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The synergistic effect of honey and cinnamon against *Streptococcus mutans* bacteria

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ABSTRACT

Objective: To investigate the effect of Iranian honey, cinnamon and their combination against *Streptococcus mutans* bacteria.

Methods: Nine experimental solutions were examined in this study, including two types of honey (pasteurized and sterilized), two types of cinnamon extract (dissolved in distilled water or dimethyl sulfoxide) and five different mixtures of cinnamon in honey (prepared by admixing 1%–5% w/w of cinnamon extract into 99%–95% w/w of honey, respectively). Meanwhile, each of mentioned agent was considered as the first solution while it was diluted into seven serially two-fold dilutions (from 1:2 to 1:128 v/v). Therefore, eight different concentrations of each agent were tested. The antibacterial tests were performed through blood agar well diffusion method, and the minimum inhibitory concentration (MIC) was determined. Ultimately, the data were subjected to statistical analysis incorporating Two-way ANOVA and Bonferroni *post hoc* tests ($\alpha = 0.01$).

Results: The highest zone of inhibition was recorded for the mixtures of honey and cinnamon while all the subgroups containing 95%–99% v/v of honey were in the same range ($P < 0.01$). The MIC for both honey solutions were obtained as 500 mg/mL whereas it was 50 mg/mL for both cinnamon solutions. Moreover, the MIC related to all honey/cinnamon mixtures were 200 mg/mL.

Conclusions: A profound synergistic effect of honey and cinnamon was observed against *Streptococcus mutans* while there was no significant difference among extracts containing 99%–95% v/v of honey admixing with 1%–5% v/v of cinnamon, respectively.

1. Introduction

Dental caries is an infectious disease that is started by biofilm formation on tooth surface [1]. Among various causative bacteria in this biofim, *Streptococcus mutans* (*S. mutans*) has been proved to be the main corresponding species for carious lesion [2]. Therefore, any antibacterial agents against *S. mutans* could be incorporated as a preventive strategy against dental caries [3]. Although various antibacterial compounds such as chlorhexidine mouth rinse have been prescribed broadly, several side effects have been reported for these chemicals [4]. For that reason, many investigations have been performed to seek for adjunctive materials that could prevent plaque formation on tooth surfaces [3,5,6].

In the wake of increasing interest in complementary and alternative medicine, herbal extracts are attracted recently [7,8]. Appropriately, numerous literatures have reported a strong antibacterial effect for different plants and natural products against *S. mutans* [3,5,6]. Among these natural antibiotics, the unique potential of either honey or cinnamon has been documented frequently [9–14]. However, to the best of our knowledge, there is no available data about incorporating the combination of honey and cinnamon on cariogenic bacteria.

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On the other hand, mixing different plants against the target bacteria were encouraged drastically in previous publications because the combination would assure the exposure of any microorganism to various chemical compounds and lead to profoundly enhanced activity [15,16]. Accordingly, it was argued that the combined treatment with honey and some plants showed enormous synergism effect against bacterial species comparing to their pure extracts [11]. Therefore, since both honey and cinnamon extracts are strongly effective against *S. mutans* [9–14], it could be hypothesized that the honey/cinnamon mixture would be more favorable.

Moreover, the broad-spectrum antibacterial potential of honey is directly depended on its component which is vastly affected by the type of producer bee and its geographic condition; because each type of bee would provide different additional factors available in its honey [17,18]. In view of that, some studies obtained that local honey products have more antibacterial efficacy comparing to commercially available ones [19].

Therefore, this lack of adequate data on the honey/cinnamon synergism as well as the variations in honey extracted from different regions has prompted us to investigate the effect of Iranian honey, cinnamon and their combination against *S. mutans* bacteria.

