

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative evaluation of antimicrobial properties of four different extracts of *Polyalthia Longifolia* (Ashoka) 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. The antibacterial activity of *Polyalthia Longifolia* has been obtained. Hence we undertook a study of *Polyalthia Longifolia* and chlorhexidine against salivary microflora, (deft-3-4) from mixed dentition. Aim was To evaluate and compare antimicrobial properties of *Polyalthia Longifolia* and 0.2%chlorhexidine against acidogenic salivary microflora from mixed dentition age group. Saliva samples were collected by asking the subject to spit in funnel. Microbial inhibition assay was prepared 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 inhibition of growth and were measured in millimetres. The zone of inhibition was measured for extract and 0.2%chlorhexidine. The results confirmed the antimicrobial potential of the plant when comparable with gold standard chlorhexidine.

Key words: Caries, herbel medicines, chlorhexidine, saliva

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INTRODUCTION

Oral health is an important aspect of the overall health of an individual. The diseases produced by a number of micro-organisms are manifested in the oral cavity. Dental caries, prevalence as high as 60-80% in children, is major health problem in India [1]. 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 [2,3]. These research based products come with naturally occurring active ingredients, that achieve the desired antibacterial and anti inflammatory effect [3]. 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 diarrhea. These drawbacks justify the search for new effective anticariogenic compounds that could be employed in caries prevention [4,5].

Polyalthia longifolia is a member of the Annonaceae family and is large genus of shrubs and trees distributed in many tropical countries around the world. It is commonly known as False Ashoka. *Polyalthia Longifolia* var. *Pedula* has been used in traditional system of medicine in the treatment of fever, skin diseases, mouth ulcers, hypertension, helminthiasis, gonorrhoea, uterine ailments, leucorrhoea and menorrhagia.

In this sense, efforts have been made to evaluate & compare the antimicrobial properties of four different extracts of *Polyalthia Longifolia* with 0.2% chlorhexidine gluconate 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. Authentication was performed, Pune, Maharashtra, India.

Criteria for selection of patients

Patients in mixed dentition period in age group of 6–12 years are selected. They should have good general health with no history of antibiotic therapy and use of chemical anti plaque agents prior to six months of study initiation. They should have moderate caries (DMFT=3-4) (modified WHO criteria 2003) [6].

Exclusion criteria

Patients with history of antibiotic and oral drug therapy, chemical anti-plaque agents prior to six months of study initiation, physically and mentally handicapped patients were excluded from the study.

Microbial flora

The saliva samples from the dental caries patients were collected using sterile cotton tipped swabs placed in the floor of the mouth. It was then placed in a sterile container with saline (2 ml) and was used to inoculate on the agar plates.

Antimicrobial assay

The agar well diffusion method was employed. Samples of each acetone, ethyl acetate, chloroform and ethanol extracts (200mg) were dissolved in respective solvents. Sterile 8.0 mm diameter of well were impregnated with different extracts. The salivary flora were 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 millimeters. 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 [8]. 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 [9,10].

In the present study, the plant viz., Polyalthia Longifolia(Ashoka) was selected based upon its traditional medicinal uses in the treatment of oral diseases. It is commonly known as False Ashoka, the Buddha tree, Indian Fir tree, Ashoka or Devadaru in sanskrit, Debdaru in Bengali, Deodar in Hindi, Asopalav (Gujarati), Glodogantiang (Indonesian), devdar in Marathi and NettiLinkam in Tamil(Singh and Karthikeyan, 2000; Kirtikar and Basum, 1995).Traditionally the plant has been used in Bangladesh and India for several medicinal purposes. Literature survey revealed that various parts of the plant possess different biological activities. The plants containing clerodane diterpenoids and alkaloids have found to be active against a wide variety of microorganisms (Faizi et al., 2008)[11]. This paper reports the antibacterial activity and the effectivity of different extracts of the above mentioned plant against salivary microflora.

Four different extracts of Polyalthia Longifolia 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.

The results of the antimicrobial assay of the acetone,ethyl acetate,chloroform and ethanol extracts of Polyalthia Longifolia are presented in **Table No. 1**.

