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Evaluation of the effect of Kandovan propolis against Streptococcus Mutans

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Short Communication

Abstract

BACKGROUND AND AIM: In recent years, propolis has been introduced as one the most efficient agents against cariogenic bacteria. However, due to the lack of data on the propolis collected from Kandovan (Easthern Azarbaijan, Iran), this study was designed to investigate the effect of this type of propolis on Streptococcus mutans (S. mutans).

METHODS: In this experimental study, the ethanolic extraction of propolis (EEP) was prepared with different concentrations (1%, 3%, 5%, and 10%) while the distilled water was incorporated as control. The antibacterial efficacy was tested via two standard methods including the agar disk diffusion and minimum inhibitory concentration (MIC) tests. Finally, the resulting data were analyzed using one-way analysis of variance (ANOVA) and Bonferroni post-hoc tests ($\alpha = 0.05$).

RESULTS: The obtained MIC was 2.5 mg/ml. However, in disk diffusion test, the 1% and 3% EEP solutions did not exhibit any zone of inhibition, however the 5% EEP showed very strong antibacterial effect ($P < 0.001$).

CONCLUSION: The EEP extracted from Kandovan had significant antibacterial effect against S. mutans when prepared in 5% concentration. Therefore, this type of propolis could be considered as one of the most efficient propolis against S. mutans.

KEYWORDS: Propolis; Streptococcus Mutans; Antibacterial

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Various anti-microbial substances have been extensively utilized in dentistry for caries prevention due to microbiological etiology of tooth decay.[1-3] Accordingly, numerous chemical antibacterial agents have been marketed in the form of mouth rinses, varnishes, chewing gums, etc., which are recommended for caries prophylaxis.[4-6]

In recent years, numerous investigations had focused on the incorporation of natural products in preventive dentistry due to their cost effectiveness and also the lack of systemic adverse effects.[3,7,8] Among these natural gifts, propolis has been introduced as one of the most successful materials.[9-14] Honeybees produce the propolis as a semisolid substance in the entrance of the hive and employ it to seal the hive and the defensive techniques.[15,16] Beside these beneficial properties of propolis, it has been shown to have unique antibacterial capacity against a wide range of bacteria including the cariogenic microbes.[9-14] Since Streptococcus mutans (S. mutans) has been introduced as the main bacterium responsible for dental caries, the majority of the studies have been accomplished regarding these bacteria. In this

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regard, it has been frequently documented that the propolis has effective suppressive effect against S. mutans.[9-14] However, the chemical composition of propolis is directly related to its geographic origin due to the variation in honeybee's nutrition that is obviously dependent on the host plant.[16,17] Moreover, some publications also confirm that even the year of collection would significantly influence the propolis properties.[18] Hence, investigating the propolis collected from different geographical areas would be beneficial and necessary in dentistry field to validate the most effective propolis against the S. mutans.

Only very few studies have been carried out evaluating the antibacterial potency of Iranian propolis against S. mutans.[19,20] Although all these studies confirm a significant effectiveness for Iranian propolis, the results presented in these studies are controversial regarding the required dose for controlling S. mutans.[19,20] Furthermore, there is no available data about the Kandovan propolis.

Therefore, this study was conducted to evaluate the antibacterial effect of Kandovan propolis against S. mutans.

Methods

Propolis extraction: The propolis was harvested by hand in spring season (April-May 2015) from beehives situated in Kandovan area, a region in Easthern Azarbaijan Province roughly located in the north-west of Iran. The samples were desiccated and stored at 4 °C prior to the start of the study.

The ethanolic extraction of propolis (EEP) was prepared and adjusted to the Bosio et al. method.[12] Accordingly, the propolis was added to ethanol 95% (v/v) and shacked for 7 days at room temperature. Then, the whole mixture was centrifuged and filtered using a Whatman filter paper. In the next step, the solution was desiccated and a powder was obtained.

Finally, the powder was diluted by ethanol to produce 1.3% and 5.0% solutions for disk diffusion test while the 10% solution was serially diluted for the minimum inhibitory concentration (MIC) tests.

