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Research Article

EVALUATION OF ANTIBACTERIAL EFFICACY OF *CHIA* (*SALVIA HISPANICA*) SEEDS EXTRACT AGAINST PORPHYROMONAS GINGIVALIS, FUSOBACTERIUM NUCLEATUM AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS-AN IN-VITRO STUDY

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ABSTRACT

Background: *Chia* (*Salvia hispanica* L.) seeds are used widely because of its nutritional value, antioxidant and antibacterial activity against various microorganisms but there are no studies that relate the antibacterial activity of *Chia* seeds against periodontal pathogens. Hence, this study aimed to determine the antimicrobial activity of *Chia* seeds extract on Porphyromonas gingivalis, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans. **Materials and Methods:** Extract of *Chia* seeds in ethanol and distilled water were obtained by cold maceration. The crude residue was obtained by evaporation at room temperature. The antimicrobial efficacy of *Chia* seeds in all extracts were assessed against oral microorganisms by finding out zone of inhibition and minimal inhibitory concentration. **Results**: *Chia* seeds (*Salvia hispanica*) in ethanol and distilled water were found to be effective against A.actinomycetamcmitans, P.gingivalis and F.nucleatum by highest zone of inhibition being 18mm for aqueous extract of *Chia* seeds against A.actinomycetamcomitans. Minimum inhibitory concentration for aqueous extract of *Chia* seeds for A.actinomycetamcomitans and P.gingivalis was established at 50% and for F.nucleatum at 12.5%.The minimum inhibitory concentration of the ethanolic extract of *Chia* seeds for A.actinomycetamcomitans, P.gingivali and F.nucleatum was 12.5%, 6.25% and 50 % respectively. Conclusion: From results of the study, it is suggested that an ethanolic and aqueous extract of *Chia* seeds (*S.hispanica*) has antimicrobial activity against P. gingivalis A. actinomycetemcomitans and F.nucleatum.

KEYWORDS: *Chia* seeds, Aggregatibacter actinomycetemcomitans; Porphyromonas gingivalis; Fusobacterium nucleatum, Salvia hispanica.

INTRODUCTION

Periodontal diseases have been considered as one of the major health problems affecting humans.[1] Periodontal disease initiated by pathogenic bacteria like *Porphyromonas gingivalis, Fusobacterium species* causes infiltration of Polymorphonuclear leucocytes. The PMNs engaged in phagocytosis release Reactive Oxygen Species (ROS) which can result in an imbalance between production of ROS and antioxidant defense mechanism thus increasing oxidative stress.[2] Thus, the main treatment target remains the effective reduction of the supra- and subgingival pathogenic flora, mostly by mechanical means and by variety of chemical means, like antibiotics and antiseptics as an adjunct to mechanical therapy but they are known to cause antimicrobial resistance and emergence of uncommon infections probably due to the inappropriate or widespread overuse of antimicrobials.[3-5] Hence there is search for newer and safer adjunctive therapeutic agents which has both antimicrobial and antioxidant property to combat periodontal disease.

There has been a change in thinking globally, with a growing tendency to "GO NATURAL".[6] 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, many based on their use in traditional medicine.[7] These plant-based traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care and still hold their own unique place, by showing no side effects. [8,9]

Salvia hispanica L. a biannually cultivated plant, is categorized under the mint family (*Labiatae)*, super division of *Spermatophyta*, and kingdom of *Plantae, n*ative to central and southern Mexico, Guatemala and India. It is prominently grown for its *Chia* seeds, a nutritional powerhouse packed with 25% to 40% oil with 60% of it comprising (omega) ω-3 alpha-linolenic acid and 20% of (omega) ω-6 linoleic acid, protein (15–25%), fats (30-33%), carbohydrates (26–41%), high dietary fiber (18-30%), ash (4-5%), minerals, vitamins, and dry matter (90-93%). Another key feature of *Chia* seed is that it does not contain gluten.[10] The available human and non-human studies showed possible effectiveness for allergies, athletic performance enhancement, cancer, coronary heart disease (CHD), endocrine disorders, hyperlipidemia, hypertension and stroke. Some evidence also suggests possible anticoagulant, antioxidant and antiviral effects of Salvia hispanica.[11] *Chia* seed products also have been springing up in the market over the past few years.

However reports are not available on anti-microbial property of *Chia* seeds against oral microbiota.

