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IN VITRO ANTIMICROBIAL ACTIVITY OF *CASSIA AURICULATA (L)* AGAINST MICROBES PRESENT IN HUMAN SALIVA

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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. The aim of the present study is to compare antimicrobial activity of active compound of *Cassia auriculata* with 2% chlorhexidine, 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 can be used as preventive and therapeutic measure in dentistry.

KEYWORDS: *Cassia auriculata*, Anti-microbial activity, Human saliva, Chlorhexidine.

INTRODUCTION

Since ancient times, mankind has used plants to treat common diseases and some of these traditional medicines are still included as part of the habitual treatments of various maladies.[1] Folk medicine, mainly based on plants, enjoy a respectable position today, specially in the developing countries, where the availability of modern health services is limited. However, in the absence of a scientific base, such practices may generate serious adverse effects. The analyses of the pharmacological activity of plant extracts may therefore make possible the design of less expensive therapies to be used in economically unprivileged regions.[2] Mainstream medicine is increasingly receptive to the use of antimicrobials and other drugs derived from plants, as traditional antibiotics become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extraction.[3] However, the full acceptance of phytopharmaceuticals and the integration of phytotherapy into the concept of classical medicine can be achieved only if they meet the same criteria of quality as synthetic pharmaceuticals. Moreover, the ideal procedures for standardization of the phytopharmaceuticals are based on knowledge of the major pharmacological and toxicological assays.[4] In addition, the overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes.[5] Antimicrobial agents play a vital role in eliminating pathogenic bacteria that invade gingival tissue. In clinical practice amoxicillin, metronidazole, tetracycline, and azithromycin are the most frequently used as adjunctive therapy for the treatment of periodontitis cases.[6–8] The exponentially rising multidrug-resistant (MDR) bacteria to present antibiotics is a very critical issue as it represents the predominant cause of treatment failure and increased the percentage of mortality.[9] Thus, it becomes a necessity to develop antibacterial agents that not only prevent the process of drug resistance but also improve the results of the infectious disease treatment. Studies have revealed that long-term use of chlorhexidine mouthwash is associated with a number of adverse effects like staining of the teeth, loss of taste sensation, degeneration of tongue papilla, and in rare cases parotid swelling.[10] Plants and their extracts are known to be used for therapeutic purpose since the time immemorial, due to the facts that there use is safe, economical, effective, and easily available. Salvadora persica (S. persica) is a small tree or shrub belongs to the family Salvadoraceae. Plant root, stem and twig have been widely used by many people in Africa, the Middle East, and Asian subcontinents for oral hygiene maintenance. It is recommended as an effective tool for oral health care by the World Health Organization (WHO).[11] *Cassia auriculata* is a legume tree belonging to the subfamily Caesalpinioideae. It is commonly known by its local names matura tea tree, ranawara or avaram, (Tamil:

avarai) or the English version avaram senna. It is the State flower of Telangana .It occurs in the dry regions of India and Sri Lanka. *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 at increasing concentration in order to contribute to the bigger picture of Ayurveda an alternative to synthetic drugs against microbes present in human saliva.

1. MATERIALS AND METHODS

1.1 Collection of 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. The fresh flowers of cassia auriculata were collected from local area of sulur. All solvents used were grade ethanol, aqueous, acetone were obtained from college store. The flowers were collected and healthy flowers were shade dried and then powdered using electric blender to get a coarse powder.

1.2 Extraction

The powdered material was extracted with solvents like acetone, ethanol and water by cold maceration process. The extract were prepared by taking 50g of dried flower powder in separate containers and to this 200ml of each solvent was added and kept it for 48hrs.The extracts were collected by filtered through 5 layers of muslin cloth. Then the extract was collected.

Fig. 1: Ethanol, Acetone, Water Extract of *Cassia auriculata L.*

1.2 Phytochemical screening

The phytochemical screening for the extracts was carried out by standard protocols Alkaloids (Mayer's test), Glycosides (Legal's test), Saponins (Froth formation test), Carbohydrates (Molisch's test) Proteins (Xanthoproteic test), Aminoacids (Ninhydrin test) Flavanoids (Lead acetate test), Steroids (salkowski test) Tannins (Ferric chloride test), Volatile oils (hydro distillation method), were analysed.

