

Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against Streptococcus mutans isolated from dental caries

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Abstract

Effective control of pathogenic bacteria and dental plaque is key to the prevention and treatment of Dental caries. Worldwide, hundreds of plants are used in traditional medicine as treatment for bacterial infections. Since the discovery of *S. mutans* as an etiological agent of dental caries, much attention was focused on this bacterium, as a target for the prevention of disease through the use of **antimicrobial** agents and vaccine preparation. The **antimicrobial activities** of 4 traditional plant extract against *Streptococcus mutans* by agar diffusion methods were tested. The results of the present study showed that alcohol & aqueous extract of green tea had **antimicrobial activity** against *Streptococcus mutans*. This indicates the presence of potent antibacterial activity, which confirms its use as antibacterial agents. Both the ethanol and water extract of green tea and clove produced inhibitory actions against *Streptococcus mutans*, but the zones of inhibition of alcohol extract were less than aqueous extract. This tends to show that the active ingredients in the leaves were better extracted with ethanol than water. The alcohol and aqueous extracts of the four plants used in the present study have antibacterial activity against *S. mutans*, therefore, can be used in mouth rinses and toothpastes and can be beneficial in controlling dental caries.

INTRODUCTION

Dental caries is a chronic infection that affects populations worldwide. In the United States alone, health care costs associated with treating dental caries have topped 64 billion USD annually (1). In the developing world, dental caries is more prevalent, especially among pediatric patients and may be a result of a switch to 'Western' diets that feature high levels of dietary sugars (2-4).

Streptococcus mutans gives its name to a group of seven closely related species collectively referred to as the mutans streptococci. The primary habitats for *S.*

mutans are mouth, pharynx, and intestine (5). Several factors, such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves and the ability to synthesize extracellular polysaccharides are present in dental caries (5, 6). *S. mutans* and *Streptococcus sobrinus* have a central role in the etiology of dental caries (7, 8), because these can adhere to the enamel salivary pellicle and to other plaque bacteria (9). Mutans streptococci and lactobacilli are strong acid producers and hence cause an acidic environment creating the risk for cavities (10). Usually, the appearance of *S. mutans* in the tooth cavities is followed by caries after 6-24 months (11).

Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against *Streptococcus mutans* isolated from dental caries

Since the discovery of *S. mutans* as an etiological agent of dental caries, much attention was focused on this bacterium, as a target for the prevention of disease through the use of antimicrobial agents and vaccine preparation. Application of antibiotics for prevention and treatment of dental caries would be harmful for the patients and the development of multidrug-resistant strains of bacteria has been widely reported (12-14). Effective control of pathogenic bacteria and dental plaque is key to the prevention and treatment of Dental caries. Worldwide, hundreds of plants are used in traditional medicine as treatment for bacterial infections. Although natural products are not necessarily safer than synthetic antibiotics, some patients prefer to use herbal medicines. Oil of cloves, also known as clove oil, is an essential oil from the clove. It is a natural analgaesic and antiseptic used primarily in dentistry for its main ingredient eugenol. It can also be purchased in pharmacies over the counter, as a home remedy for dental pain relief, mainly toothache; it is also often found in the aromatherapy section of health food stores. The oil produced by cloves can be used in many things from flavouring medicine to remedies for bronchitis, the common cold, a cough, fever, sore throat and tending to infections. The main oil-producing countries are Madagascar and Indonesia (15). Capsicum, also known as red or chili pepper, used to make medicine. Chilli tinctur was previously reported to be able to inhibit the growth of *Staphylococcus* sp., *Escherichia coli*, *Bacillus cereus* and *Bacillus subtilis*(16). Tea leaves infusion (*Camelia sinensis*) is the most popular beverage containing large amounts of polyphenols, and it could also be important source of those constituents in human diet. There are many studies providing strong evidence that these compounds possess antioxidant capacity preventing many diseases. Green tea is a non-fermented tea. The tea is an infusion of flavorful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids,

saponins, tannins, catechin and polyphenols (17). Toda et al (18) also showed that moderate daily consumption of green tea killed *Staphylococcus aureus* and other harmful bacteria. Arak, a tree used for Miswake, is also known as "tooth brush tree" and "mustard plant". Although the Miswake is usually obtained from the roots of the Arak tree, some sticks are made from its branches and Bark (19). Studies have indicated that *Salvadora persica* contain substances that possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria which are frequently found in the oral cavity. The growth and acid production of these bacteria is thus inhibited (20).

Materials and methods

Samples : Ten samples were collected from patients suffering from dental caries attending dental clinics at Baghdad Hospital.