Hence the present study was planned to evaluate antimicrobial efficacy with the objective to evaluate the minimum inhibitory concentration and zone of inhibition of *Chia* (*Salvia hispanica*) seeds extract in ethanol and distilled water against Porphyromonas gingivalis, Fusobacterium nucleatum and *Aggregatibacter* actinomycetemcomitans. The null hypothesis was ethanolic and aqueous extract of *Chia* (*Salvia hispanica*) seeds has no antibacterial effect against Porphyromonas gingivalis, Fusobacterium nucleatum and *Aggregatibacter* actinomycetemcomitans.

MATERIALS AND METHODS

Study design

Experimental design. In- vitro study. Laboratory setting.

Method

The methodology for preparation of extract was adopted from Agarwal P.[12]

Collection of the samples and drying the plant material

Salvia hispanica L species was identified based on the taxonomic features by a botanist. The seeds of the plants were separated, washed with sterile water and shade dried. The dried seeds were then pulverized to coarse powder using a sterilized mixer grinder and stored in air tight bottle.

Preparation of Extract

100 grams of the seeds powder was weighed and macerated in 600 ml of distilled water in a sterile glass container for aqueous extract and similarly 100 grams of seeds powder were weighed and macerated in 600ml of ethanol in a sterile glass container for ethanolic extract respectively. Cold maceration was performed for two days by shaking at regular intervals. It was subjected to filtration using Whatman filter paper to obtain a clear filtrate. This was kept on a water bath set at 60^{o} C to obtain crude extract of *Chia* seeds.

Stock solution

Stock solution of ethanolic *Chia* (*Salvia hispanica* L.) seeds extract and aqueous *Salvia hispanica* L (Chia) seeds extract was prepared. The stock solution was stored in a sterile container and used for microbiological procedures.

Cultivation of microorganisms

Blood agar media with hemin, vitamin K and Kanamycin was used to culture Porphyromonas gingivalis and crystal violet erythromycin blood agar was used to culture Fusobacterium nucleatum and Thiyoglycollate broth with 1% horse serum was used to culture *Aggregatibacter* actinomycetemcomitans

Assessment of antibacterial activity

The antibacterial activity of extracts was evaluated by using the disc diffusion method and the Minimum Inhibitory Concentration (MIC) of the test solutions was determined by the tube dilution method. Double dilution was made from a higher dilution to a lower dilution in a series of test tubes. The MIC values of the test extracts were compared against the MIC of 0.2% chlorhexidine solution (positive control) against Porphyromonas gingivalis, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans respectively.

RESULTS

The results of aqueous and ethanolic extract of chia seeds (*S.hispanica*) are presented in table 1 and table 2. From the data presented in Table 1, the minimum inhibitory concentration of aqueous extract of chia seeds for A.actinomycetamcomitans was established at 50% and ethanolic extract at 12.5%. The minimum inhibitory concentration of the aqueous extract of chia seeds for P.gingivalis, was at 50 % and ethanolic extract was at 6.25%. For F.nucleatum, the minimum inhibitory concentration of the aqueous extract of chia seeds was at 12.5% and ethanolic extract at 50%.

From the data presented in Table 2, the aqueous and ethanolic extracts of chia seeds (S.hispanica) showed antimicrobial inhibitory activity against all three microorganisms In aqueous extract of chia seeds (S.hispanica), highest zone of inhibition was found at 18 mm for A.actinomycetamcmitans, 15 mm for P.gingivalis and 13 mm for F.nucleatum. In ethanolic extract of chia seeds (S.hispanica), zone of inhibition was found at 14 mm for A.actinomycetamcomitans, 15 mm for P.gingivalis and 16 mm for F.nucleatum.

Table 1: The Minimum Inhibitory Concentration of Distilled Water and Ethanolic Extracts of Salvia Hispanica Extraxt Against Selected Oral Microorganisms on Specific Media, Determined by Serial Tube Dilution Method and Compared with the Standards