Bacterial strain and growth condition: S. mutans Persian Type Culture Collection, Iranian Research Organization for Science and Technology (IROST), (PTCC) 1683 was employed in this study and the bacteria were cultured overnight in 5 ml of Mueller-Hinton Broth (Liofilchem, Italy) at 37 °C. Ultimately, the bacterial suspension was adjusted to 0.5 McFarland standards incorporating the sterile normal saline.

Susceptibility test: The susceptibility test was accomplished via disk diffusion method. In this process, 200 µl of bacterial suspension was uniformly spread on the Mueller Hinton Agar by means of a sterile swab. In this way, the standard paper disk was wiped by 10 µl of 1%, 3%, or 5% solutions while the same amount of distilled water was used as negative control. Finally, the disks were immediately placed on the prepared agar medium, which were incubated at 37 °C, and the inhibition zone around each were measured in mm scale after 24 hours. This test was triplicate for every sample.

MIC: 1 ml of 10% EEP solution was inserted in a tube and it was serially two fold diluted into 6 other tubes using ethanol. Then, 1 ml of the prepared bacterial suspension (~ 1.5×10^8 bacteria/ml) plus 1 ml of Nutrient Broth (Merck, Germany) were added in each tube. After 24 hours of incubation at 37 °C, the minimum concentration, which inhibited bacterial growth (according to the liquid turbidity) was considered as MIC. Moreover, the distilled water and the pure bacterial suspension were incorporated as negative and positive controls, respectively.

After exploring the normal distribution using Kolmogorov-Smirnov test, the data were examined using one-way ANOVA and Bonferroni post-hoc tests. A P value of 0.050 was considered as the significance level.

Results

1 sample of the Mueller Hinton Agar

cultured media is shown in figure 1 representing the obtained inhibition zones. In addition, the mean ± standard deviation (SD) related to inhibition zone for groups 1%, 3% and 5% EEP were 0, 0 and 14.5, respectively. As can be seen, it was discovered that the 1% and 3% EEP solutions did not have any significant difference with distilled water. Therefore, 1% and 3% EEP could not be considered as antibacterial agents against S. mutans.

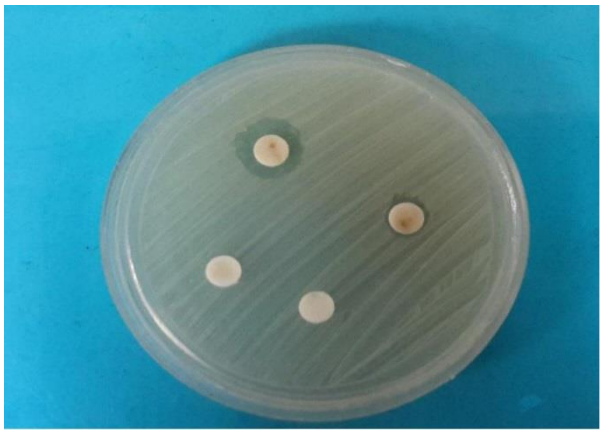

Figure 1. The observe bacterial growth inhibition zone around each sample in agar medium
The upper most, right, bottom and left disks contain 5%, 3%, 1% and 0% of EEP solution respectively.

In contrast, the 5% EEP showed noticeably higher inhibition zone that had a statistically significant difference with other groups ($P < 0.001$).

Moreover, the MIC of propolis samples in the present study was obtained as 2.5 mg/ml.

Furthermore, figure 2 displays micro-tubes incorporated in MIC test. As can be seen, the MIC was obtained as 2.5 mg/ml according to the liquid turbidity.

Discussion

The results of the current study revealed significant antibacterial effect of Kandovan propolis against S. mutans in 5% concentration.

Although the antibacterial potency of propolis has been frequently documented in previous studies, this study was the first one examining the Kandovan propolis against S. mutans.