Fig. 2: Phytochemical screening of ethanolic extract of *Cassia auriculata L.*

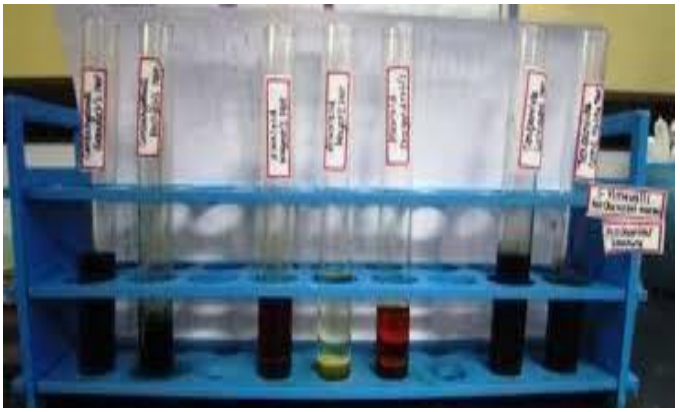

Fig. 3: Phytochemical screening of Acetone extract of *Cassia auriculata L.*

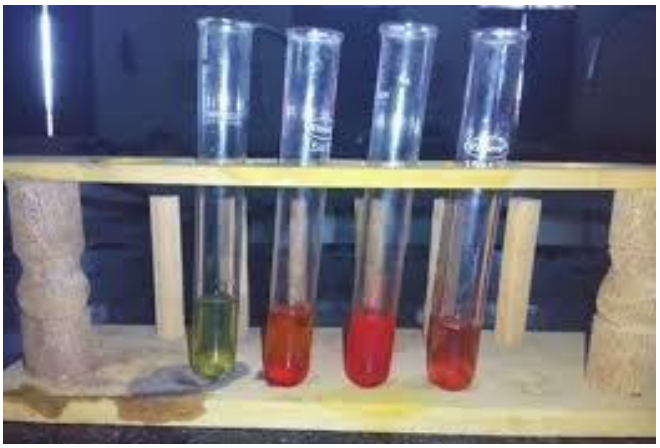

Fig. 4: Phytochemical screening of Water extract of *Cassia auriculata L.*

Table 1: Preliminary phytochemical screenning of Flower Extracts of *Cassia auriculata L*.

S.NO	CONSTITUENTS	TESTS	INTERFERENCE		
			ETHANOL	ACETONE	WATER
1	Alkaloids	Dragendroff's test	+ve	-ve	+ve
		Hager's test	+ve	-ve	+ve
		Wagner's test	+ve	-ve	+ve
		Mayer's test	+ve	-ve	+ve
2	Carbohydrates	Anthrone test	+ve	+ve	+ve
		Benedict's test	+ve	+ve	+ve
		Fehling's test	+ve	+ve	+ve
		Molisch's test	+ve	+ve	+ve
3	Starch	Iodine test	-ve	+ve	+ve
4	Glycosides	Kedde's test	+ve	-ve	-ve
		Killer killani test	+ve	-ve	-ve
5	Flavanoids	Shinoda's test	+ve	+ve	-ve
		Lead acetate test	+ve	+ve	-ve
		Ferric chloride test	+ve	+ve	-ve
6	Terpenoids	Libermann Burchard'stest	-ve	-ve	+ve
7		Resins	+ve	+ve	+ve
8		Saponins	+ve	+ve	+ve
9	Steroids	Libermann Burchard's test	-ve	+ve	-ve
		Salkowaski reaction	-ve	+ve	-ve
10	Proteins	Millon test	+ve	-ve	+ve
		Biuret test	+ve	-ve	+ve
11	Tannins	Ferric chloride test	+ve	-ve	+ve

+ indicates -Positive
- indicates-Negative

1.3 Thin layer Chromatography Analysis

Silica gel plate was prepared (layer thickness 0.20mm). The lower end was marked with a slim line with a HB pencil to identify spotted end. 1mg of the extract was dissolved in 1ml of methanol as solvent. 0.5ml was drawn in a capillary and spotted in the labelled spots. The plate were allowed to run in the mobile phase (ethyl acetate: n- butanol: formic acid: water) (10:6:2:2 v/v/v/v) vanillin sulphuric acid reagent was used as spray reagent for colour, development reaction of tannins. The spot were located on the plate under uv lamp. The Rf values were calculated.

Fig. 5: TLC Screening of Ethanol,Acetone and Water Extract of *Cassia auriculata L.*

2. Determination Of *In-Vitro* Anti Microbial Activity Of Cassia Auriculata(L)

2.1 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 ± 0.1oC 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.

2.2 Method of Saliva Collection and Storage

The early morning saliva was collected from children of age group 6-12 years having DMFT value four or above four and it was stored in a sterile vial for the determination of antimicrobial activity against human saliva.

