Emerging Medical Science
Journal Homage: https://emspub.com
Volume 1 issue 1 (September 2022)

Comparison between the effect of Propolis and honey on oral flora: An in vivo Study

Abdulrhman Alkhaled
Tishreen university

Abstract:

Background: Oral rinses are one of the means of the oral health care, and natural and plant-based mouth rinses are a good alternative to synthetic rinses with chemical composition such as Chlorhexidine and Listerine, and because of this, interest in natural alternatives such as (honey and propolis), which have proven their effectiveness over time.

Objectives: the aim of the research is to evaluate the effect of mouthwashes honey 50% - propolis 5% on oral flora in children.

Material and methods: A laboratory study to assess the efficiency of honey and Propolis on oral flora counts. The sample included 40 children divided into: G1 (Honey 100% = 20 children)G2 (Propolis 5% = 20 children). The data was analyzed using the statistical analysis program SPSS, version 13.00, at a confidence level of 95% (P <0.05(. Paired sample T test was used to compare the variable means of the values of the studied variables.

Results: There is significant difference between two groups. The reduction ratio was 55.44% in G1 and 74.78% in G2.

Conclusion: This study demonstrated the effectiveness of mouthwashes of propolis and honey on the bacteria of oral flora counts.

Keywords : oral flora - honey - propolis.

Introduction:

The human oral cavity contains a number of places that may be a home to oral germs; such as the teeth, the gingival gutter, the tongue, the cheeks, the hard and soft palate, and the tonsils. The oral flora consists of more than 600 predominant bacterial species, with distinct subgroups prevalent at different oral sites [1]

Oral rinses are a chemotherapeutic agent that patients use to improve oral health as an effective method of home care. Many mouthwash manufacturers say they are antiseptic and anti-bacterial, and reduce dental caries, gingivitis, and bad breath caused by plaque. Oral rinses can be preventative and disinfecting[2, 3]

Honey is a natural food compound, sticky in texture, with a sweet taste, ranging in color from light brown to dark brown, depending on the area where the bees are located.

After collecting it, worker bees add compounds to it, and then place it in the hexagonal cells of the waxy beehive, until it becomes suitable for human consumption[4]

Scientific studies have shown that honey is effective against nearly 60 types of Gram-positive and gram-negative anaerobic and aerobic oral bacteria, and one of the most important of these bacteria is Streptococcus mutans, a pathogen that is widely involved in dental caries [5]

Manuka honey has been used in traditional medicine in New Zealand for a long time for its antiseptic properties. (English *et al.*, 2004), and the results of one study indicated the possible therapeutic role of mouthwashes containing Manuka honey in gingivitis and periodontal disease[6]

Propolis, commonly known as bee glue, is a complex mixture of resinous materials of dark brown or greenish color with an aromatic odor. Bees collect it from different parts of plants (twigs, flowers, buds, pollen), and from many trees (beech, pine, palm, poplar, birch). It is then treated in the beehive by worker bees by adding salivary secretions, wax and pollen to it [7] [8]

It consists of 55% colloidal materials, 3% wax balm, 10% volatile oils, 5% pollen - 14 acids from derivatives of cinnamic acid and 12 acids derived from benzoic acid, and other compounds from terpenes and some carbohydrates. The concentrations differ based on botanical and geographical origin.

Propolis contains fats, organic acids, vitamins (A, E, and B), as well as the minerals aluminum, vanadium, iron, calcium, manganese, silicon and strontium [8] [9]

Many studies confirmed the antibacterial effect of propolis against other bacteria that cause dental caries (in vivo and in vitro), as it showed that propolis inhibits the growth of Streptococcus sobrinus, Streptococcus mutans and Streptococcus cricetus, which are known to contribute to the occurrence of dental caries. It also showed that drinking water infused with propolis reduced by 50-60% the occurence of dental caries in the teeth of rats infected with S. sobrinus bacteria, and propolis also reduced the caries in rats[9]

[10] investigated the effectiveness of propolis-containing toothpastes and their effect on the oral environment of patients who had recently had implants via compensation procedure, and the study concluded the benefit of using these toothpastes in accelerating recovery after oral surgery, especially in patients predisposed to infection and periodontal diseases.

Study aim:

Evaluation of the antibacterial efficacy of mouthwash composed of 50% honey and 5% propolis, on the oral flora in children aged 6-12 years.

Materials and methods:

The study included 40 children (20 males and 20 females) ranging in age from 6 to 12 years, with good oral health, who did not suffer from infections in the supportive tissues, did not wear fixed or immobile braces, and did not take antibiotics.

The written consent was taken from the volunteers and guardians before starting the study according to a special form designed for the research that includes details about the research and the materials used.

The children were divided into two groups, each group contained 20 children (20 honey children and 20 propolis children).

