Periodic Research

Evaluation of Antimicrobial effect of Allium cepa and Zingiber officinale on Streptococcus mutans isolated from Dental Caries of humans

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Abstract

Dental caries is a localized, transmissible, pathological infection process that ends up in the destruction of hard dental tissue. *Streptococcus mutans* is considered to be the principle etiological agent of dental caries. Numerous reports have shown the close relationship between higher incidence of *S. mutans* and dental caries.

Aim: The present study renders few easily available household medicinal plants like onion bulb*(Allium cepa)* and ginger rhizome (*Zingiber officinale*) to the field of dentistry which can be used as alternative medicines and adjunct to conventional therapy in dental caries prevention.

Materials and Method: In the present study *Streptococcus mutans (S. mutans*) was tested for its antimicrobial susceptibility against both Aqueous and Methanolic plant extracts of onion bulb and ginger rhizome . The antimicrobial activity of plant extracts were assayed using agar well diffusion method .

Results: The results showed significantly maximum antimicrobial effects with Methanolic ginger extract against *S. mutans* (zone of inhibition,mean±s.d.,19.2±1.1mm at 50% concentration;15.45±0.99mm at 25% conc.;and 11.04±0.48mm at 12.5% concentration) when compared with 32% concentration of Ampicilline(18.84±0.53mm).

The antimicrobial activity by Aqueous ginger extract, Methanolic onion extract & Aqueous onion extract were 16.06±0.08mm,13.34±0.24mm,10.37±0.65mm at 50% concentration; and 11.97±0.03mm,10.41±0.37mm,8.49±0.07mm at 25% concentration respectively seen against *S.mutans* , whereas at 12.5% concentration these extracts did not exhibited any zone of inhibition except Methanolic ginger extract(11.04±0.49mm).

Conclusion: This study provides evidence for the presence of one or more soluble constituents in these plants parts used,that probably interferes with bacterial growth and their metabolism which would prevent and inhibit initiation and progression of dental caries .So that consumption of these two herbs in everyday diet should be recommended.

Keyword: antimicrobial activity, aqueous and Methanolic plant extracts, dental caries.

Introduction

Worldwide, Dental Caries has been arised as a major global oral health problem in last two –three decades. Dental caries is a localized and transmissible pathological infectious problem with microbial origin that produces the destruction of the hard dental tissue1. This tooth disease caused by the complex interaction of food, especially starches and sugars, with the bacteria that form dental plaque. The term also refers to the tooth cavities that result from the disease. Plaque bacteria produce acids that cause demineralization of enamel and enzymes that attack the protein component of the tooth. This process, if untreated, ultimately leads to the formation of deep cavities and bacterial infection of the pulp chamber, which contains blood vessels and nerves. . The development of dental caries in a debilitated patient is a concern because of the danger that infections of the teeth or gingival tissues may spread to the rest of the body. In addition, teeth that are decayed or painful inhibit mastication and can lead to dietary changes, which may in turn cause nutritional and

digestive disorders and is also associated with oral bacterial infections, endocarditis and septicemia.

Streptococcus mutans is a Gram positive cocci arranged in chains, belongs to the viridians group of streptococci and producing Alpha-haemolysis on blood agar..It is an acidogenic and aciduric microorganism colonizing the oral cavity by making a biofilm, is considered to be the main cause of dental caries.[2] The cariogenecity of this bacterium is associated with various factors including dextran production, production of high concentration of acid in the plaque and glycosial transferage activity. As glycan is the main component of dental biofilm and is directly proportional to the production of glycosyl transferase by *S.mutans*. Therefore *S. mutans* is a most common pathogen isolated from human dental plaque and its prevalence has been reported.[3,4]Different studies have shown a correlation between counts of *S. mutans* in the oral cavity and both the prevalence and incidence of caries.[2,5,6] In addition to dental caries and related pyogenic dental infection, *S. mutans* is also a very important endocarditis agent.[7,8]

