



# Article Methylglyoxal, the Major Antibacterial Factor in Manuka Honey: An Alternative to Preserve Natural Cosmetics?

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**Abstract:** Microbial safety is an essential prerequisite of cosmetics, and preservatives are required to prevent product spoilage and damage to consumers' health. Consumer concern about the safety of some cosmetic ingredients and the increasing demand for more natural beauty products has driven cosmetic industries and formulators to find natural alternatives to replace synthetic preservatives currently used. In this study, methylglyoxal (MGO, the main factor responsible for the antimicrobial activity of manuka honey) was tested for antimicrobial activity against a panel of selected bacteria and mycetes by using conventional microbiological techniques (determination of M.I.C., time-kill assay), and its potential preservative in an O/W emulsion was investigated (challenge test). MGO showed a remarkable and fast antibacterial activity (M.I.C. values 0.150–0.310 mg/mL), while the inhibitory activity against fungi was less marked (M.I.C. values 1.25–10 mg/mL); chitosan has proven to be a synergist of antimicrobial effectiveness of MGO. Results of the challenge test showed that the addition of MGO to a cream formulation was efficient against microbial contamination. On the basis of our results, MGO appears to be a good candidate as a cosmetic preservative of natural origin; further studies are needed to confirm its applicability and its safety.

Keywords: methylglyoxal; cosmetic preservatives; antibacterial activity; antifungal activity; chitosan

# 1. Introduction

Microbial contamination and growth can occur in cosmetics and personal care products during the manufacture, storage, and particularly during the period of use [1]. Multiplication of microorganisms may cause spoilage of cosmetic formulations, but can also constitute a threat to consumer health, especially when cosmetics are intended for use in areas of particular concern or when they are used by young children or immunocompromised subjects. Therefore, preservatives that are able to prevent microorganisms from growing play a crucial role, particularly in formulations containing water. In the European Union, only preservatives listed in Annex V of Regulation (EC) [2] can be used in cosmetics and personal care products. Unfortunately, exposure to some of these substances can produce undesirable effects, such as skin irritation and sensitization [3], and some of them are recognised as potential endocrine disruptors [4]. Moreover, a hypothetical connection between cosmetic preservatives—namely parabens—and breast cancer was recently suggested [5,6], although this link has been denied in a relatively recent review of literature [7]. These concerns and the rising consumer demand for more natural cosmetics are driving the cosmetic industry to find natural, safer alternatives to synthetic preservatives. In that context, a number of botanical extracts and essential oils have been tested in vitro as potential cosmetic preservatives, sometimes with promising results [8–11]. As part of the research about potential natural preservatives, a recent study [12] investigated the preservative

efficacy of manuka honey, a New Zealand honey well known for its pronounced antimicrobial activity also against clinical multi-resistant isolates [13–15], in an O/W emulsion. Manuka honey was proven to be more effective than methylparaben against *Pseudomonas aeruginosa* and showed the same efficacy of this synthetic compound against Escherichia coli; however, it did not reveal inhibitory activity against fungal contamination [12]. Methylglyoxal (MGO), a 1,2-dicarbonyl compound present in manuka honey in exceptionally high levels (38–761 mg/kg), is the main factor responsible for its bactericidal activity [16,17]; however, neutralization of MGO reduced but did not completely eliminate antibacterial activity, which proves the presence in manuka honey of other antibacterial factors [18]. The aim of the present investigation was to evaluate the potential of MGO as an alternative natural preservative intended for cosmetics and personal care products, by using conventional microbiological techniques (M.I.C. and M.B.C. evaluation, time-kill test, challenge test). Moreover, it is well known that a combination of preservatives is often a good solution to improve the efficacy of a preserving system and to reduce the side effects associated with the individual substances; in accordance with these considerations, we also investigated an eventual synergistic interaction between MGO and chitosan, a biopolymer obtained by partial deacetylation of chitin that possesses, among others, interesting antimicrobial properties [19].

#### 2. Materials and Methods

#### 2.1. Materials

Methylglyoxal (MGO) solution (40% w/vol in water) was supplied by Sigma Aldrich; before each experiment, it was diluted tenfold in sterile, distilled, MilliQ water, and the resulting solution (4%) was sterilized by filtration using sterile membrane filters (Sartorius, pore size 0.22  $\mu$ m). Chitosan (molecular weight 190–310 kDa; deacetylation degree 75–85%; viscosity Brookfield, 1% solution in acetic acid 200–800 cps; manufacturer values) was supplied by Aldrich (Milwaukee, WI). Its aqueous solution was prepared by dissolving chitosan in hydrochloric acid 0.1 M at 1% w/vol and by evaporating the resulting solution to dryness in a Rotavapor R110 (Buchi, Flawil, Switzerland) at 70 °C under vacuum; the residue was then re-dissolved in 100 mL of Milli-Q water and sterilised by filtration through 0.22  $\mu$ m Sartorious filters. The pH of this solution, evaluated with a pH meter Hanna 8417, was 2.35.