A first saliva sample was taken before using the rinse using a sterile salivary swab for this procedure. The procedure included passing the swab head over the vestibular surfaces of the teeth, the dome of the palate, the floor of the mouth and the vestibule of the cheek. After that, the children were asked to rinse their mouths with their own solution using 10 ml of the solution, for a period of 30 seconds. We then took a

second swab for each child, similar to the procedures for the first swab, and the child's data (name, age, gender, and swab before / after) was recorded on each sample and sent to the bacterial culture laboratory in Hama National Hospital, Hama city, to start the laboratory work procedures.

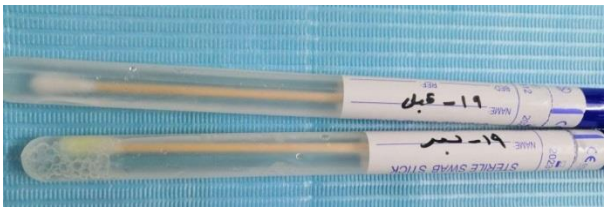


Figure 1 : sterile salivary swab (befor after)

Honey sample (20 children):

Because the honey was 100% concentrated provided by B Pharma Company for Pharmaceutical Industries, Syria, we extracted 5 ml of honey and added it to 10 ml of distilled water in a sterile sample collection container. It was then mixed and given to the child to use for rinsing his mouth.

Propolis sample: (20 children)

The mouthwash used is a ready-to-use 5% propolis solution produced by Tact Company for Essential Oils, Syria.

Distilled water: Provided by Siraj National Company, Syria

The study was done using the Agar Well Diffusion Method, and cultivation was done on blood agar.

procedures for bacterial culture in the laboratory:

Serial dilution for sample :

We dilated the saliva samples in two stages to reduce the bacterial load for ease of counting, provided that the real concentration of germs is calculated later as follows:

Stage one:

We extracted 10000 microliter (10 ml) of saline by disposable single-use syringe and put it in the glass tube. We then discarded 100 microliter thus having 9900 microliter remaining in the tube. We then added 100 microliter of the saliva sample by micropipette. Finally we got dilate of saliva sample in 1/100 ratio (10^{-2}). We then mixed the homogenous dilate saliva sample on vibrator for 30 seconds.

Stage two:

We repeated the previous stage but by adding 100 microliter (10^{-2}) of dilate solution to 9900 microliter from the saline in the other glass tube and the ratio became 1 / 10000 (10^{-4}) and then repeated the homogenization process by vibrator.

Bacterial culture:

We took 12 µl of the diluted solution (10^{-4}) using a micropipette tip and isolated it on the surface of the blood agar using a sterile platinum Inoculation loop (as shown in figure 2) in a way that allowed obtaining clear isolated colonies. Then the dish was covered and placed in the incubator at 37°C for 24 hours.

Figure 2 sterile platinum Inoculation loop

Colonies Count:

We counted the colonies under the magnifying glass of the counting device located in the laboratory and recorded the results in the search table.

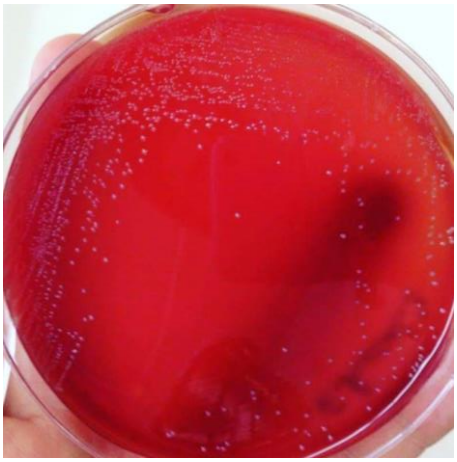


Figure 3 Salivary flora count before mouthwash

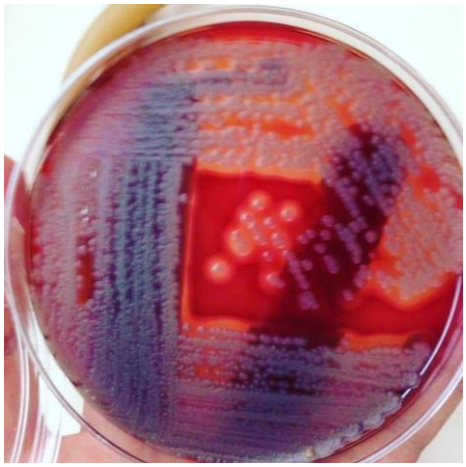


Figure 4 Salivary flora count after mouthwash

Statistical analysis:

The data were analyzed using the statistical analysis program SPSS, version 13.00 at a confidence level of 95% ($P<0.05$).

We used the Paired sample T test to compare the arithmetic averages of the values of the studied variables, and to calculate the percentages of the decrease in the general census variable.