Plants as sources of medicinal compounds have continued to play a predominant role in the maintenance of human health since ancient times. More than 80% of the world's population relies on traditional medicine for their primary healthcare needs and over 50% of all modern origin and natural products play an important role in drug development programs in the pharmaceutical industry. So trend is shifting towards naturopathy clinical drugs are of natural product. The use of Synthetic dentifrices with antimicrobial properties is known to produce harmful side effects on prolonged use. Hence, dentifrices and mouth rinses that contain extracts of medicinal plants and herbs are becoming popular.[9,10] To cope with the wide-spread problem of antimicrobial resistance, a number of strategies such as reduced antimicrobials use and antimicrobials alternatives have been proposed.[11,12,13] So the use of natural products for the control of oral diseases is considered as an interesting alternative to synthetic antimicrobials due to their lower negative impact.[14] and to overcome intrinsic (primary) resistance or secondary resistance to the drug during therapy.[15]

The present study renders few easily available household medicinal plants like onion and zinger to the field of dentistry which can be used as alternative medicines and adjunct to conventional therapy which will be a great help in developing countries with financial constraints and limited oral health care facilities for populations.

The Onion is one of the oldest cultivated vegetables in history. It is thought that bulbs from the onion family have been utilized as a food source for Millennia. Onion consists of its herbaceous plant part and its edible bulb part. It is probably a native to southwestern Asia.[16] The leaves are bluish –green and hollow. The bulbs are large, fleshy and firm. There are three main varieties- white, red and purple skinned.[17] The relative pungency of onion has both genetic and environmental components. **Sulphur** compounds in onions have also been shown to be **anti-inflammatory** both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). **Thiosulfinates** condition anti-thrombotic benefits, including antioxidant activity,[18,19] reduced serum cholesterol and enhance in vitro platelet activity.[20] This later effect is important for cardiovascular health by reducing the probability that platelets aggregate in the blood, a major cause of heart attacks and strokes.[21]Hence, thiosulphinates found in onion have been shown to inhibit in-vitro platelet aggregation.[22,23]**Flavonoids** are a second class of health enhancing compound produced by onions, an example is quercetin. Flavonoids are chemical compounds active against microorganisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of microorganisms.[24]

Ginger, *Zingiber officinale* is a common household spice originated from Southeast Asia; a city with its Sanskrit name *Shunti* was already in existence in 200 B. C. Ginger is also called as "*The Great Medicament*" in Ayurvedic medicines.[25] It belongs to family Zingiberaceae and is a perennial plant with thick tuberous rhizome which are the medicinally useful part of this plant. The medicinal history of ginger has been extensively searched throughout the world and found to possess **anti-inflammatory, cholesterol-lowering, and antithrombotic properties.**[25,26] . Important secondary metabolites present in the rhizome are **curcumene**, non-volatile hydroxyaryl compounds e.g**. zingerone**, **gingeroles and shogaoles (phenylalkanones),** volatile **sesquiterpenes (e.g. zingiberene and bisabolene) and mono-terpenoids (e.g. citral).**[27] Although, the antimicrobial activity and chemical analysis of essential oil and olioresins of this plant has been investigated**.**[28] (Singh,G.,2008).

Therefore, the present study has been designed to

1) **Isolate the *Streptococcus mutants* from the patients having Dental Caries.**
2) **Assess the antimicrobial potential of crude extracts of *Allium cepa and Zingiber officinale* against selected bacterial pathogen**.

Material and Method

In this experimental study, a total 50 subjects were selected from the Dental OPD of GM Hospital, Rewa Town during March to May 2013.

Criteria for selection of patients The selected subjects were generally in good health; individuals with diabetes, autoimmune disorders, or other disorders were excluded. All subjects had untreated dental caries.

