

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative evaluation of antimicrobial properties of two different extracts of Artemisia Pallens (Davana) and 0.2% Chlorhexidine against acidogenic salivary microflora in mixed dentition age group.

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ABSTRACT

Herbal medicines are in great demand in the developed world for primary health care because of their safety, efficacy and minimal side effects. Since centuries plants and their extracts have been analysed and reported for their significant therapeutic properties. The antimicrobial efficacy of many plants is yet to be verified. In this study, the antimicrobial property of two different extracts of Artemisia pallens have been evaluated against acidogenic salivary microflora. The salivary samples were collected from children of 6-12 years of age with moderate caries. Antibacterial assay was carried out using paper disc diffusion method in lab. The results are compared with Chlorhexidine as standard. Saliva samples were collected by passive drooling method. Microbial assay was performed using the agar “well-diffusion” method. Sterile 8 mm of well were impregnated with the extract and chlorhexidine. The plates were observed for zones of inhibitions and were measured in millimetres. The zone of inhibitions were measured for extracts and 0.2% Chlorhexidine. The results confirmed the antimicrobial potential of the plant when compared with gold standard chlorhexidine.

Keywords: Caries, herbal medicines, chlorhexidine, mix dentition, saliva

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INTRODUCTION

Dental caries is the most commonly occurring oral disease and is multifactorial in origin. The oral cavity consists of complex and highly diverse microflora containing colonies of bacteria like streptococcus, actinomycetes, staphylococcus etc. which varies in different individuals. [1] According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary health care needs. [2]

Dental caries, prevalence as high as 60-80% in children, is major health problem in India.[3]

Due to the growing evidence of relation between oral health and whole-body health dental practitioners may seek to respond to their patient's oral hygiene needs with newer products.[4,5] This research based products come with naturally occurring active ingredients, that achieve the desired antibacterial and anti-inflammatory effect.[5] Moreover, chemicals like chlorhexidine and amine fluorides have undesirable side-effects such as staining of teeth and restorations, increase in calculus deposition and imbalance of the oral and intestinal flora, thus leading to vomiting and diarrhoea. These drawbacks justify the search for new effective anticariogenic compounds that could be employed in caries prevention. [6,7]

The genus *Artemisia*, of the family Asteraceae, tribe Anthemideae, is a large genus with about 400 species widely distributed in Europe, north America, Asia and South Africa. Species of this genus are important medicinal plants and have been used by many cultures in folk and modern medicine due to their medicinal usage. [8]

The medicinal aromatic herb *Artemisia pallens* is used in floral decoration, religious offerings and for the extraction of an essential oil- Davana oil. Plants are accredited with anthelmintic, tonic and antipyretic properties. [9]

In this sense, efforts have been made to evaluate & compare the antimicrobial properties of two different extracts of *Artemisia pallens* with 0.2% chlorhexidine against acidogenic salivary microflora.

MATERIALS AND METHODS

Collection and Identification of plant material

The plant material (extracts) of the species was collected from local market and it's authentication was done by Botanical survey of India

Inclusion criteria

- Mixed dentition phase; age 6-12
- No history of antibiotic therapy and use of chemical anti plaque agents prior to one month of study initiation.
- deft/DMF= 3-6

Exclusion criteria

- History of known systemic disease
- Subjects who are not willing to participate

Saliva: Collection and transport [10]

- The informed consent was taken from the subjects.
- Subjects were asked to report from 10am to 12pm.
- Subjects were instructed not to drink or eat atleast 1 hour prior to collection of the samples.
- Subjects were asked to rinse mouth with tap water before sample collection.

- Saliva samples were collected 10 minutes after rinsing to avoid dilution by passive drooling method into sterile glass tube.
- Saliva collection was done with the patient being seated in well-lit room.
- Samples were transferred to a calibrated cylindrical flask to measure the volume of each collected salivary sample.
- 5ml of saliva sample were transferred to sterile vials.
- The salivary samples labelled according to the patient's name sent to the laboratory within 1 hour after saliva collection in an ice box.

Antimicrobial assay [10]

The microbial inhibition assay was prepared using the agar 'well-diffusion' method. Samples of each acetone and ethanol extracts (200 μ g) were dissolved in respective solvents. Sterile 8.0 mm diameter of well were impregnated with different extracts. The salivary flora was inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1 °C. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid medium in plates. Following this, the sterile discs impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37 ± 0.1 °C for 48 hours. After incubation, all the plates were observed for zones of inhibition and the diameters of these zones were measured in millimetres. All tests were performed under sterile conditions. Chlorhexidine was used as positive control.