Results:

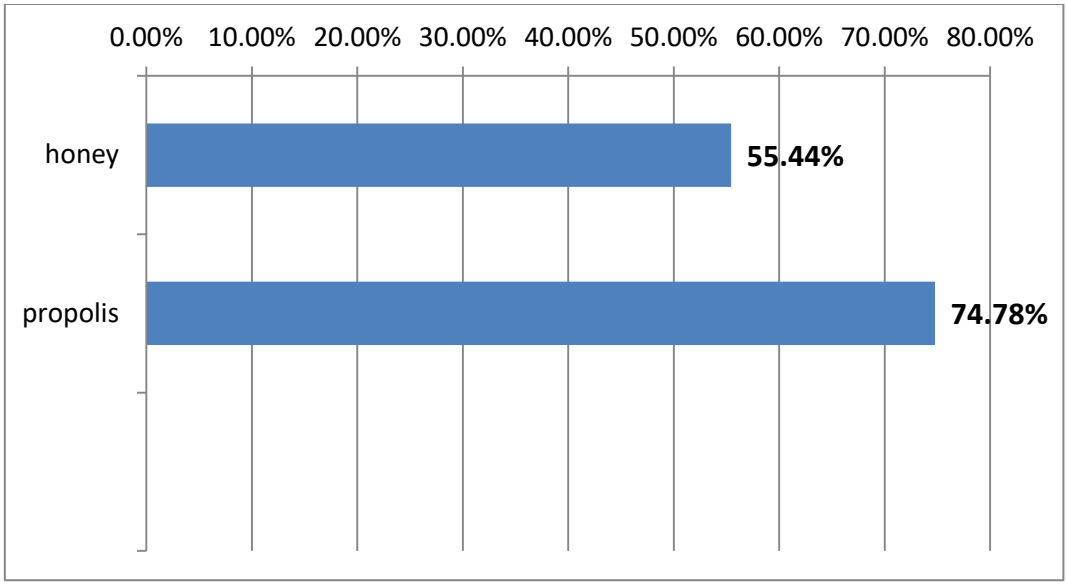
Table (1) Descriptive statistical measures of the oral flora census variable before and after using the substance in the three experimental groups.

Material	Sample	Studied variables	mean	SD	minimal value	max value
honey	20	number of oral flora before using honey	167.85	53.025	100	258
		number of oral flora after using honey	74.80	23.460	44	115
propolis	20	number of oral flora before using propolis	196.70	57.576	109	299
		number of oral flora after using propolis	49.60	13.108	31	74

Table (2) The results of using a double-sampled student's T-test.

Materials	Comparisons		difference between arithmetic means	T Test value	df	P-value	description
honey	Pair 1	number of oral flora before using honey - number of oral flora after using honey	93.05	14.059	19	0.000	There are statistically significant differences
propolis	Pair 1	number of oral flora before using propolis - number of oral flora after using propolis	147.10	14.316	19	0.000	There are statistically significant differences

Figure (5) The percentages of the decrease in the variable total number of oral flora between the two times (before using the substance and after using the substance) among the three experimental groups.



Discussion:

Bacteria have developed antibiotic-resistant strains, and pharmaceutical factories have found it difficult to develop new antibacterial agents to meet this new challenge due to the high costs of drug research, which prompted researchers to search for an alternative from inexpensive natural materials [11]

In developed countries, especially in Europe and North America, about 50% of all medicines used in various treatments are natural products. In large areas of the world, people believe in medicines of natural origin, mostly in the form of plants or plant products [12]

The use of honey has emerged as a promising alternative to industrial pharmaceutical products, after studies have shown the effectiveness of honey against a wide range of clinically resistant multi-organisms [11]

Saliva is a reasonable indicator of complete bacterial load in the oral cavity, and the enumeration of Streptococcus mutans or lactobacilli in total plaque samples does not explain the variance in caries incidence as well as it does when counting them in saliva [13]

In this study, the culture method used solid nutrients to study the oral microbiota, because this method allows qualitative and quantitative evaluation. This allows us to conduct an enumeration of microorganisms, that is referred to as colony forming units (CFU). This is the lowest number of germ cells - a pair, a chain, clusters, or an entire colony - that is found in the center of the surface of the culture medium, and grows to form a colony that can be seen with the naked eye [14]

Blood agar was used as a culture medium, because its properties make it a general growth medium [15]

Figure (5) shows that the rate of decrease in the general bacterial colony count of oral flora was statistically significant after one minute of oral rinses.

The rate of decrease when using propolis rinsing was 74.78%, and when using honey rinsing was 55.44%, at a confidence level of 95%.

The results of this study showed that the effectiveness of propolis was direct and higher on bacterial colony count than honey.

