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Inhibition of *Streptococcus mutans* adhesion to buccal epithelial cells by an aqueous extract of *Thymus vulgaris*

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Hammad M, Sallal A-K, Darmani H. Inhibition of *Streptococcus mutans* adhesion to buccal epithelial cells by an aqueous extract of *Thymus vulgaris*.

Abstract: *Objectives:* The aim of this study was to investigate the effect of an extract of *Thymus vulgaris* (thyme) on the growth of *Streptococcus mutans* (*S. mutans*) and the adhesion of this bacterium to human buccal epithelial cells. *Methods:* Different concentrations of an aqueous extract of thyme were prepared and the effects investigated on growth of *S. mutans*. Furthermore, the effect of these extracts on adhesion of *S. mutans* to buccal epithelial cells was also investigated and compared with the effects of chlorhexidine digluconate. *Results:* The data revealed that exposure of *S. mutans* to thyme extract showed a time and concentration-dependent decrease in bacterial viability. The greatest effect was observed when *S. mutans* had been exposed to 20% thyme extract for a period of 48 h which resulted in 96% inhibition of bacterial growth. Furthermore, the adhesion of *S. mutans* to buccal epithelial cells was also reduced when either buccal epithelial cells or *S. mutans* had been pre-incubated with different concentrations of aqueous thyme extracts (83–98% and 75–89% inhibition respectively). There was also greater reduction in the adherence of bacterial cells to buccal epithelial cells after mouth rinsing with 20% aqueous thyme extract compared to rinsing with chlorhexidine digluconate (45% and 89% inhibition of bacterial adhesion respectively). *Conclusion:* The diminished adherence of *S. mutans* to buccal epithelial cells after exposure to various concentrations of aqueous thyme extract as well as the antimicrobial properties of this plant may have clinical relevance.

Key words: adhesion; chlorhexidine digluconate; *Streptococcus mutans*; *thymus vulgaris*

Introduction

Dental plaque is a host-associated biofilm, which is initially formed through bacterial interactions with the tooth and then through physical and physiological interactions among different species within the microbial mass (1). Dental plaque formation begins with the initial colonization of the pellicle by *Streptococcus gordonii*, *S. oralis* and *S. mitis*. These bacteria adhere to the pellicle by specific ionic, hydrophobic and lectin-like interactions (2). This is followed by co-aggregation of other types of bacteria during the formation of dental plaque.

Dental caries forms through a complex interaction over time between acid-producing bacteria and fermentable carbohydrate, and many host factors including teeth and saliva. The main strategies for preventing dental caries include minimal ingestion of sucrose, daily brushing and mouthwashes (2) such as chlorhexidine to prevent both plaque formation and development of gingivitis (3–6).

In recent years interest in herbal medicine and the use of essential oils and extracts of many plant species have become popular. Although the antimicrobial activities of essential oils isolated from many plants have been recognized for centuries, only recently have such properties been confirmed (7–11). Thyme is stated to possess carminative, antispasmodic, antitussive, expectorant, secretomotor, bactericidal and astringent properties. At present, the essential oils of many *Thymus* species are widely used as flavouring agents in food processing and many pharmacological preparations, and thyme oil is still among the world's top 10 most used essential oils (12).

Essential oils from *Thymus vulgaris* (*T. vulgaris*) have been reported to be fungitoxic and shown to inhibit the radial growth, conidial germination and production of *Penicillium digitatum* completely (7). The antibacterial properties of *T. vulgaris* extracts have also been investigated (8–10). Furthermore, essential oils from *T. vulgaris* have been found to exert significant bacteriostatic activity against many Gram-positive and -negative bacteria, although the activity is reported to be more marked against Gram-positive bacteria (11).

In this study, we report the effect of an aqueous extract of *Thymus vulgaris* on the growth of an oral pathogen *S. mutans* and on the adhesion of this bacterium to buccal epithelial cells (BEC).

