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Comparative evaluation of antimicrobial properties of Five different extracts of *Amaranthus cruentus* (Lal Math) and 0.2% chlorhexidine against acidogenic salivary microflora in mixed dentition age group.

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ABSTRACT

Herbal medicines are in great demand for primary health care. *Amaranthuscruentus* is an important medicinal plant. The extracts of this plant were found to possess anti-inflammatory, anti-oxidant activities. Hence investigating antimicrobial activity against salivary microflora of caries active children in mixed dentition age group (deft 3-4). Chlorhexidine has often been used as a positive control during assessment of anticariogenic potential of other agents. Aim is to evaluate and compare antimicrobial properties of *Amaranthuscruentus* 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 compared with gold standard chlorhexidine.

Keywords: *Amaranthus cruentus*, chlorhexidine, antimicrobial, dentition

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INTRODUCTION

Herbal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. However, during this time of transition, the synthesized, purified or extracted active ingredients of pharmaceutical drugs were observed to exhibit significant adverse side effects. The potential risk of using synthetic form of phytochemicals has been reported [1].

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. In recent years, prevalence of dental caries in most western countries is steadily declined. In the oral cavity, saliva serves as a reservoir for normal commensals as well as pathogenic micro flora causing infectious diseases. Dental decay is a chemo-parasitic process in which the oral microorganisms play a very pivotal role. For prophylactic purposes, it seems reasonable to target processes involved in formation of single or mixed bacterial communities that have the potential to cause or favor initiation of dental caries, without perturbing the balance of the normal flora. [2]

About the plant

- a) Name of plant - Lalmath
- b) Common names – Red Amaranth, African-spinach, blood amaranth, bush greens, caterpillar amaranth, grain amaranth, purple amaranth
- c) Scientific name – *Amaranthus cruentus*
- d) Family- Amaranthaceae (Amaranth family)
- e) Origin- Mediterranean

Amaranth is a tall annual herb topped with clusters of dark pink flowers. The *Amaranthus cruentus* from the family Amaranthaceae is commonly found in India. The amaranth grain is prized due to its amino-acid composition [3], superior to conventional grains. Besides, Amaranth seed contains considerable amounts of mineral salts, vitamins and fiber [4], oil rich in unsaturated fatty acids [5] and squalene [6], polyphenols, anthocyanins and flavonoids [7], tocopherols and tocotrienols [8]. The amaranth seed positively affects treatment of hypercholesterolemia in animal studies [9], or nutrition of people with celiac disease [10], although this is disputable since there are no clinical research plainly affirming influence amaranth products on their state of health. Apart from high nutritional value of its grain, antioxidant activity of different Amaranth species (*Amaranthus cruentus* and *Amaranthus blitum* [11], *Amaranthus hypochondriacus* [7], *Amaranthus caudatus* [6]) seed is known.

METHODS

Inclusion criteria

Patients of 6-12 year-old in mixed dentition age group with moderate caries (decayed, missing, filled teeth=3-4) (modified WHO criteria 2003) having good general health. [12]

Exclusion criteria

Patients with a history of antibiotic and oral drug therapy, chemical anti-plaque agents prior to 6 months of study initiation, physically and mentally handicapped patients were excluded from the study. [12]

Plant extracts

Plant materials used in this study were procured from the local market of Pune, Maharashtra, India.

Saliva collection

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 (3 ml) was collected in vial. 3

samples were collected in the early morning time. These salivary samples were diluted (3:1) in a sterile vial containing 1 ml of normal saline and were used to inoculate on the agar plates. [12]

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 different extracts.

Adequate amount of Muller-Hinton Agar was dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1 ml) 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 (8.0 mm). The well was filled with different extract and plates were incubated at 37±0°C for 24 hrs. After incubation, the plates were observed for zones of inhibition of growth and the diameters of these zones were measured in millimeters using bacterial inhibition zone reading scale.[12]