The results of the current study agreed with the results of [16]which confirmed the presence of antibacterial properties in New Zealand honey mouthwash, and the results of the laboratory study [17]on Slovenian honey. This antibacterial effect was attributed to the oxidative action of honey. The obtained results also agreed with the results of a study [18] who studied the antibacterial activity of two types of local honey in South Africa, and with the results of a study [19] in which they compared the antibacterial effect of honey and propolis, and found that propolis has an in vitro antibacterial effect on the general salivary bacteria count. Honey caused bacterial growth at lower concentrations, while at high concentrations it had an inhibitory effect on bacterial growth in the laboratory, where the total salivary count of germs decreased within one hour after applying honey. The antibacterial effect of the tested honey can be attributed to the effect of the osmotic pressure of honeyFinally, the obtained results also agreed with the results of a study [20] in which they tested the antibacterial activity of 11 different samples of propolis from different geographical regions, where all samples were found to be effective against gram-positive strains of bacteria. Australian propolis was 30% and American propolis was 20% (non-alcoholic) effective in inhibiting periodontal pathogenic microorganisms, but the same concentrations were toxic to periodontal fibroblast cells. Therefore, when preparing propolis solutions, we recommend to maintain a minimum concentration because it is effective and safe and will not harm the gingival fibroblasts.

Conclusion

This study demonstrated the effectiveness of 5% propolis and 50% honey mouthwashes on salivary flora. Propolis showed the maximum effectiveness, followed by honey in reducing the general oral flora.

Acknowledgment:

None

Referance :

1. Dewhirst, F.E., et al., *The human oral microbiome.* 2010. **192**(19): p. 5002-5017.
2. Takenaka, S., T. Ohsumi, and Y.J.J.D.S.R. Noiri, *Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis.* 2019. **55**(1): p. 33-40.
3. Yadav, A.R., S.K. Mohite, and C.S.J.A.J.o.R.i.P.S. Magdum, *Preparation and evaluation of antibacterial herbal mouthwash against oral pathogens.* 2020. **10**(3).
4. Fakhlaei, R., et al., *The toxic impact of honey adulteration: A review.* 2020. **9**(11): p. 1538.
5. Ramsay, E.I., et al., *Honey in oral health and care: A mini review.* 2019. **61**(1): p. 32-36.
6. Nayak, P.A., U.A. Nayak, and R.J.C.c.d. Mythili, *Effect of Manuka honey, chlorhexidine gluconate and xylitol on the clinical levels of dental plaque.* 2010. **1**(4): p. 214.
7. Kapare, H.S., L.J.R.J.o.P. Sathiyanarayanan, and Technology, *Nutritional and Therapeutic potential of Propolis: A Review.* 2020. **13**(7): p. 3545.
8. Ripari, N., et al., *Propolis antiviral and immunomodulatory activity: a review and perspectives for COVID-19 treatment.* 2021. **73**(3): p. 281-299.
9. Zulhendri, F., et al., *The use of propolis in dentistry, oral health, and medicine: A review.* 2021.
10. Morawiec, T., et al., *The biological activity of propolis-containing toothpaste on oral health environment in patients who underwent implant-supported prosthodontic rehabilitation.* 2013. **2013**.
11. Zainol, M.I., et al., *Antibacterial activity of selected Malaysian honey.* 2013. **13**(1): p. 1-10.
12. Dias, D.A., S. Urban, and U.J.M. Roessner, *A historical overview of natural products in drug discovery.* 2012. **2**(2): p. 303-336.
13. Sullivan, Å., et al., *Number of mutans streptococci or lactobacilli in a total dental plaque sample does not explain the variation in caries better than the numbers in stimulated whole saliva.* 1996. **24**(3): p. 159-163.
14. Krieger, R.I., *Hayes' handbook of pesticide toxicology.* 2010.

15. Council on Dental Materials, I. and E.J.T.J.o.t.A.D. Association, *Infection control recommendations for the dental office and the dental laboratory.* 1988. **116**(2): p. 241-248.
16. Rupesh, S., et al., *Evaluation of the effects of manuka honey on salivary levels of mutans streptococci in children: A pilot study.* 2014. **32**(3): p. 212.
17. Kunčič, M.K., et al., *Antibacterial and antimycotic activities of Slovenian honeys.* 2012. **69**(4): p. 154-158.
18. Basson, N.J., S.R.J.B.C. Grobler, and A. Medicine, *Antimicrobial activity of two South African honeys produced from indigenous Leucospermum cordifolium and Erica species on selected micro-organisms.* 2008. **8**(1): p. 1-4.
19. Steinberg, D., G. Kaine, and I.J.A.j.o.d. Gedalia, *Antibacterial effect of propolis and honey on oral bacteria.* 1996. **9**(6): p. 236-239.
20. Sonmez, S., et al., *The effect of bee propolis on oral pathogens and human gingival fibroblasts.* 2005. **102**(3): p. 371-376.