Isolation of the test microorganism (*Streptococcus mutans*):

Ten swabs were taken from patient attending dental clinics at Baghdad Hospital and cultured on sheep blood agar, and incubated at 37 C for 24 hours with 10% CO₂. Colonies with α -haemolysis and characteristic features of *S. mutans* were further diagnosed using biochemical tests (Table-1).

Preparation of plants extract:

Seeds of four plants (red pepper, miswake, carnation, and green tea) were procured from local market in Baghdad. The seeds were put in blender for 10 minutes and the powder were dissolved with water or ethanol, and concentrated at reduced pressure to afford the various crude extracts of plants samples (21). Filtered juice was then poured in dishes and placed in an incubator at 37C to dry. Two different solvent (Ethanol and Water) used to make different concentrations. Once the constant of volume of the solvent was achieved, the extraction efficiency was

Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against *Streptococcus mutans* isolated from dental caries

calculated as percent volume of solvent of the initial quantity (10 ml) as in (Table-2).

The samples were kept in the refrigerator at 5 C° and frequently were shaken before use.

Antibacterial assay:

The clinical isolates were inoculated into nutrient broth and incubated at 37 C overnight. The bacterial broth culture was checked to provide 10⁸ cfu/ml which is equivalent to the standard opacity of a barium sulfate suspension (density 0.5 on the McFarland scale). Antibacterial assay of the plant extracts were performed using agar well diffusion methods (22). Plates of muller-hinton agar were prepared and 0.1 ml of culture broth were added and spread with a sterile spreader. Wells were made in the plates with a cork borer (0.65 cm). 100 µl test compound were introduced into the wells and the plates incubated overnight at 37 C. The antimicrobial activities were interpreted by measuring the diameter of the zone of inhibition in mm.

Results

Figure-1 shows the inhibition zone of aqueous extract of the four plants used in the present study. It is clear that the aqueous extract of green tea at different concentrations (5, 10, and 15%) were more effective than other plant, though the inhibition zone were ranged between 29 to 35 mm at different concentration of aqueous solution of green tea. Clove was second to green tea in its activity against *Streptococcus mutans* isolates and the inhibition zones were ranged between 23 to 28 mm (Fig-1). While the aqueous extracts of chilli and miswake have little effect on the bacterium, since the inhibition zone of chilli and miswake were 10 mm at the concentrations used in the present study (Fig.1).

Figure 2 shows the effect of alcohol extracts of the plants on *Streptococcus mutans* isolates. It is obvious that alcohol extract of

clove was better than other plants according to the zone of inhibition, followed by green tea. While chilli and miswake have less activity against the test bacterial isolates (Fig 2).

Discussion

In this study, the antimicrobial activities of 4 traditional plant extract against *Streptococcus mutans* by agar diffusion methods were tested. The results of the present study showed that alcohol & aqueous extract of green tea had antimicrobial activity against *Streptococcus mutans* (Fig 1 and 2). This indicates the presence of potent antibacterial activity, which confirms its use as antibacterial agents. Both the ethanol and water extract of green tea and clove produced inhibitory actions against *Streptococcus mutans*, but the zones of inhibition of alcohol extract were less than aqueous extract. This tends to show that the active ingredients in the leaves were better extracted with ethanol than water. Toda et al reported that daily consumption of green tea can kill gram positive *S. aureus* and other harmful bacteria (18). Also Ahn et al and Wakayama et al found that the green tea contains catechin and polyphenols (23, 24). These compounds have been found to possess antibacterial and antiviral action as well as anti-carcinogenic and anti-mutagenic properties. Tea polyphenols have previously been shown to have antibacterial activities against human and animal disease-related bacteria, phytopathogenic bacteria and food-borne bacteria (25).

In the present study, aqueous and alcohol extracts of clove inhibited bacterial growth, but their effectiveness varied. The zones of inhibition of aqueous extract were less than ethanol extract. The use of natural herbs as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise of the last few decades, one of such plants was clove (26) active constituents of clove (biflorin, kaempferol, rhamnocitrin, yricetin, gallic

Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against *Streptococcus mutans* isolated from dental caries

acid, ellagic acid and oleanoic acid) possess antibacterial activities against periodontal oral pathogens, including *Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas* and *Prevotella intermedia* (27).