RESULTS AND DISCUSSION

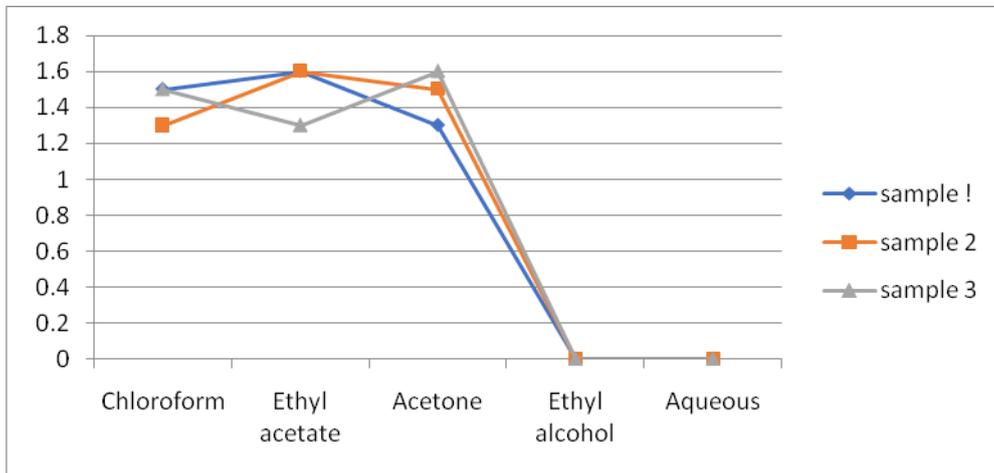
The experiments are performed with various extracts of the *Amaranthuscruentus*. The results depict that all the extracts have marked activity against the tested microorganisms. Results of test samples are reported after twenty four hours and indicate its dose dependent activity. The results of antimicrobial assay of *Amaranthuscruentus* of Chloroform, ethyl acetate, acetone, ethyl alcohol, aqueous is given in table 1.

Sr no	Sample	Extract	Zone of inhibition (mm)			mean (mm)
1.	Sample 1	Chloroform	1.5	1.5	1.5	1.5
		Ethyl acetate	2	1	2	1.6
		Acetone	1	1.5	1.5	1.3
		Ethyl alcohol	Nil	Nil	Nil	Nil
		Aqueous	Nil	Nil	Nil	Nil
2.	Sample 2	Chloroform	1	1.5	1.5	1.3
		Ethyl acetate	1	2	2	1.6
		Acetone	1.5	1.5	1.5	1.5
		Ethyl alcohol	Nil	Nil	Nil	Nil
		Aqueous	Nil	Nil	Nil	Nil
3.	Sample 3	Chloroform	1.5	1.5	1.5	1.5
		Ethyl acetate	1	1.5	1.5	1.3
		Acetone	2	1	2	1.6
		Ethyl alcohol	Nil	Nil	Nil	Nil
		Aqueous	Nil	Nil	Nil	Nil

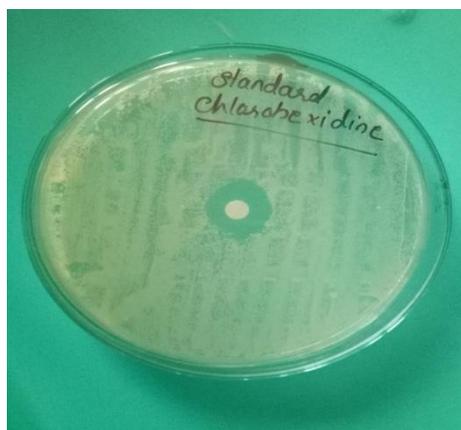
The mean value of average zone of inhibition of active compound of *Amaranthuscruentus* is 1.5 mm in three different extracts viz, Chloroform, ethyl acetate, acetone as compared to 0.2% chlorhexidine in which the zone of inhibition is 3mm in five salivary samples. The extract of *Amaranthuscruentus* ethyl alcohol, aqueous has shown very less zone of inhibition as compared to chloroform, ethyl acetate, acetone. The extract of *Amaranthuscruentus* used in this study is without any additives has shown zone of inhibition against acidogenic salivary microflora. Thus concluding antimicrobial property of *Amaranthuscruentus* it can be used in various oral health prevention with increased concentration and additives.

Primary prevention among children and adolescents plays an important role in India, because of high population and wide social- economic disparities amongst its population [10]. Oral and dental health awareness has improved tremendously over the last century but the prevalence of dental caries in children remains a significant clinical hurdle [11].

Dental caries and root canal infections being polymicrobial in nature [12] can be delayed in their process via using susceptible antimicrobial agent which acts against specific bacteria respectively. Though, Chlorhexidine is used as standard antimicrobial drugs for such use and hindering the carious process [12], medicinal plants like *Amaranthus creuntus* due to their herbal properties have the dual action of having fewer side effects actively pronounced by the chemical agents which are the prime ingredients of chlorhexidine as well as being an effective antimicrobial product [12].



Amaranthus creuntus



Chlorhexidine

CONCLUSION

The resistance in many dental pathogens to currently used antibiotic drugs is ever increasing. The extract derived from *Amaranthus cruentus* plants used in this study are herbal, they are eco-friendly and do not produce any side-effects as well as are effective and economical, when compared to the synthetic drugs. This study has confirmed the antimicrobial potentials of the plant without any additives. The extract of *Amaranthus cruentus* can be used with increased concentration as intracanal medicaments, mouth wash, tooth paste etc. Thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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