Sampling of Carious bacteria Microbiological sampling was carried out by inserting and then rubbing sterile paper points to the bottom of the carious tooth for 10 seconds. Those Paper points were immediately placed in a screw capped vial containing 5 ml sterile Nutrient Broth and transported to the laboratory and kept under aerobic conditions at 37ºC for 24 h. Bacteria from Nutrient broth were then sub-cultured on Sheep blood-agar plates and inoculated plates were again placed in aerobic

condition at 37^{o}C for 48-72h. After 48h incubation, the green pigmented colonies showed alpha- hemolysis were subcultured for achieving of pure culture and identification (morphologically by Gram's staining and biochemically through standard biochemical test).

Extraction of The Plant Materials

Onion and Ginger Extraction

The onions and gingers were washed with clean sterile distilled water and allowed to air dry for one hour. The outer covering of the onions and gingers were manually peeled off. The onion bulbs and ginger rhizomes being separated were washed and extracted in the following ways:29

1. **Aqueous Extraction**

Exactly 200gms of fresh onion bulbs and 200gms of fresh ginger rhizomes were blended into fine pulp and soaked in 100mls of distilled water for 24hrs. The pulps obtained were left in the clean, sterile glass containers and shaken vigorously at room temperature for 24 h on shaker with 150 rpm for proper extraction. Solutions were filtered through muslin cloth and then refiltered by passing through Whatman Filter No. 1. The filtrates thus obtained were concentrated by complete evaporation (air dried) of water at room temperature to yield the pure extract and stored below ambient temperature until use.

2. **Organic Solvent Extraction -**

Exactly 200g of fresh onion bulbs and 200gms of ginger rhizomes were blended and soaked in 100mls of 95% methanol for 24hrs. The pulps obtained were left in the clean, sterile glass containers and shaken vigorously at room temperature for 24 h on shaker with 150 rpm for proper extraction. Solutions were filtered through muslin cloth and then refiltered by passing through Whatman Filter No. 1. The filtrates thus obtained were concentrated by complete evaporation (air dried) of solvent at room temperature to yield the pure extract and stored below ambient temperature until use.

Preparation of different concentrations w/v (50%, 25% and 12.5%) of onion and ginger extract: 50 mg of medicinal herb extract (onion or ginger) mixed with 100 ml of distilled water in a tube gives a 50% concentration. 25 mg of medicinal herb mixed with 100 ml of distilled water gives a 25 % concentration. 12.5 mg of medicinal herb mixed with 100 ml of distilled water gives a 12.5 % concentration.[30]

In the same way, 50%, 25% and 12.5% concentrations of both herbs with 95% methanol were also prepared.

Antibacterial assay

Antibacterial activities of all aqueous and organic extracts of bulb of *Allium cepa* and rhizome of *Zingiber officinale* were determined by standard agar well diffusion assay.[31] Petri dishes (100 mm) containing 18 ml of Mueller Hinton Agar (MHA) seeded with 100 µl inoculum of bacterial strain, inoculum size was adjusted (Exactly 0.5 McFarland standard) so as to deliver a final inoculum of approximately 10^{8} CFU/ml).[32] Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized cup-borer. 50 µl of each extract was poured in the respective well and the plates were incubated at 37°C for overnight. Distilled water and Organic solvent(95% methanol) were used as nega tive control in aqueous and alcoholic plates respectively while Ampicilline antibiotic (32 %) was used as positive control. Antibacterial activity in terms of zones of inhibition (mm) was recorded after 24 h. of incubation at 37^{o}C under aerobic condition. The diameter of the zone of growth inhibition around each well was measured.

Observations

The present study involved 50 Patients attending Dental OPD of GM Hospital, Rewa town with complaints of Dental caries.

Following observations were obtained from this study.

Table No. 1

Incidence of Dental Caries with S.mutans infection in different gender

Male	Percent	Female	Percent
20	40%	30	60%

Table No. 2

Incidence of Dental Caries with S.mutans infection in different age groups.