2. Materials and methods

2.1. Preparation of experimental agents

2.1.1. Honey

The honey was harvested by hand in spring season from beehives situated in Hajiabad area, a region situated in Ghom Province that is roughly situated in the center of Iran. The collected honey was diluted by distilled water to produce a 200 mg/mL solution. In order to avoid bacterial or yeast contamination, we had to pasteurize or sterilize the honey. However, for evaluating the effect of these process in the antibacterial effect of honey, we conducted the study in two separate groups of honey, including pasteurized (30 min at 65 °C) and sterilized (autoclaved at 121 °C and 15 atm for 20 min) honey.

2.1.2. Cinnamon

Ethanolic extract of cinnamon was prepared by immersing 200 g of cinnamon in 1000 mL of ethanol (70%) prior. After 72 h, the whole solution was filtrated using Whatman No. 1 paper (150 μm diameter hole). Subsequently, the ethanol was evaporated by means of water bath device (Gesellschaft für Labortechnik mbH, Burgwedel, Germany) while the extract was lyophilized and stored at 4 °C until the test was performed. Since the obtained extract was not soluble in water, we incorporated dimethyl sulfoxide (DMSO) (Merck Co., Darmstadt, Germany) to produce 20 mg/mL solution of cinnamon hydro-alcoholic extract for the rest of the study.

2.1.3. Honey/cinnamon mixtures

Five different mixtures of honey and cinnamon were prepared by admixing 1%–5% w/w of cinnamon extract into 99%–95% w/w of honey respectively.

2.1.4. Preparation of the dilutions

Ultimately, each of the mentioned agent was considered as the first solution while it was diluted into seven serially two-fold dilutions (from 1:2 to 1:128 v/v). Therefore, eight different concentrations of each agent were tested. However, it should be emphasized that the honey extract was diluted into distilled water while the cinnamon into DMSO and their mixture, respectively.

2.2. Antibacterial tests

2.2.1. Bacterial strain and growth condition

S. mutans PTCC 1683 (Persian Type Culture Collection, IROST, Iran) was employed in this study. The bacteria were cultured overnight in 5 mL of Mueller–Hinton broth (Liofilchem, Roseto Degli Abruzzi, Italy) at 37 °C. Ultimately, the bacterial suspension was adjusted to 0.5 McFarland's standard incorporating the sterile normal saline.

2.2.2. Susceptibility test

The susceptibility test was accomplished via blood agar well diffusion method. In this process, 200 μL of bacterial suspension was spread on each plate of blood agar medium by means of a sterile swab and the plates were put on the bench for 1 h prior to punch some wells with the dimension of 6 mm diameter × 8 mm depth using the sterile cork-borer while the wells were at least 30 mm apart from each other. Consequently, each well was filled with 30 μL solution and the plates were incubated at 37 °C and the inhibition zone around them was measured in mm scale after 24 h.

2.2.3. Minimum inhibitory concentration (MIC)

Briefly, 1 mL of the prepared bacterial suspension (~1.5 × 10^8 bacteria/mL) was inserted into the tubes containing 1 mL of nutrient broth (Merck Co., Darmstadt, Germany). Afterward, 1 mL of each mentioned two-fold dilutions (ranged from 1:1 to 1:512 v/v) were added into the tubes and incubated at 37 °C for 24 h. Finally, the minimum concentration which inhibited bacterial growth (according to the liquid turbidity) was considered as MIC for each agent.

2.3. Statistical analysis

After exploring the normal distribution using Kolmogrov–Smearnov test, the data were subjected to Two-way ANOVA in order to evaluate the effect of the agent as well as its concentration simultaneously on the zone of inhibition. Meanwhile, Bonferroni *post hoc* test was incorporated for pairwaise comparisons while the level of significance was adjusted as 0.05.

3. Results

3.1. Susceptibility test

The mean amount of inhibition zone and the SD related to all subgroups are depicted in Table 1. It showed that the highest value was recorded for the mixtures of honey and cinnamon while all the subgroups containing 95%–99% v/v of honey were in the same range. Therefore, honey and cinnamon showed strong synergistic antibacterial effect against *S. mutans* because their pure solutions were not as much effective comparing to their combination.

The pairwise *P* values of serially two-fold dilutions of each agent are demonstrated in Tables 2–6. As it is evident, the least