

## Microbiological Comparison of Royal Jelly and Chlorhexidine 0.2%<sup>1</sup>

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### Abstract

The aim of this paper is to evaluate the antibacterial property of royal jelly and chlorhexidine 0.2%. As a methodology, in our study, we used piastres in blood agar, where the holes in the agar field were made through a glass pipette, sterile "Paster", in a diameter of 7 mm. Used a bacterial culture of Streptococcus gr. D (Enterococcus faecalis) in a concentration of  $10^5$ , which was distributed in sterile condition, using a sterile tampon, according to the method of diffusion in agar. As a result, we used a ruler for the measurement of inhibition areas: -in the royal jelly's hole, the radius of inhibition resulted 14 mm, -in the chlorhexidine's hole, the radius of inhibition resulted 20 mm. Based on the results obtained from our study, presented facts to use the royal jelly and chlorhexidine 0.2% in the dental practice. As a conclusion, we can say that the royal jelly contains important elements with antibacterial action compared to the chlorhexidine one.

**Keywords:** chlorhexidine, diffusion, inhibition, royal jelly.

### Introduction

Nowadays, royal jelly has considerable commercial appeal and is utilized in many sectors, ranging from the pharmaceutical and food industries to the cosmetic and manufacturing sectors. Numerous studies have been dedicated to royal jelly since as far back as the late 19<sup>th</sup> century [7,8,9]. Additional complicating factors are the multiplicity of experimental conditions, as well as the diversity of the analytical methods used and their continual evolution [14,15]. Knowledge of the composition of recently produced royal jelly is essential in order to define a standard composition, evaluate the quality of commercial products and detect the presence of royal jelly in other products which containing it [13,16,17].

Chlorhexidine is extensively used in various medical fields [3,4]. Chlorhexidine is a bisbiguanide formulation with cationic properties. The molecule is symmetric with two chlorophenyle rings and two bigunide groups connected by a central hexamethylene chain. It is a strong base and is most stable in the form of salts. The most common preparation is the digluconate salt because of its water solubility [2,6]. Chlorhexidine was developed in late 1940s as a result of search for antiviral agents. It was found that chlorhexidine does not possess antiviral activity but instead it possesses antibacterial

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activity. The use of chlorhexidine was begun as a general disinfectant with a broad antimicrobial spectrum. Its antimicrobial spectrum include most of the microbials such as gram positive and gram negative organism including bacterial spores, lipophilic viruses, yeasts and dermatophytes etc [10,11,12].

### Aim

This study was done to evaluate the antibacterial effect of the royal jelly and chlorhexidine 0.2%.

### Methodology

#### Diffusion in Agar

Isolation of bacteria used in this research was done by the extraction of a tooth root (premolar) that presented an apical granuloma situated in a cultural field sterile (heart - brain) for microbiology study.

The two bacteria isolated at the coloring Gram resulted as coccus gram positive and negative catalase. Then agglutination test was done (Biomerieux) to identify the group: one of which resulted to be Streptococcus Group D (Enterococcus faecalis), which was chosen as sample to our tests after microscopically identified (Fig.1a,b,c).

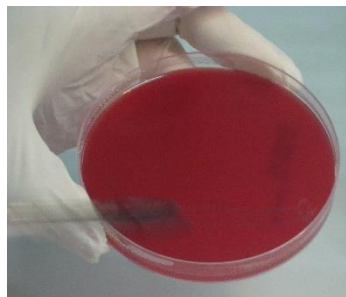


**Fig.1 a,b,c:** These three pictures are for the agglutination test done to identify the group: one of which resulted to be Streptococcus Group D (*Enterococcus faecalis*), which was chosen as sample to our tests, after microscopically identified.

For the purpose of the study we used a bacterial culture of Streptococcus gr. D (*Enterococcus faecalis*) with a concentration of  $10^5$  (Fig.2), which was distributed in sterile condition, using a sterile swab, according to the diffusion method in agar (Fig.3). We used blood agar plates and through a sterile glass pipet "PASTER" with a 7 mm diameter we made holes in the agar.



**Fig.2:** 50  $\mu$ l of bacterial concentration of  $10^5$ .



**Fig.3:** The bacteria (*E.faecalis*) distribution in sterile condition, using a sterile swab, according to the diffusion method in agar.

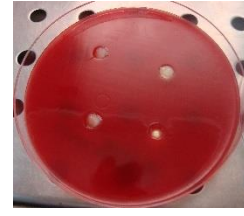
In one of the holes was added chlorhexidine (positive control) at a concentration of 0.20% (Fig.4), then royal jelly (positive control) was added in the other holes (Fig.5). Royal jelly appeared gelatin, of white color, aromatic flavor and taste acidic (pH 3.5 to 4.5). While in the last hole was added physiological solution (negative control). Plates were incubated at 37°C for 48 hours in a thermostat (Fig.6).