Microorganism	Samples	Concentration to which test agent was diluted									
		100%	50%	25%	12.5%	6.25%	3.12%	1.6%	0.8%	0.4%	0.2%
A.actinomy-cetamcomitans	*Chia* seeds (Aqueous)	S	S	R	R	R	R	R	R	R	R
	Chia seeds (Ethanol)	S	S	S	S	R	R	R	R	R	R
	0.2% CHX	S	S	S	S	S	S	R	R	R	R
P.gingivalis	*Chia* seeds (Aqueous)	S	S	R	R	R	R	R	R	R	R
	Chia seeds (Ethanol)	S	S	S	S	S	R	R	R	R	R
	0.2% CHX	S	S	S	S	S	R	R	R	R	R
F.nucleatum	*Chia* seeds (Aqueous)	S	S	S	S	R	R	R	R	R	R
	Chia seeds (Ethanol)	S	S	R	R	R	R	R	R	R	R
	0.2% CHX	S	S	S	S	S	R	R	R	R	R

S = Sensitive; R = Resistant

Table 2: Zone of Inhibition (In Millimeters) for Different Volumes of Aqueous and Ethanolic Extracts of S.Hispanica (Chia Seeds). Compared to Positive Controls

Microorganisms	Type of extract	Volume of test agent in each well					0.2% Chlor hexidine
		75 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	
A.Actinomycetam-comitans	*Chia* seed extract (Aqueous)	18mm	13mm	10mm	R	R	17mm
	Chia seed extract (Alcoholic)	14mm	12mm	9mm	5mm	R	
P.gingivalis	*Chia* seed extract (Aqueous)	15mm	12mm	8mm	R	R	15mm
	Chia seed extract (Alcoholic)	15mm	8mm	R	R	R	
F.nucleatum	*Chia* seed extract (Aqueous)	13mm	11mm	8mm	R	R	16mm
	Chia seed extract (Alcoholic)	16mm	10mm	9mm	7mm	4mm	

S = Sensitive; R = Resistant

DISCUSSION

Most oral diseases are primarily caused, or at least modified, by bacteria that inhabit the oral cavity. Among them, caries and periodontitis represent the most common pathological conditions, which are caused by the biofilm-forming bacteria and yeasts of dental plaque. Accordingly, most of the prophylactic and therapeutic interventions aim to reduce the bacterial load in such a way that oral health can be maintained or obtained. Antimicrobials are useful to support these efforts by effectively inhibiting the formation or removing established biofilms. [13]

However synthetic antimicrobial agents have resulted in considerable side effects, antimicrobial resistance and the emergence of previously uncommon infections owing to their improper usage. Instead, herbal extracts may prove to be better and safer alternatives if they are supported by scientific-based evidence. Several common natural products like lemon peel and Tulsi have been tested for their antibacterial properties. [14,15]

Chia seeds has shown antioxidant, antiviral, anticoagulant and antineoplastic property however studies pertaining to antimicrobial property is limited.In the present study the invitro antimicrobial property of aqueous and ethanolic extracts of *S hispanica* L. (*Chia* seeds extract) was assessed against P.gingivalis, F.nucleatum and A.actinomycetamcomitans. Both aqueous and ethanolic extracts were effective against all the three microorganisms. Cold maceration was selected to prepare *S.hispanica* L (*Chia* seeds extract) extract to ensure that the heat sensitive active agents present in A. coynei are not degraded during the extraction process.

The present study shows that minimum inhibitory concentration of ethanolic extract of S.hispanica (*Chia* seeds) against A.actinomycetamcomitans and P.gingivalis were exhibited at lower concentrations (i.e. at 12.5 % and 6.25%) and for F.nucleatum species, aqueous extract of S.hispanica (*Chia* seeds) showed Minimum inhibitory concentration at lower concentrations (i.e.12.5%).

The antimicrobial efficacy of S.hispanica in aqueous and ethanolic extracts showed Zone of inhibition against all the three microbial organisms (A.actinomy-cetamcomitans, P.gingivalis and F.nucleatum) which is almost similar to the positive control (0.2% Chlor-hexidine).

The antimicrobial action of chia seeds probably is due to its composition. It has approximately 30% oil, which is reported to contain 17-26% linoleic acid and 50-57% linolenic acid. *Chia* seeds also reported to contain significant quantities of macro and micronutrients like protein content is approximately 23.4% and also it contains calcium, iron, potassium, vitamin C, magnesium, folate, vitamins B, zinc, selenium, and vitamin A.[16] The presence of antioxidants like omega 3 fatty acids are proven to show antimicrobial effect. The antimicrobial activities can be enhanced if the photoactive components are purified and its adequate dosage is determined for proper administration.