Figure 2. The minimum inhibitory concentration (MIC) test, which were accomplished by seven, serially diluted concentrations of propolis solution
Three different rows represents triplicate of the test for confirmation, while the distilled water (the most left column) and the pure bacterial suspension (two most right columns) were incorporated as negative and positive controls respectively. Noticeably, the forth column from the left (in every rows) that had 2.5 mg/ml concentration, obtained as the MIC according to its turbidity

This type of propolis is quite important as one of the most important aims of the studies in natural medicine include discovering the most effective origin of each natural product for the specific property.[9-14,19,20] In fact, it has been strongly documented that the geographic origin of the propolis could have noticeable effect on its antibacterial property.[21] This fact is directly related to the nutrition of the honeybees that influence the chemical composition of the produced propolis.[16,17]

The disk diffusion test in this study showed 14 mm zone of bacterial growth inhibition around the disks containing 5% EEP, however the 1% and 3% solutions did not show any inhibition zone. Therefore, regarding to observations in the present study, only the 5% EEP had antibacterial property and the other two solutions were too weak to have antibacterial effect.

Comparing the results of the present study with the literature confirm that the Kandovan propolis have superior effect compared to the other Iranian propolis types against S. mutans.

In this regard, Jafarzadeh Kashi et al. accomplished a study using propolis samples collected from Tehran, Iran.[19] In this study, the inhibition zone around EEP was 16 mm

while they used 20% EEP. Therefore, the results of the present study showed a considerable stronger activity of Kandovan propolis compared to the samples in the latter study, which were collected from north-east of Tehran. During the extraction process, the propolis samples of the latter study were immersed in ethanol for only 48 hours, however, the samples in the present study experienced a longer period (7 days) of submersion, which could be reason for stronger effect in the present study.

Moreover, in an investigation by Moumen Beyt Elahi et al., the inhibition zone was only 7.8 mm in 30% EEP, which were collected from Hamedan, Iran.[20] Despite immersion of the samples into ethanol for 10 days in this study, which was too longer than the present study, the extracted solution was weaker even in higher doses like 30%.[20] In addition, the clinical mutans samples form the gingival sulcus were incorporated in the latter study, however the standard bacterial samples were used in the present study.

Moreover, stronger effect of EEP against S. mutans has been reported in other studies worldwide. Accordingly, Dziedzic et al. argued that the S. mutans growth was completely stopped in 4-hour activity of 3.0% EEP. However, in 24-hour activity, even the 1.6% EEP also inhibited the bacterial growth.[15] It is noteworthy that the propolis samples in this study were collected from a region situated in the south of Poland.[15]

In addition, a randomized clinical trial was accomplished by Anauate Netto et al. on Brazilian propolis.[14] 2% ethanolic propolis mouthwash was used in this study and the results revealed considerable quantitative reduction of S. mutans and Lactobacilli bacteria among patients.

Therefore, the antibacterial capacity of propolis is strongly supported by available evidences, however this property is directly related to the chemical composition of the propolis, which is drastically dependent on its geographic origin.[16,17,21]

The possible mechanism correspondence for antibacterial effect of propolis is related to its various compositions including flavonoids, caffeic acid, and cinnamic acid.[15,22-25] These ingredients would disrupt the bacterial cytoplasmic membrane and cause bacteriolysis. Additionally, it has been documented that these chemicals could inhibit the process of protein synthesis in bacterial cell.[15,26] However, the comprehensive mechanism behind the antibacterial property of propolis is not discovered yet.

In general, although the in-vitro tests do not reproduce the real clinical situations, it was demonstrated in the present study that the propolis extracted from the Kandovan region could be incorporated as an effective agent in preventive dentistry. However, further studies are strongly suggested to resemble the clinical effectiveness of Kandovan propolis. Furthermore, complementary investigations are recommended to detect the detailed chemical composition of different Iranian propolis samples and compare their antibacterial effects with each other.

Conclusion

Despite the limitations in this study, significant anti-bacterial effect against S. mutans was reported for 5% EEP, however the 1% and 3% EEP did not show any antibacterial capacity. In addition, the MIC was obtained as 2.5 mg/ml.

Conflict of Interests

Authors have no conflict of interest.

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