The test organisms used in this study were as follows: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Streptococcus mutans* (ATCC 35668), *Candida albicans* (ATCC 10231), *Rhodotorula mucilaginosa* (ATCC 66034), *Aspergillus brasiliensis* (*niger*) (ATCC 16404) and *Geotrichum candidum* (ATCC 34614) (all purchased from Oxoid-Thermofisher Scientific, Rodano, Italy), and three *Candida* spp. clinical strains (respectively isolated from a vaginal, rectal, and pharyngeal swab, kindly supplied by Dr. M. Tidore, Laboratory of Clinical and Microbiological Analysis of Policlinic Hospital of Sassari, Italy). Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Liquid Medium (SLM), Sabouraud Dextrose Agar (SDA), Peptone Water (PW), Blood Agar Base n°2, sterile defibrinated horse blood, and phosphate-buffered saline tablets (PBS, Dulbecco A, pH 7.3) were purchased from Oxoid-Thermofisher Scientific (Rodano, Italy). Culture media, PBS, and other solutions were prepared with MilliQ water.

#### 2.2. Antibacterial Activity of MGO

The antibacterial activity of MGO was determined as Minimum Inhibitory Concentration (M.I.C.) by using a broth microdilution test performed in 96-well microplates [20]. Twofold dilutions of the 4% solution of MGO, ranging from 1% to 0.007%, were prepared in MHB; control wells contained only liquid medium. All assays were performed at least in triplicate. Microplates were inoculated with about  $1 \times 10^4$  bacteria/well and aerobically incubated at 35 °C for 24 h. After incubation, plates were visually checked for bacterial growth, and the M.I.C. of MGO was defined as the lowest concentration at which no growth was observed. To determine the M.B.C. (Minimum Bactericidal Concentration), aliquots of 2 µL of medium from each well with no visible growth were subcultured onto MHA plates

(or blood agar 5% for *Streptococcus mutans*), which were then incubated at 35 °C for 24 h; M.B.C. was defined as the lowest concentration at which no growth was detectable. Results are reported in Table 1.

#### 2.3. Antifungal Activity of MGO

The antifungal activity of MGO was assessed on *Candida albicans, Rhodotorula mucilaginosa, Geotrichum candidum,* and *Aspergillus brasiliensis* standard strains and on *Candida* spp. clinical isolates. Fungi were grown on SDA plates at 35 °C (*Candida* and *Rhodotorula*) or 25 °C (other fungi), and stock cultures were maintained at 4 °C during all the experiments. M.I.C.s of MGO for *Candida* and *Rhodotorula* strains were determined by using the same broth microdilution assay described in the previous paragraph; twofold dilutions of the 4% solution of MGO, ranging from 1% to 0.007%, were prepared in SLM. All assays were performed at least in triplicate. Microplates were inoculated with about  $1 \times 10^4$  yeasts/well and aerobically incubated at 35 °C for 24 h. After incubation, plates were visually checked for fungal growth, and the M.I.C. of MGO was defined as the lowest concentration at which no growth was observed. To determine the M.F.C. (Minimum Fungicidal Concentration), aliquots of 2 µL of medium from each well with no visible growth were subcultured onto SDA plates, which were then incubated at 35 °C for 24 h; M.F.C. was defined as the lowest concentration at which no growth was detectable. Results are reported in Table 1.

M.I.C.s and M.F.C.s of MGO against *Aspergillus brasiliensis* and *Geotrichum candidum* were determined using an agar macrodilution method [21]. Twofold serial dilutions of MGO in SDA were made in 5 mm Petri dishes (final volume 10 mL) in order to obtain final concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL. The experiments were all performed in triplicate. Control plates containing only SDA were run simultaneously. The agar surface of the plates was then inoculated into the center with  $1-3 \mu L$  of a conidial or yeast cell suspension prepared in sterile distilled water +0.05% Tween-80, containing  $10^3-10^4$  conidia/cells. Plates, wrapped with Parafilm to maintain the correct water activity in the medium, were inverted and incubated at room temperature (about 25 °C). Five days later, plates were visually checked for fungal growth; results are reported in Table 1.