RESULTS AND DISCUSSION

As per the World Health Organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care. [11] Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by oral pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[12,13]

In the present study, the plant viz.,Artemisia Pallens (Davana) was selected based upon its traditional medicinal properties. Literature survey revealed that various parts of the plant possess different biological activities. Artemisia pallens is a small and aromatic herbaceous plant which is native to the southern parts of India, especially to the states of Karnataka, Tamil Nadu, Andhra Pradesh and in Maharashtra.

In the regional languages of the south, it is known by several names as "davanam" in Tamil, "davanamu" in Telugu and "davana" in Kannada. The extract of Artemisia pallens showed antibacterial activity against gram positive and gram-negative bacteria.[10]

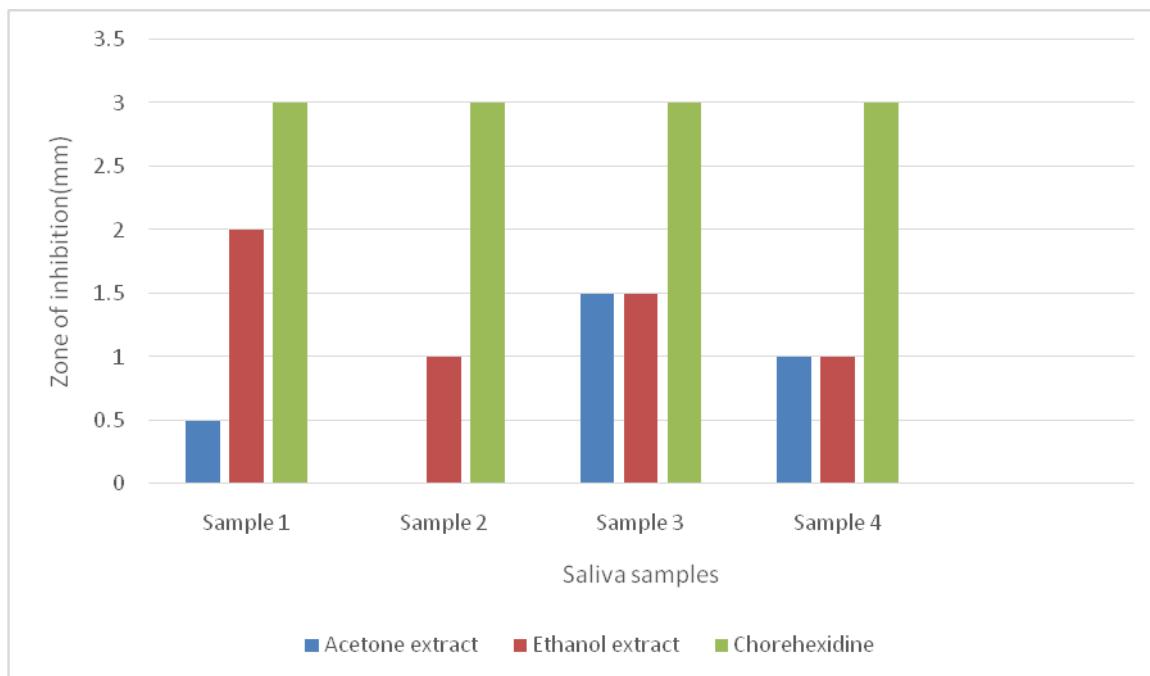
This paper reports the antibacterial activity and the effectiveness of different extracts of the above-mentioned plant against salivary microflora.

It is given in **Table no.1 and Graph no.1**

Table no 1: Zones of inhibition of two different extracts on salivary samples in mm

Sr no.	Acetone extract	Ethanol extract	Chlorhexidine (control group)
1	0.5	2	3
2	Zero	1.0	3
3	1.5	1.5	3
4	1.0	1.0	3

Graph no. 1: Zones of inhibition(mm) of two different extracts against Chlorhexidine



Two different extracts of Artemisia pallens is evaluated for its antimicrobial properties(zone of inhibition) in triplicate and their mean value has been calculated. The gold standard ,0.2% chlorhexidine has been tested for its antimicrobial property in triplicate and mean value will be calculated.

Acetone and ethanol shows 0.5 mm and 2mm zone of inhibition respectively. Ethanol extract represents maximum zone of inhibition compared to acetone extract.Acetone extract shows average Zone of Inhibition (1mm) which is comparable to gold standard.

Ethanol extract had significant inhibitory effect on the growth of microorganisms.

These results are effective against different strains present in the mouth. Further study is needed to check the plant potency against Streptococcus Mutans which is the main organism responsible for caries.

CONCLUSION

This study reveals the antimicrobial properties of Artemisia pallensand thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

The ethanol extract and acetone extract can be used as a therapeutic agent with different forms. We may use the ethanol and Acetone extract as well, but this study indicates that the ethanol extract has a superior antimicrobial activity.

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