Materials and Methods

Organism and growth conditions

Streptococcus mutans was isolated from patients attending the periodontology clinic at Jordan University of Science and Technology. The organism was grown on blood agar and on brain–heart infusion medium at 37°C. Identification was carried out according to Cowan and Steel (13) and then confirmed using API strep kit (API Laboratory products Ltd, Basingstroke, England).

Preparation of aqueous thyme extract

Forty grams of *T. vulgaris* leaves were soaked in 100 ml hot distilled water (70°C) for 1 h, then filtered using a double layer of cheese cloth. The filtrate was then sterilized using a 0.45 μm Millipore filter (Millipore, Billerica, MA, USA).

Effect of leaf extracts of *T. vulgaris* on growth of *S. mutans*

Aliquots of 1.0 ml (10^6 cells ml^{-1}) of a log phase culture of *S. mutans* were inoculated into 100 ml of the brain–heart infusion containing different concentrations of leaf extracts of *T. vulgaris* (5%, 10% and 20% w/v) and incubated at 37°C in a rotary shaker water bath. Growth was measured at different time intervals by measuring the absorbance at 420 nm.

Preparation of buccal epithelial cells

Buccal epithelial cells were collected from six healthy students by gently rubbing the mucosal surface of the cheeks with a sterile tongue depressor. The epithelial cells were washed twice with Hanks balanced salt solution (HBSS) and centrifuged at 500 *g* for10 min to remove any saliva and other contaminating oral secretions. The cells were then resuspended in HBSS at a concentration of 10^5 cells ml^{-1}.

Exposure of *S. mutans* to aqueous thyme extract

The effect of aqueous thyme extract on the adherence of *S. mutans* to human BEC was studied by incubating the bacterial cells (10^6 cells ml^{-1}) in the presence of 0%, 5%, 10% and 20% aqueous thyme extract, in a rotary shaker for 1 h at 37°C. Bacterial cells were then harvested and washed twice with HBSS. These cells were used in the adhesion assay.

Exposure of buccal epithelial cells to aqueous thyme extract

Buccal epithelial cells were collected as described above and suspended in 8 ml of HBSS. This cell suspension was divided into four equal samples of 2 ml (10^5 cells ml^{-1}). Samples were then exposed to different concentrations of aqueous thyme

extract (final concentrations of 0%, 5%, 10%, 20%) at 37°C for 1 h in a shaking water bath.

In another experiment the effect of mouth rinse with aqueous thyme extract (5%, 10%, 20%) was studied using the method of Tobgi *et al.* (14). Buccal epithelial cells were collected, as described previously, from healthy adult students by gently rubbing the right cheek with a sterile tongue depressor, which was then agitated in 5 ml HBSS. This acted as a control for each experiment. Subsequently, the mouth was rinsed with 10 ml aqueous thyme extract (5%, 10%, 20%) for 1 min, followed by a 10 ml tap water rinse for 5 s. Buccal epithelial cells were immediately harvested from the left cheek and suspended in 5 ml HBSS.

In the third experiment, the mouth was rinsed with 5 ml chlorhexidine digluconate for 1 min, and then BEC were collected and processed in the same way as described for the aqueous thyme extract.

Adherence assay

Adherence assays were performed as described by Ghannoum *et al.* (15). Equal volumes of BEC (1×10^5 cells ml^{-1}) and an overnight culture of *S. mutans* (1×10^6 cells ml^{-1}) were incubated at 37°C for 2 h in a rotary shaker water bath (speed of 100 rev/min). Bacterial adhesion to the BEC was assayed microscopically by observing adhesion of *S. mutans* to 50 randomly selected BEC.

Results and Discussion

The effect of various concentrations of thyme extract on the growth of *S. mutans* was studied as shown in Fig. 1. The figure shows that exposure of *S. mutans* to aqueous thyme extract resulted in a time and concentration-dependent decrease bacterial viability. Exposure of *S. mutans* to 20% aqueous thyme extract for a period of 48 h, resulted in 96% inhibition of bacterial viability.

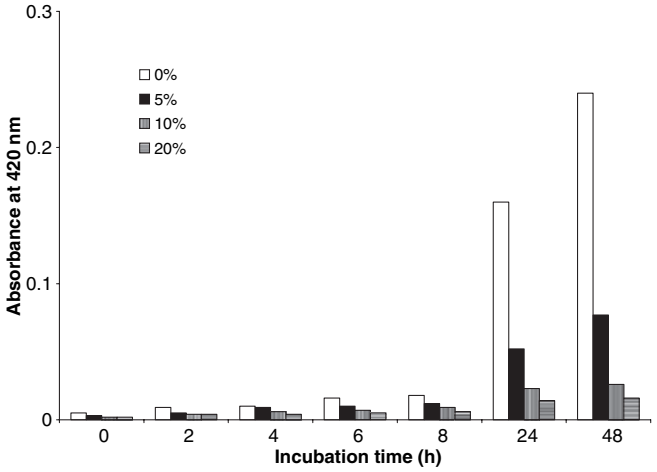

Fig. 1. Effect of different concentrations of *Thymus vulgaris* extract on the growth of *Streptococcus mutans*. □0%, ■5%, ▥10%, ▤20%.

Table 1 shows the effect of preincubation of BEC with various concentrations of aqueous thyme extract on adherence of *S. mutans*. Preincubation of BEC with 5%, 10% and 20% aqueous thyme extract for 1 h resulted in 83%, 90% and 98% reduction, respectively, in adherence of *S. mutans* to BEC.

Table 2 shows the effect of pretreatment of bacterial cells with aqueous thyme extract on adhesion of *S. mutans* to BEC. Preincubation of *S. mutans* with 5%, 10% and 20% aqueous thyme extract for 1 h resulted in 75%, 87% and 89% reduction, respectively, in adherence of *S. mutans* to BEC.

The effect of mouth rinsing with various concentrations of aqueous thyme extract on the adherence of *S. mutans* to BEC is presented in Table 3. There was a concentration-dependent reduction in bacterial adherence to buccal epithelial with the use of an aqueous thyme extract mouth rinse. When BEC were collected after 1 min of an oral rinse with 20% aqueous thyme extract, bacterial adhesion to these cells was inhibited by 85%, compared with 45% inhibition of adhesion when an oral rinse with chlorhexidine digluconate had been used for the same time duration.

The results obtained in this study indicate that, in addition to its antibacterial effects, aqueous thyme extract greatly inhibited the adherence of *S. mutans* to BEC. The current study found that oral rinsing with chlorhexidine was found to

Table 1. **The effect of preincubation of buccal epithelial cells (BEC) with various concentrations of aqueous thyme extract on adherence of *Streptococcus mutans***

Extract (%)	Adherent bacterial cells per BEC	Reduction in adhesion (%)
0.0 (control)	70	
5.0	12	83
10.0	7	90
20.0	1	98

Numbers represents an average of duplicate readings with SD 4%.

Table 2. **Adherence of *Streptococcus mutans* to human buccal epithelial cells after incubation of bacterial cells with different concentrations of aqueous thyme extract**

Extract (%)	Adherent bacterial cells per BEC	Reduction in adhesion (%)
0.0 (control)	181	
5.0	45	75
10.0	23	87
20.0	20	89

Numbers represents an average of duplicate readings with SD 4%.

Table 3. **Adherence of *Streptococcus mutans* to BEC collected after an oral rinse with various concentrations of aqueous thyme extract or chlorhexidine digluconate**

	Adherent bacterial cells per BEC	Reduction (%)
Extract (%)		
0.0 (control)	82	
5.0	62	24
10.0	42	49
20.0	12	85
Chlorhexidine digluconate	45	45

Numbers represents an average of duplicate readings with SD 4%.

have almost an equal activity as that of rinsing with 10% aqueous thyme extract on preventing *S. mutans* adherence to BEC (Table 3).

Although of all chemical plaque control agents, chlorhexidine digluconate has proven to be the most effective and safe (5), various adverse effects, such as teeth staining and increased calculus formation, have been observed.

The findings of the current study suggest that aqueous thyme extract has a good potential as plaque control agent with less adverse effects and may be more acceptable to consumers and the regulatory agencies in comparison to synthetic chemical compounds.

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