# 2.4. Killing Time Test

The antimicrobial activity of MGO was also characterized by a "killing time" assay performed on *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *C. albicans* ATCC 10231; this test evaluates the reduction of viable microorganism count when a standardized inoculum is incubated with different MGO concentrations in a liquid medium that does not support cell growth. The assay was performed in agreement with Juliano et al. [22]. Microorganisms in the logarithmic phase of growth were centrifuged at 1500 *rpm* for 10 min, washed in PBS, and then re-suspended at a density of  $5 \times 10^5$ – $1 \times 10^6$  colony-forming units (cfu)/mL in appropriate volumes of PBS containing a suitable concentration of MGO (equal or greater than M.B.C.). Control tubes (microorganisms suspended in PBS) were included in each assay. Test tubes were incubated at 35 °C. At time zero and at predetermined intervals (30, 60, and 120 min), 0.5 mL of the suspensions were removed and subjected to serial tenfold dilutions in PBS; aliquots of 0.5 mL of the appropriate dilutions were thoroughly mixed in Petri plates (50 mm diameter) with molten SDA or MHA (45 °C). Plates were then incubated for 24 h at 35 °C; after this time, the number of viable microorganisms at each time was evaluated by counting plates with 30–300 colonies.

#### 2.5. Challenge Test

A challenge test consists of inoculating selected microorganisms (bacteria, fungi) individually into a formulation and determining the log reduction of viable counts at prescribed time intervals in order to evaluate the effectiveness of an antimicrobial preservative. In this investigation, several preliminary challenge tests were performed according to European Pharmacopoeia [23]. The formulation challenged in our experiments was an O/W emulgel composed of 87% (w/w) distilled sterile water, 10% sweet almond oil, and 3% Sepigel 305 (Farmalabor, Canosa di Puglia, Italy) as an emulsifier. To obtain the emulgel, Sepigel 305 was dispersed in water at room temperature, and the oil phase was incorporated in the resulting suspension under mechanical agitation; the result was an emulsion of creamy, gel-like consistency. Samples of emulgel of 50 g were directly prepared in the final containers. Formulations (with suitable MGO concentrations and without MGO as control) were contaminated with 10<sup>5</sup>–10<sup>6</sup> microorganisms/g. Immediately after inoculation, 1 g of each sample was transferred under sterile conditions in a beaker with a magnetic stirring bar, brought to 10 mL with PW, and thoroughly mixed at room temperature on a magnetic stirrer. Finally, tenfold dilutions of the resulting suspension were prepared in saline and spread on plates of appropriate solid media (MHA for bacteria, SDA for mycetes); plates were incubated at 37°C, and the number of colony-forming units (cfu) was evaluated in each plate after 24 h. After the first count (zero time count), inoculated formulations were maintained at 25°C and subjected to the same enumeration of viable microorganisms at time intervals of 24 h, 7, 14, and 28 days. The evaluation of antimicrobial activity was expressed in terms of the log reduction in the number of viable microorganisms compared to the initial inoculum.

## 2.6. Enhancement by Chitosan

The inhibitory activity of chitosan solution against *Ps. aeruginosa*, *E. coli*, and *C. albicans* strains was previously evaluated as M.I.C. and M.B.C./M.F.C. by using the techniques described in the Sections 2.2 and 2.3. Chitosan solution was tested at twofold serial dilutions ranging from 2 to 0.0039 mg/mL in MHB or SLM (pH value of these did not change after the addition of chitosan solution; data not shown). Afterwards, M.I.C. and M.B.C./M.F.C. of MGO were evaluated as previously described in the presence of concentrations of chitosan equal to one-half and one-quarter of its M.I.C. value.

# 3. Results

## 3.1. Antimicrobial Activity of MGO

A preliminary assessment of the antimicrobial activity of MGO was made with conventional microbiological assays (M.I.C. and M.B.C. determination in liquid or solid medium). The analysis of Table 1 points out that MGO presents a good antimicrobial activity against bacterial strains examined, particularly against Gram+ microorganisms (*S. aureus, Str. mutans*), with M.I.C. values of 0.150 mg/mL, while slightly higher concentrations were required to inhibit Gram– bacteria (*E. coli, Ps. aeruginosa*). On the other hand, MGO was found to be rather less active against yeasts (M.I.C.s 0.63–1.25 mg/mL) and against filamentous fungi (7.5–10 mg/mL); these results are in agreement with the lower activity shown by manuka honey against fungi compared with its antibacterial activity [12].

**Table 1.** Inhibitory activity of MGO (expressed in mg/mL and as %w/vol) against bacterial and fungal strains. M.I.C. = Minimum Inhibitory Concentration; M.B.C. = Minimum Bactericidal Concentration; M.F.C. = Minimum Fungicidal Concentration. Results are the average of at least three independent determinations.

Strain	M.I.C.	M.B.C./M.F.C.
Escherichia coli ATCC 8739	0.220 mg/mL (0.022%)	0.310 mg/mL (0.031%)
Pseudomonas aeruginosa ATCC 9027	0.310  mg/mL (0.031%)	0.310 mg/mL (0.031%)
Staphylococcus aureus ATCC 6538	0.150 mg/mL (0.015%)	0.310 mg/mL (0.031%)
Streptococcus mutans ATCC 35668	0.150 mg/mL (0.015%)	0.310 mg/mL (0.031%)
Candida albicans ATCC 10231	0.630 mg/mL (0.063%)	1.25 mg/mL (0.125%)
<i>Candida</i> spp. from rectal swab (1)	1.25 mg/mL (0.125%)	5 mg/mL (0.5%)
<i>Candida</i> spp. from pharyngeal swab (2)	1.25 mg/mL (0.125%)	5  mg/mL (0.5%)
Candida spp. from vaginal swab (3)	1.25 mg/mL (0.125%)	5  mg/mL (0.5%)
Rhodotorula mucilaginosa ATCC 66034	1.25 mg/mL (0.125%)	3.75 mg/mL (0.375%)
Aspergillus brasiliensis ATCC 16404	10 mg/mL (1%)	10 mg/mL (1%)
Geotrichum candidum ATCC 34614	7.5 mg/mL (0.75%)	7.5 mg/mL (0.75%)

#### 3.2. Killing Time Test

The results of this assay are summarized as shown in Figure 1. MGO was tested at concentrations equal or greater than M.B.C. Time–kill curves show that MGO is able to reduce the viability of the microbial population very quickly in a dose-dependent manner (Figure 1). After 60 min of contact with MGO 2 mg/mL, the viable count of *E. coli* was virtually zero, while with MGO 1 mg/mL, it took 120 min to achieve the same result. After this time, MGO 0.310 mg/mL did not kill all the bacteria, but it reduced their number to a very large extent. *S. aureus* viable counts were reduced at a slower rate since MGO 1 mg/mL required 120 min to kill the standard inoculum; moreover, after 120 min, MGO 0.310 mg/mL reduced bacterial viability by over 70%. In the same test, after a 120 min contact with MGO at 1.25 and 5 mg/mL, only 19% and 9% of *C. albicans* survived, respectively; the rate of killing of yeasts was slower than that of bacteria, as indicated by the lower slope of the curves.



Figure 1. Time–kill curves of *E. coli, S. aureus,* and *C. albicans* in PBS in the presence of different concentrations of MGO.

#### 3.3. Challenge Test

Results obtained in the challenge test showed that, as expected, control creams (without any preservative) did not meet the European Pharmacopoeia microbiological standards (data not shown). The addition of 1 mg/mL of MGO to the cosmetic emulsion allowed effective control of the bacterial strains tested (*E. coli, S. aureus*) (Figure 2). In fact, MGO led to the total eradication of bacterial populations after 7 days of testing, meeting criterion A of European Pharmacopoeia referring to bacterial contamination of preparations for cutaneous application (this criterion is satisfied when the total number of microorganisms is reduced in 2 to 3 logarithmic units between the second and the seventh day, and an increase in the bacterial number does not occur to the end of the test, which is after 28 days) [23]. The same criterion referring to fungi is met when the log reduction of microorganisms after 14 days is of two units and no growth occurs at the end of the test; therefore, MGO at 2.5 mg/mL was also found to be effective against *C. albicans* (Figure 2).



**Figure 2.** Challenge test: growth inhibition of different microorganisms in a cosmetic formulation containing MGO.

#### 3.4. Interaction of MGO with Chitosan

When evaluated in the presence of sub-inhibitory concentrations of chitosan (1/4 and 1/2 of M.I.C. value), M.I.C. and M.B.C. of MGO were reduced in a concentration-dependent manner (Table 2); this effect was particularly evident for *E. coli* (M.B.C. of MGO was reduced by a factor of ten in the presence of a concentration of chitosan equal to 1/2 M.I.C.), while in the same condition, M.B.C. of *Candida* spp. strain 1 was reduced by four times. These results show that, in our experimental conditions, the combination of MGO and chitosan leads to their synergistic interaction, with an improvement in antimicrobial efficiency.

**Table 2.** Effect of sub-inhibitory concentrations of chitosan on the antimicrobial activity of MGO. M.I.C. = minimum inhibitory concentration; M.B.C. = minimum bactericidal concentration; M.F.C. = minimum fungicidal concentration. Results are the average of at least three independent determinations.

	Escherichia coli	Pseudomonas aeruginosa	Candida spp. Strain 1
M.I.C. Chitosan	0.063 mg/mL	0.25 mg/mL	1 mg/mL
M.B.C. Chitosan	0.063 mg/mL	0.5 mg/mL	1 mg/mL
M.I.C. MGO	0.220 mg/mL	0.310 mg/mL	1.25 mg/mL
M.B.C/M.F.C. MGO	0.310 mg/mL	0.310 mg/mL	5 mg/mL
M.I.C. MGO + 1/2 M.I.C. Chitosan	0.015 mg/mL	0.15 mg/mL	1.25 mg/mL
M.B.C. MGO + 1/2 M.I.C. Chitosan	0.031 mg/mL	0.15 mg/mL	1.25 mg/mL
M.I.C. MGO + 1/4 M.I.C. Chitosan	0.07 mg/mL	0.310 mg/mL	2.5 mg/mL
M.B.C. MGO + 1/4 M.I.C. Chitosan	0.07 mg/mL	0.310 mg/mL	2.5 mg/mL

# 4. Discussion

This preliminary work aimed to evaluate the antimicrobial activity of MGO and its preservative potential in a cosmetic formulation, with the objective to find out whether this compound can provide a viable alternative to the conventional preservatives currently used. Our results showed that MGO possesses a pronounced antibacterial activity with minor antifungal properties; its inhibitory effect occurs in rather short times, as demonstrated by the killing time test. In the preliminary challenge tests carried out, MGO, at the concentrations used, conformed to the criteria of the European Pharmacopoeia against the microorganisms tested; therefore, MGO appears to be eligible for a future possible use as an alternative cosmetic preservative. In view of this, further investigations are mandatory. The first issue to be addressed concerns safety. MGO is a molecule produced endogenously in the body in several metabolic pathways (primarily through anaerobic glycolysis) [24]; it is also found in food products of both animal and plant origin, with particularly high levels reported in Manuka honey and other honeys, brewed coffee, soya sauce, toast, and soft drinks [25]. MGO is of low acute toxicity; there is in vitro evidence that MGO is genotoxic, but the in vivo relevance is unclear [25], and the International Agency for Research on Cancer (IARC) has determined MGO to be not classifiable as a

carcinogen [26]. Moreover, manuka honey, containing exceptionally high levels of MGO, has a long history of safe use also in wound management; taken together, literature data suggest that MGO can be considered safe, even if further toxicological investigations into its topical application will be indispensable. From a technological point of view, MGO presents some advantages. It is characterised by an interesting antibacterial activity, and it significantly reduces the microbial population in the challenge test in compliance with the criteria of European Pharmacopoeia. It is also inexpensive and is characterised by high water solubility, being therefore able to protect the aqueous phase of emulsions from microbial contamination. Moreover, the association with sub-inhibitory concentrations of chitosan increases the synergistically antimicrobial efficacy of MGO. This is of great interest because chitosan is a biopolymer widely employed in cosmetics and personal care products for its many and specific properties, such as antimicrobial, film-forming, antioxidant, moisturizing, and conditioning [27], and its combination with MGO could lead to an efficient preservative system of natural origin. On the other hand, specific investigations would be required to verify the compatibility of MGO with other ingredients of formulations and with packaging materials in order to assess MGO stability over time and to determine appropriate storage conditions.

To conclude, to the best of our knowledge, this study was the first attempt to apply MGO as a preservative of natural origin in cosmetics. The results obtained are promising, but further studies are obviously needed to confirm the preservative activity of MGO in different cosmetic formulations, as well as its safety.

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## References

- Halla, N.; Fernandes, I.P.; Heleno, S.A.; Costa, P.; Boucherit-Otmani, Z.; Boucherit, K.; Rodrigues, A.E.; Ferreira, I.C.F.R.; Barreiro, M.F. Cosmetic preservation: A review on present strategies. *Molecules* 2018, 23, 1571. [CrossRef] [PubMed]
- 2. European Commission (EC). Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. *Off. J. Eur. Union* **2009**, *27*, 59–209.
- 3. Yim, E.; Baquerizo Nole, K.L.; Tosti, A. Contact dermatitis caused by preservatives. *Dermatitis* **2014**, *25*, 215–231. [CrossRef] [PubMed]
- 4. Nohynek, G.J.; Borgert, C.J.; Dietrich, D.; Rozman, K.K. Endocrine disruption: Fact or urban legend? *Toxicol. Lett.* **2013**, 223, 295–305. [CrossRef] [PubMed]
- 5. Golden, R.; Gandy, J.; Vollmer, G. A review of the endocrine activity of parabens and implication for potential risks to human health. *Crit. Rev. Toxicol.* **2005**, *35*, 435–458. [CrossRef]
- Darbre, P.D. Environmental oestrogens, cosmetics and breast cancer. *Best Pract. Res. Clin. Endocrinol. Metab.* 2006, 20, 121–143. [CrossRef]
- 7. Witorsch, R.J.; Thomas, J.A. Personal care products and endocrine disruption: A critical review of the literature. *Crit. Rev. Toxicol.* **2010**, *40* (Suppl. 3), 1–30. [CrossRef]
- 8. Maccioni, A.M.; Anchisi, C.; Sanna, A.; Sardu, C.; Dessì, S. Preservative systems containing essential oils in cosmetic products. *Int. J. Cosmet. Sci.* **2002**, *24*, 53–59. [CrossRef]
- Muyima, N.Y.O.; Zulu, G.; Benghu, T.; Popplewell, D. The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. *Flavour Fragr. J.* 2002, 17, 258–266. [CrossRef]
- 10. Ibarra, F.; Johnson, G.H. Natural preservatives from concepts in nature. Cosmet. Toiletries 2008, 123, 81–90.
- 11. Herman, A.; Herman, A.P.; Domagalska, B.W.; Mlynarczyk, A. Essential oils and herbal extracts as antimicrobial agents in cosmetic emulsions. *Indian J. Microbiol.* **2013**, *53*, 232–237. [CrossRef] [PubMed]

- 12. Juliano, C.; Gavini, E.; Giunchedi, P.; Magrini, G.A. Evaluation of Manuka honey as an adjuvant antimicrobial preservative in a O/W emulsion. *J. Appl. Cosmet.* **2016**, *34*, 87–98.
- George, N.M.; Cutting, K.F. Antibacterial honey (Medihoney<sup>TM</sup>): In-vitro activity against clinical isolates of MRSA, VRE and other multiresistant Gram-negative organisms including *Pseudomonas aeruginosa*. Wounds 2007, 19, 231–236. [PubMed]
- 14. Lin, S.M.; Molan, P.C.; Cursons, R.T. The in vitro susceptibility of *Campylobacter* spp. to the antibacterial effect of manuka honey. *Eur. J. Clin. Microbiol. Infect. Dis.* **2009**, *28*, 339–344. [CrossRef] [PubMed]
- 15. Majtan, J.; Bohova, J.; Horniakova, M.; Klaudiny, J.; Majtan, V. Anti-biofilm effects of honey against wound pathogens *Proteus mirabilis* and *Enterobacter cloacae*. *Phytother. Res.* **2014**, *28*, 69–75. [CrossRef] [PubMed]
- Adams, C.J.; Boult, C.H.; Deadman, B.J.; Farr, J.M.; Grainger, M.N.; Manley-Harris, M.; Snow, M.J. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* 2008, 343, 651–659. [CrossRef] [PubMed]
- Mavric, E.; Wittmann, S.; Barth, G.; Henle, T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol. Nutr. Food Res.* 2008, 52, 483–489. [CrossRef] [PubMed]
- Kwakman, P.H.S.; te Velde, A.A.; de Boer, L.; Vandenbroucke-Graus, C.M.J.E.; Zaat, S.A.J. Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE* 2011, 6, e17709. [CrossRef]
- 19. No, H.K.; Park, N.Y.; Lee, S.H.; Meyers, S. Antibacterial activity of chitosans and chitosan oligomers with different molecular weight. *Int. J. Food Microbiol.* **2002**, *74*, 65–72. [CrossRef]
- 20. Thrupp, L. Susceptibility testing of antibiotics in liquid media. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Lorian, V., Ed.; Williams and Wilkins: Baltimore, MD, USA, 1986; pp. 93–158. ISBN 0-683-05167-9.
- McGinnis, M.R.; