was recorded.(Table.1) it shows the zones of inhibition attained at various concentrations.

Table 1: Various concentrations of acetone extract of cassia auriculata with the average zones of inhibitions.

Sr.no	Concentrations	Zones of inhibition(mm)
1	62.5µg	0
2	125 µg	0.2
3	250 µg	7.4
4	500 µg	10.8
5	1000 µg	12.4
6	2000 µg	13.1
7	2500 µg	15.1
8	3000 µg	16
9	4000 µg	16.2
Control	2%CHX	20

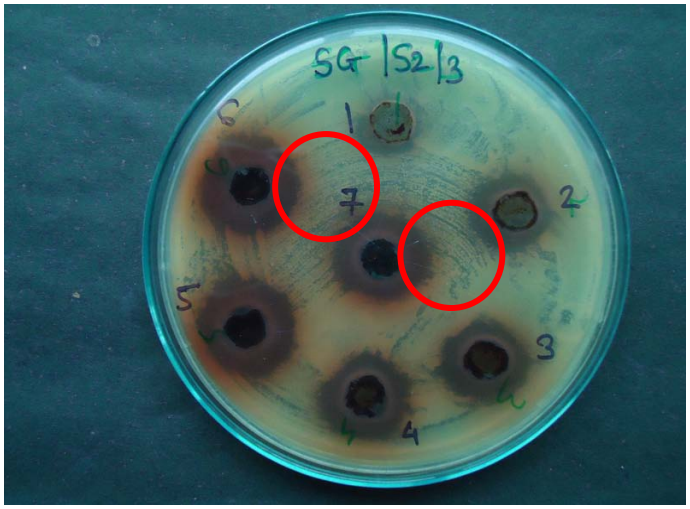

Fig 1: Muller Hilton agar plate showing the Zones of inhibition

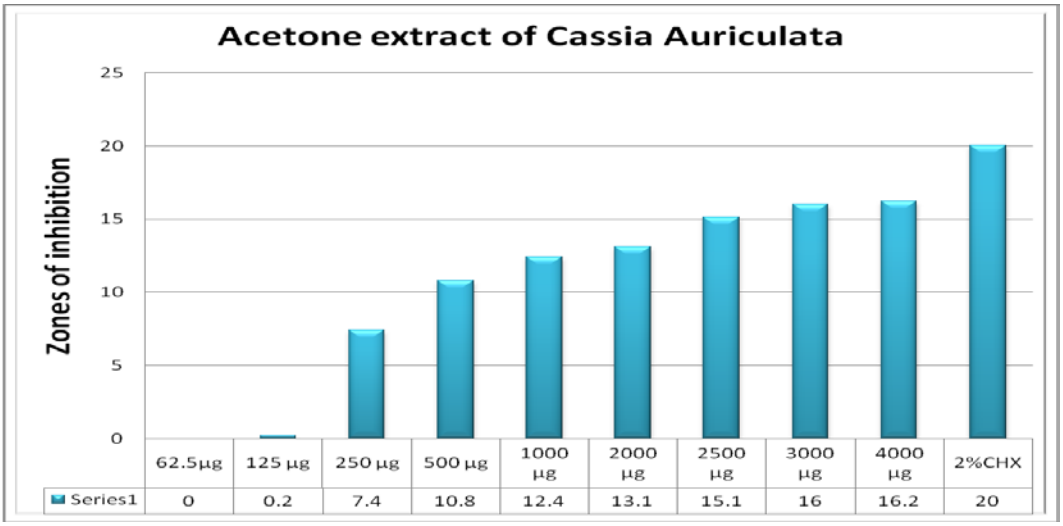

Fig 2: It Shows The Acetone Extract Of Cassia Auriculata With The Average Zones Of Inhibition.

1. **Series 1 are the average zones of inhibition in mm.**
2. **3000µg and 40000µg of acetone extract shows the zones of inhibition of 16 and 16.2mm which is comparable to the chlorhexidine having zones of inhibition of 20mm.**
3. **Chlorhexidine which is used as a control has the maximum zones of inhibition 20mm.**

RESULT/ DISCUSSION

The results of the anti-microbial assay of the acetone extract of Cassia Auriculata are reported in fig.1. concentration of 3000µg and 4000 µg of acetone extract displays the maximum anti-microbial activity with an inhibition zone of 16mm

As dental caries is now regarded as a disease process the concept of '***Primordial prevention***' comes into play. Primordial prevention involves the halting the caries process and preventing the establishment of new carious lesions.

For prophylactic purposes, it seems reasonable to target processes involved in the actual biofilm formation of single- or mixed-bacterial communities that have the potential to cause or favor disease[7], without perturbing the balance of the normal flora. Any chemical agents that affect microbial cells may have some adverse effects on against target structure or metabolic pathway is unique to microbial cell. Thus Cassia Auriculata can be used in various chemical and mechanical plaque control agents to plaque formation and thus preventing the formation of dental caries. For effectivity of Cassia Auriculata to be an effective antimicrobial agent it must be used in a solvent which is soluble in water and saliva. It has been seen that acetone is miscible with water that is it forms a homogenous solution with water[8]. Thus acetone extract of Cassia Auriculata can be effectively used as an antimicrobial agent to prevent caries process and as an antiplaque agent.

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