2.3 Preparation and sterilization of culture media and agar plate

Medium is defined as any substrate or material that will enable the micro organism to grow and multiply. A common nutrient medium used for cultivating the bacteria consist of beef extract- 0.35, peptone- 0.5%, sodium chloride-0.1% in water and it is called liquid medium or nutrient broth. All medium should provide carbon, nitrogen sources in addition to mineral and other growth factors for the growth and multiplication microbes, the basal nutrient medium can be supplemented with different substance such as sugar, proteins, alcohol or inorganic salts to satisfy the requirement of particular organisms. Accurately weighed amount of ingredients are added to required quantity of water in a conical flask. Gently stir and keep it on the boiling water bath. Add 2% of agar to convert the liquid medium in to solid nutrient agar medium. Make sure that all ingredients were dissolved. Take out the flask from the water bath and check the pH for 7-2 by using pH meter. If necessary brings out the pH to 7-2 by adding buffers. Carry out sterilization by autoclaving.

2.4 Preparaton of agar plates

Label the petri plates and organisms kept on the inoculation cabin. Hold the bottom of the conical flask on your right hand. Remove the cotton plug from your flask by using your left hand in proper way. Hold your petri plate in your left hand with a specified position. Flame the mouth of the conical flask. Pour the sterilized agar liquid medium into the bottom of the petri plate just to form one circle. Again flame the mouth of the flask and replace the cotton. Immediately place the plate on the table from the edge. Leave the plates for solidification.

2.5Antibacterial activity

The extracts sample were dissolved in a solvent at final concentration of 10mg/1ml was pipette into the different wells in a sterilized environment at different volumes (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6) in plates using a micro pipette. And Control wells were inoculated by ciprofloxacin for bacteria (E.coli, Staphylococcus aures) The plates were incubated for 24h for bacteria and the Zone of inhibition were measured.

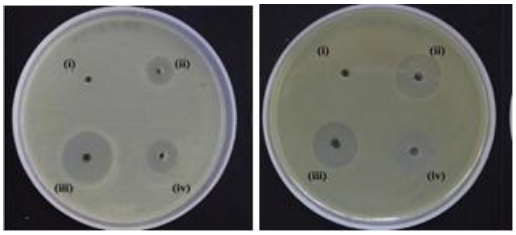

Fig. 6: Determination of Inhibition Zone Diameter (For Water Extract).

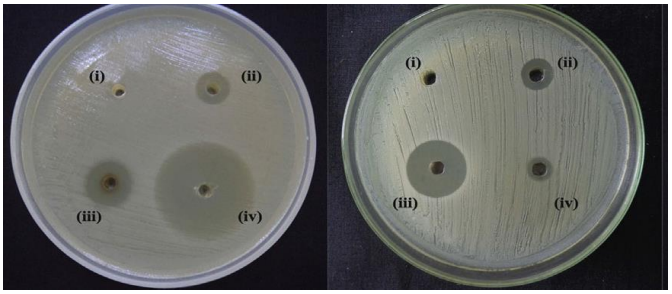

Fig. 7: Determination of Inhibition Zone Diameter(for Ethanolic Extract).

Table 2: Antibacterial activity of flower extract of *Cassia auriculata L*.

Bacterial strain	Zone of Inhibition(mm)				
	Ethanol	Aqueous	Acetone	DMSO	Ciproflaxacin
E.coli	18	10	14	0	31
Slaphylococcusaureus	15	10	15	0	32

2.6 Antifungal activity

The extracts sample were dissolved in a solvent at final concentration of 10mg/ml was pipette into the different wells in a sterilized environment at different volumes (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6mg/ml) in plates using a micro pipette. And Control wells were inoculated by griseofulvin for fungi (A.niger, C.albicans). The plates were incubated for 72hrs for fungi and the Zone of inhibition were measured.

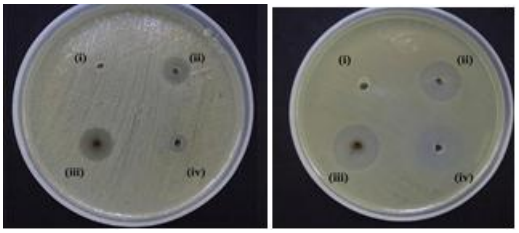

Fig. 8: Determination Of Inhibition Zone Diameter (For Water Extract)

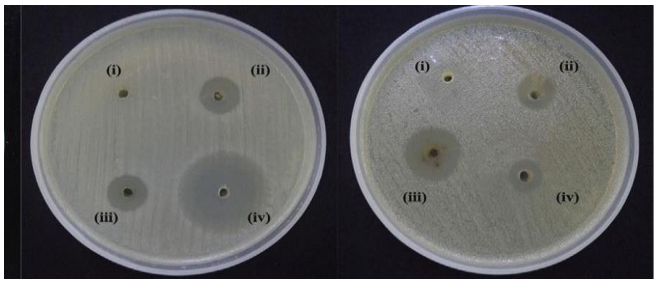

Fig. 9: Determination Of Inhibition Zone Diameter (For Ethanolic Extract).

Table 3: Antifungal activity of flower extracts of Cassia auriculata.