S. No.	Age group(in years)	No. of patients	Percentage
1	0-15	5	10%
2	16-30	12	24%
3	31-45	25	50%
4	46-60	8	16%
5	61-75	-	0%

Table No. 3

Occurance of Dental Caries with S.mutans infection in the patients with different dietary habits.

Vegetarian	%	Non Vegetarian	%	Vege. And Tobacco user	%	Non Vege.And Tobacco user	%
20	40 %	10	20 %	20	40 %	0	0 %

Table No. 4

Sensitivity pattern of aqueous extract of Onion bulb (Allium cepa) against S.mutans isolated from Dental Caries.

No. of isolates from carious patients(n= 50)	Zone of Inhibition in mm				
	Concentration(in %) of Aqueous extract of Onion bulb				
	50%	25%	12.5%	Distilled water(negative control)	32% Ampicilline (positive control)
mean±S.D.	10.37±0.65	8.49±0.072	0.0	0.0	18.84±0.53

S.D=Standared deviation

Table No. 5

Sensitivity pattern of aqueous extract of Ginger Rhizome (Zingiber officinale) against S.mutans isolated from Dental Caries.

No. of isolates from carious patients (n=50)	Zone of inhibition in mm				
	Concentration(in %) of Aqueous extract of Ginger Rhizome				
	50%	25%	12.5 %	Distilled water(negative control	32% Ampicilline (positive control)
Mean±S.D	16.06±0.08	11.97±0.03	0.0	0.0	18.84±0.53

Table No.6

Sensitivity pattern of Methanolic extract of Onion bulb(Allium cepa) against S.mutans isolated from Dental Caries.

No. of isolates from carious patients (n=50)	Zone of inhibition in mm				
	Concentration(in %) of Methanolic extract of Ginger Rhizome				
	50%	25%	12.5 %	95% Methanol (negative control	32% Ampicilline (positive control)
Mean±S.D	13.34±0.24	10.41±0.37	0.0	≤ 8	18.84±0.53

Table No.7

Sensitivity pattern of Methanolic extract of Ginger Rhizome (Zingiber officinale) against S.mutans isolated from Dental Caries.

No. of isolates from carious patients (n=50)	Zone of inhibition in mm				
	Concentration(in %) of Methanolic extract of Ginger Rhizome				
	50%	25%	12.5%	95% Methanol (negative control	32% Ampicilline (positive control)
Mean±S.D	19.2±1.1	15.45±0.99	11.04±0.49	≤ 8	18.84±0.53

Results and discussion

The present study, analyzed the number of persons affected with dental caries in different gender, age, dietary habit, among the patients studied. The occurrence of dental caries was found to be slightly higher in females (60%) than in males(40%) **(Table 1)** suggesting that females are more prone to caries than males.[33] This can be attributed to number of facts, including early teeth eruption in girls in comparison to boys, differences in dental attendance due to lack of financial independence on the part of females and fear of dentist among male and female and also to difference in dietary pattern between housewives and working men.[34] This can also be well correlated with the fact that, persons who experience caries first, usually become aware for the prevention of caries. In Indian scenario females are still not in contact with this information due to male dominating society and lack of financial independence among females. But, it has been reported that, condition of oral and dental health is relatively improving in developing countries. Dental caries is a multi-factorial disease influenced by many factors including age. The incidence among different age group shown the highest occurrence of dental caries among 31–45 years (50%) population followed by16-30years(24%) population**(Table 2).** It might be suggested that, the development of dental caries is a long term process, and the habit of sugar consumption is relatively high among the teenagers (In the form of chocolates and other sticky sugar rich food), but the peoples usually don't attend the hospitals, until and unless they feel unbearable pain in the mouth and ultimately results in caries.[33] One more possible reason may be the increasing tendency of consuming instant or fast food and juices instead of whole grains or fruits which are rich in fibres that diminishes the oral health n hygiene due to lack of exercise of teeth and gums of working age group people.. So we have found most numbers of dental caries patients among this group. But the number of cases continually increased up to the age of 31–40 (50%) and later on these cases got declined. But a more detailed and long term study is required to analyze the long term effect of age on the development of dental caries among the population.