Alcohol and aqueous extracts of Miswakee were also show antibacterial activity against *S. mutans* (Fig. 1 and 2), but their activity were less than that of green tea and miswakee. Previous studies have reported that miswakee extracts were effective against *S. mutans* (28). The antimicrobial and cleaning effects of miswakee may be attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadoura and salvadorine, saponins, tannins, vitamin C, silica, and resin, in addition to cyanogenic or lignan glycosides, alkaloids, terpenoids, and oleic, linoleic, and stearic acids (29).

According to chili, its alcohol and aqueous extracts showed antibacterial activity against the tested microorganism (Fig. 1 and 2). Chili peppers are highly demanded and consumed around the world; their secondary metabolites (capsaicinoids) have been largely associated with hot taste but also with antimicrobial and antifungal properties (30).

Conclusion

The alcohol and aqueous extracts of the four plants used in the present study have antibacterial activity against *S. mutans*, therefore, can be used in mouth rinses and toothpastes and can be beneficial in controlling dental caries.

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Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against *Streptococcus mutans* isolated from dental caries

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Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against *Streptococcus mutans* isolated from dental caries

Table 1: Biochemical tests used for the diagnosis of *Streptococcus mutans* isolates.

Tests	Results	Tests	Results
Catalase	-ve	Sorbitol	+ve
Motility	-ve	Maltose	+ve
Arginine dihydrolase	-ve	Lactose	+ve
Aceton	+ve	Sucrose	+ve
Urea	-ve	Glucose	+ve
Mannitol	+ve	Esculin hydrolysis	+ve

Table-2: Optimization of Sample-to-Solvent ratio.

Volume of solvent (Ethanol or Water)	Weight of plants dried extracts (gm)	Concentration of the Extracts
10 ml	0.5	5
10 ml	1	10
10 ml	1.5	15

Antimicrobial activity of the aqueous & alcohol extracts of four herbal medicines against Streptococcus mutans isolated from dental caries

Figure 1: The zone of inhibition of the aqueous extract of the four plants at different concentration (5, 10, and 15%).

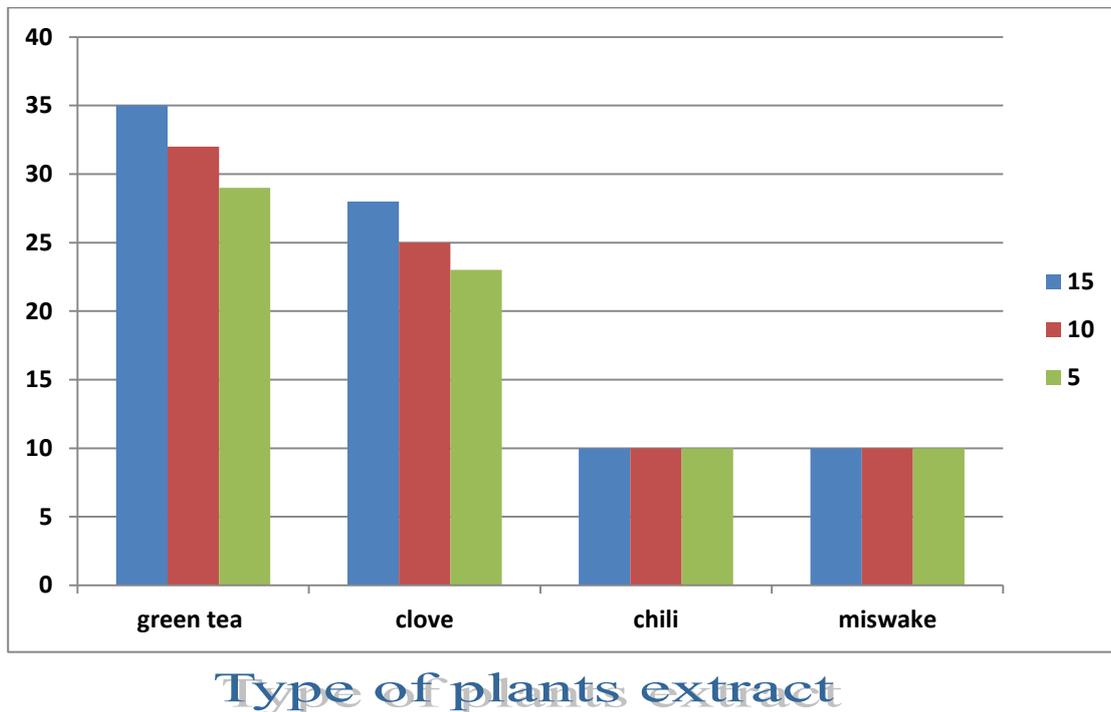


Figure 2: The zone of inhibition of the alcohol extract of the four plants at different concentration (5, 10, and 15%).