Fungal strain	Zone Of Inhibition(mm)				
	Ethanol	Aqueous	Acetone	DMSO	Griseofulvin
Aspergillus niger	10	09	10	0	26
Penicillium	12	10	10	0	28

2.7 Determination of antimicrobial activity against human saliva

The extracts sample were dissolved in a solvent at final concentration of 10mg/1ml was pipette into the different wells in a sterilized environment at different volumes (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6) in plates using a micro pipette. The freshly collected human saliva was swapped on solidified media in petri plates. Control wells were inoculated by 2% chlorhexidine for microbes present in human saliva. .The plates were incubated for 24 hrs for fungi and the Zone of inhibition were measured. The diameter zone of inhibition of each disk was measured by keeping the lid of the plate in place use a ruler to measure the diameter of the disk plus the surrounding clear area in millimeters (mm).

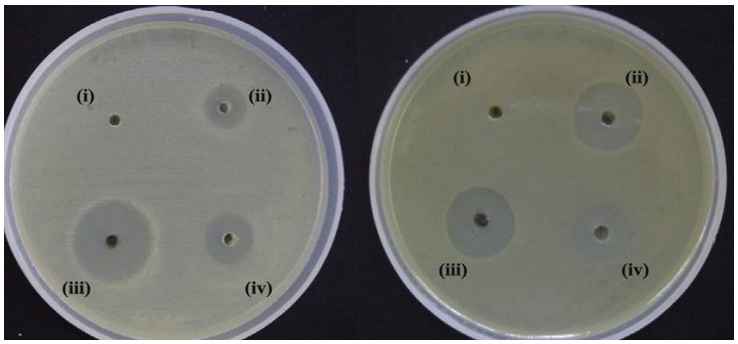

Fig 10: Determination of Inhibition Zone Diameter (For Ethanol Extract) Against Microbes Present In Human Saliva.

Table 4: Antimicrobial activity of flower extract of *Cassia auriculata L* against saliva of human.

Bacteria	Zone Of Inhibition(mm)		
	Ethanol	DMSO	2% Chlorhexidine
Microbes in humansaliva	18	0	20

3. RESULTS AND DISCUSSION

The results of preliminary phytochemical screening revealed the presence of various phytoconstituents. (Table:1). The carbohydrates and alkaloids were present in aqueous and ethanol extracts. The steroids were present in acetone extracts. The tannins were present in ethanol extracts and flavonoids were observed in acetone extract. The presence of saponins was observed in ethanol, aqueous and acetone extract. The present study shows the evaluation of in vitro antimicrobial activity of cassia auriculata against Gram-positive, Gram-negative and fungi, and microbes present in human saliva using agar well diffusion method. The results of antibacterial activity were given in (Table: 2), which clearly show that all the extracts have shown antimicrobial activity equivalent to that of the standard against entire tested organisms. Antimicrobial activity was confirmed by the presence of zone of inhibition.

The diameter of zone of inhibition range from 09- 18 mm for bacteria and 09-12 mm for fungi. The acetone extract exhibited prominent antimicrobial activity as compared to that of other extracts. The anti-microbial activity of ethanol and aqueous extracts is moderate and aqueous extract is lesser. The acetone extract exhibited maximum activity against S.aureus (15mm), and moderate activity againt E. coli (14mm). Ethanol extract exhibited maximum activity against E.coli (18mm) and moderate activity against S.aureus (15mm). The results of Antifungal activity were given in (Table: 3), the aqueous extract exhibited maximum activity against E.coli (10mm). The ethanol extract of the plant was exhibited prominent antifungal activity, acetone extract exhibited moderate activity, though the aqueous extract demonstrated less activity. The ethanolic extract exhibited maximum antifungal activity against A.niger (10mm) ethanol extract against penicillium (12mm).The Result of Antimicrobial activity against microbes present in human saliva were given in (Table:4). The diameter zone of inhibition for ethanol extract of cassia auriculata was measured (18mm) and the diameter zone of inhibition for 2% chlorhexidine was measured (20mm).

CONCLUSION

There has been a change in global awareness, with a growing tendency to ‘go natural ‘and due to the side effects of conventional mouthwashes towards caries prevention. Herbal products have been gaining much importance. Thus we had made an attempt to outline commonly available herbal leaves which can be used effectively as dental therapeutic agents. With further research in this field, we would get much safer alternatives for caries prevention the zone of inhibition obtained from ethanollic extract of cassia auriculata was approximately equal to that of the zone of inhibition obtained from 2% chlorhexidine. Hence, ethanol extract of *cassia auriculata* can be a potential alternative to 2% chlorhexidine in dental products. Thus the *cassia auriculata* can be used as one of the ingredient in herbal dental products in future.

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