Sr no	Sample	Extract	Zone of inhibition			mean
1.	Sample 1	EA	2	1.5	1.5	1.6
		Chloroform	1	NI	NI	1
		Acetone	0.1	2	1	1
		Ethanol	2	2	2	2
2.	Sample 2	EA	NI	NI	NI	NI
		Chloroform	NI	NI	NI	NI
		Acetone	3	3	2	2.6
		Ethanol	2	1	1	1.3
3.	Sample 3	EA	NI	NI	NI	NI
		Chloroform	NI	NI	NI	NI
		Acetone	1.5	1.5	1.5	1.5
		Ethanol	1	1	NI	0.6
4.	Sample 4	EA	NI	NI	NI	NI
		Chloroform	NI	NI	NI	0.6
		Acetone	2	2	2	2
		Ethanol	1	1	1	1.5

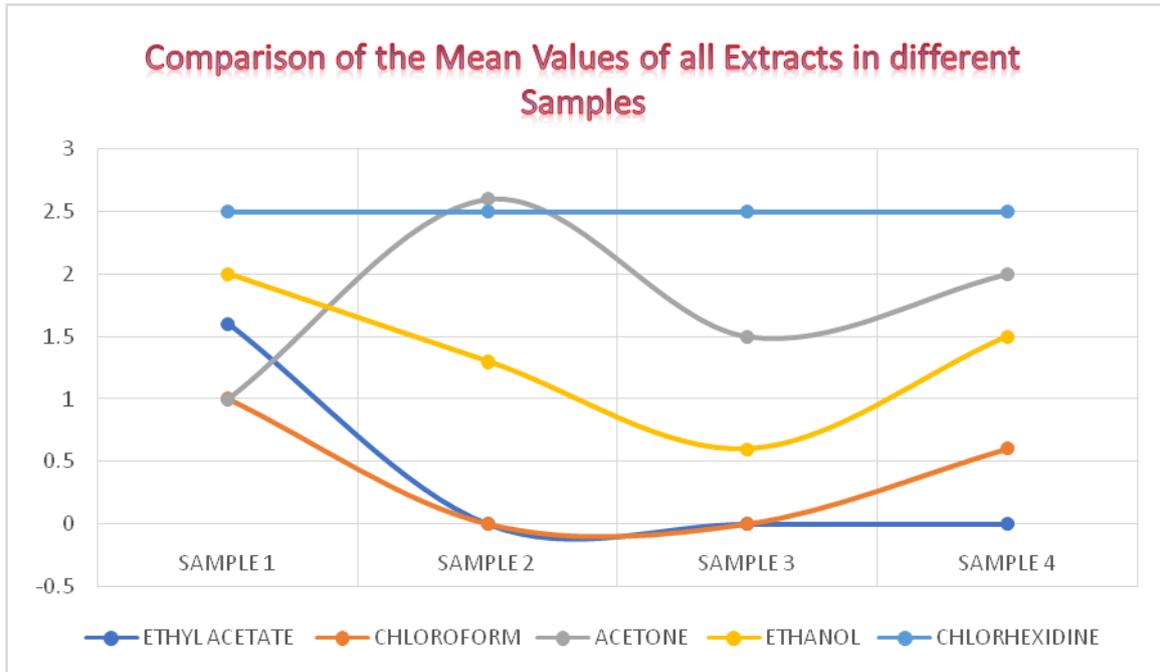
EA, chloroform shows 1.6 and 1mm zone of inhibition respectively. Acetone extract represents maximum zone of inhibition amongst all extracts (2.6mm).Ethanol shows average Zone of Inhibition (2mm) which is comparable to gold standard.

Acetone extract had significant inhibitory effect on the growth of microorganisms. Acetone extract exhibited zones of inhibition against all the tested samples; whereas chloroform extract is active for selected

samples with comparatively smaller zones of inhibition. The same procedure can be followed for four other different concentrations and minimum inhibitory concentration (MIC) can be evaluated.

This 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.

This study compares the antimicrobial properties obtained by a plant and which is easily available to the common man. It may have fewer side effects as it falls in the category of natural medicine. The Acetone extract can be formulated in the form of a mouth wash, or as an intracanal medicament where an antimicrobial agent is required. We may use the ethanol and acetate extract as well, but this study indicates that the acetone extract has a superior antimicrobial activity.



CONCLUSION

The results of the present work, and for its inexpensive cost, show that the use of Polyalthia Longifolia can help developing countries limit oral infections as an effective antibacterial agent. Thus acetone

extract of *Polyalthia Longifolia* can be used efficaciously in various chemotherapeutic agents to suppress the growth of oral bacteria and cariogenic pathogens.

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