CONCLUSION

In the present study, disc diffusion test demonstrated antibacterial effect of *Chia* (*S.hispanica*) seeds extract against all the three main pathogens of periodontal disease (i.e. A.actinomycetamcomitans, F.nucleatum and P.gingivalis). Similarly, in micro dilution assay test, both the aqueous and ethanolic extract of S.hispanica exhibited antibacterial effect against at A.actinomycetamcomitans, F.nucleatum and P.gingivalis. Further in vitro and in vivo studies should be conducted to know the effectiveness of *Chia* seeds extract as a tooth paste, gel or mouth rinse to know the ability of Salvia hispanica to prevent oral infections. Hence, if proven effective, it can be a very useful indigenous antimicrobial agent for periodontal infections.

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Figure 1: *Chia* Seeds

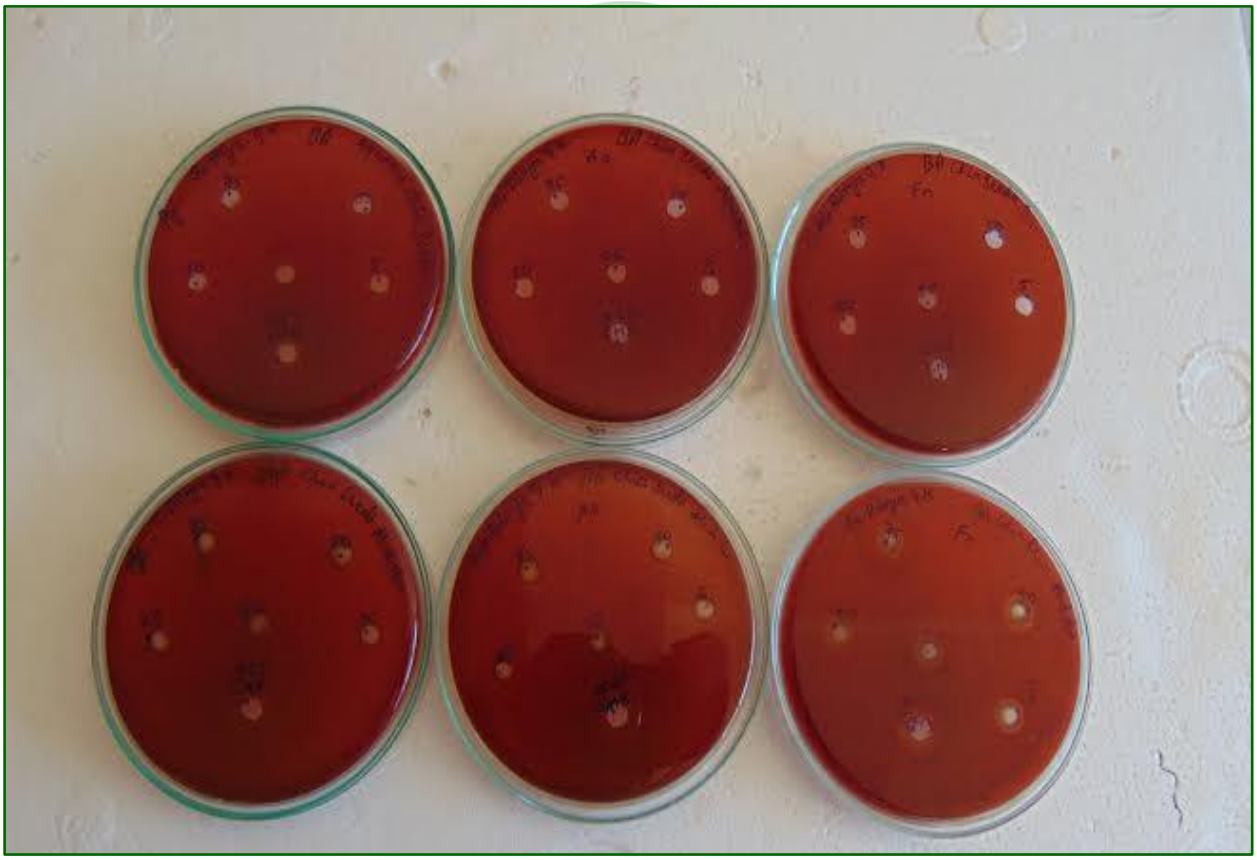

Figure 2: Zone of Inhibition (in Millimeters) for Different Volumes of Aqueous and Ethanolic Extracts of S.Hispanica (*Chia* Seeds). Compared to Positive Controls