Among the persons studied with different dietary habit, we found the highest number of caries patients with vegetarian dietary habit (40%), vegetarian plus tobacco user (40%) followed by non vegetarian (Mixed diet) (20%), respectively **(Table 3).** It has been verified that dental caries is a process due to formation of acid by fermentation of sugar and starch (maximum part of vegetarian diet) through acidogenic bacteria that lead to decalcification of dental enamel, which usually results in unhealthy gingiva, and it might result in dental caries in these areas.[33]

The results of antimicrobial properties of methanolic and aqueous extracts of Onion bulb (*Allium cepa*)and Ginger rhizome (*Zingiber officinale),* the positive control Ampicilline and the negative control (Methanol and Distilled water) are presented in **Table 4,5,6,7**. The antimicrobial activity of onion and ginger extracts on the agar plates varied for both the solvents(Methanol and Distilled water). The positive control produced significantly larger inhibition zones against the *S.mutans* (Ampicilline at 32% concentration). However, the negative control produced no observable inhibitory effect. When the Aqueous and Methanolic extract of onion and ginger were

screened for antibacterial activity out of which methanolic extracts showed significant maximum activity against the 10 isolates of bacteria *(S. mutans)*. However, aqueous extracts showed comparatively less activity against the *S.mutans* **(Table 4,5,6,7).** Onion and ginger extracts had more antibacterial activity against Streptococcus mutans .The antibacterial activity of the extracts has been increased by increasing the concentrations.

It was clear from this work that the solvent of extraction affected the degree of antibacterial activity of the extracts. It was observed that the methanolic extract of ginger gave the widest zone of inhibition (19.2±1.1mm) using the concentration of 50% while the methanolic extract of onion gave 13.34±0.24 mm with 50% each against *S.mutans*. This credit to methanol extraction was supposed to methanol being an organic solvent and will dissolve organic compounds better, hence liberate the active components required for antimicrobial activity.[24]

The aqueous extract of ginger also presented marked zone of inhibition(16.06±0.08mm) than aqueous extract of onion(10.37±0.65mm) at 50% concentration. It could be said that water as an extractant could liberate the active constituents of ginger better compared to onions.[35] Flavonoids are chemical compounds present in onion active against microorganisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of microorganisms.[24] Onion bulb also contain a large amount of sulfur compounds responsible for antimicrobial activity.[36] similarily , Important secondary metabolites present in the ginger rhizome are **curcumene**, non-volatile hydroxyaryl compounds e.g**. zingerone**, **gingeroles and shogaoles (phenylalkanones),** volatile **sesquiterpenes (e.g. zingiberene and bisabolene) and mono-terpenoids (e.g. citral).**[27] which are responsible for antimicrobial activity.

Conclusion

Both the selected herbs in their aqueous and methanolic extract preparations showed antimicrobial activity against *Streptococcus mutants*. Because this study provides evidence for the presence of one or more soluble constituents in these plants parts used, that probably interferes with bacterial growth and their metabolism which would prevent and inhibit initiation and progression of dental caries . Specifically, the methanolic extract of Ginger *(Zingiber officinale*) showed the remarkable activity . Aqueous extract of ginger showed the average activity against *Streptococcus mutans* followed by the methanolic extracts of onion and then aqueous extract of onion bulb. Using of these plant extracts as home remedies creates an oral environment which is unfavorable for microbes. This indirectly reduces the microbial colonization and modifies oral environment. Although phytochemicals (plant derived metabolites) are antimicrobial in nature but they also produce other biological activities in oral cavity like induction of immunity, which indirectly reduces the risk of oral diseases thus prevent plaque accumulation, periodontal diseases and dental caries. So that consumption of these two herbs in everyday diet should be recommended or their methanolic and aqueous extract should be used in toothpastes, mouthwashes mouthfreshnars and other mouth preparations.

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