**Fig.4:** Chlorhexidine 0.2% taken for the test, an active ingredient that fights bacteria in the mouth.



**Fig.5:** A sample of royal jelly used for our microbiological study.



**Fig.6:** Plate before incubation.

## Results

Zones of inhibition were measured by a ruler after the incubation time of 48 hr (Fig.7):

-in the chlorhexidine's hole, the radius of inhibition resulted 20 mm (Fig.8),

-in one the royal jelly's hole (biggest one), the radius of inhibition resulted 14 mm (Fig.9).

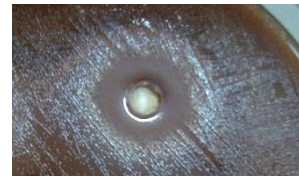
The hole with physiological solution resulted with no inhibition radius (negative control).



**Fig.7:** This is the incubated plate at 37°C after 48 hours in a thermostat.



**Fig.8:** This is the hole with chlorhexidine 0.2% where the radius of inhibition resulted as 20 mm (positive control).



**Fig.9:** This is the hole with royal jelly where the radius of inhibition resulted as 14 mm.

## Discussion

Microorganisms such as *E. faecalis*, *S aureus*, *S. mutans*, *E. coli*, and *C. albicans* are known to cause wide range of oral infections such as dental caries, periodontal diseases, and periimplant diseases due to the formation of biofilm. [1,3,5]

*In vitro* the agar diffusion technique was done to evaluate the antibacterial properties of royal jelly and chlorhexidine 0.2%. The results of this study confirmed the antibacterial efficacy of these extracts. Being in different physical conditions (royal jelly as a gelatinous and the chlorhexidine 0.2% as a liquid one), microbiological results showed, although in the gelatinous concentration, the inhibited radius of royal jelly was approximately to the chlorhexidine's one.

## Conclusion

We arrived a conclusion that royal jelly contained important elements with antibacterial property in comparison with that of chlorhexidine 0.2%. This significant result can be as a basic help for the treatment of the dental inflammations.

## Bibliography

- [1] Filoche S, Wong L, Sissons CH. Oral biofilms: Emerging concepts in microbial ecology. *J Dent Res* 2010;89:8-18.
- [2] Jarvinen, H., Tenovuo, J. and Huovinen, P.: *In vitro* susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents. *Antimicrob Agents Chemother* 37: 1158-1159, 1993.
- [3] Kolenbrander, P.E.: Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol* 54: 413-437, 2000.

- [4] Leikin, Jerrold B.; Paloucek, Frank P., eds. (2008), "Chlorhexidine Gluconate", *Poisoning and Toxicology Handbook* (4th ed.), Informa, pp. 183–184.
- [5] Lercker, G (2003) La gelatina reale: composizione, autenticità ed adulterazione. In Atti del Convegno "Strategie per la valorizzazione dei prodotti dell'alveare". Università degli Studi del Molise; Campobasso; pp. 67-81.
- [6] Lercker, G; Caboni, M F; Vecchi, M a; Sabatini, a G; Nanetti, a (1993) Caratterizzazione dei principali costituenti della gelatina reale. *Apicoltura* 8: 27-37.
- [7] LI, J K; WANG, T; PENG, W J (2007) Comparative analysis of the effects of different storage conditions on major royal jelly proteins. *Journal of Apicultural Research* 46 (2): 73-80. DOI: 10.3896/IBRA.1.46.2.02
- [8] Marsh PD. Dental plaque as a biofilm and a microbial community-implications for health and disease. *BMC Oral Health* 2006;6 Suppl 1:S14.
- [9] Marsh, P.D.: Microbiological aspects of the chemical control of plaque and gingivitis. *J Dent Res* 71: 1431-1438, 1992.
- [10] National Committee for Clinical Laboratory Standards: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. Wayne, PA: NCCLS. 2000.
- [11] Pourtallier, J; Davico, R; Rognone, M C (1990) Les analyses dans le contrôle de pureté de la gelée royale. *Abeille de France et l'Apiculteur* 753: 405-407.
- [12] Ricciardelli D'albore, G; Battaglini, M; Bernardini, M (1978) Origine géographique de la Gelée royale. *Apidologie* 9: 1-17.
- [13] Serra Bonvehi, J; Escola Jorda, R (1991) Studie über die mikrobiologische Qualität und bakteriostatische Aktivität des Weiselfuttersaftes (Gelée Royale): Beeinflussung durch organische Säuren. *Deutsche Lebensmittel-Rundschau* 87: 256-260.
- [14] Stocker, a (2003) Isolation and characterisation of substances from Royal Jelly. PhD Thesis; Université d'Orléans; Orléans (France).
- [15] Takenaka, T; Echigo, T (1980) General chemical composition of the royal jelly. *Bulletin of the Faculty of Agriculture, Tamagawa University* 20: 71-78.
- [16] Jenkins S, Addy M, Wade W (August 1988). "The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo". *J. Clin. Periodontol.* **15** (7): 415–24.
- [17] Helms JA, Della-Fera MA, Mott AE, Frank ME (October 1995). "Effects of chlorhexidine on human taste perception". *Arch. Oral Biol.* **40** (10): 913–20.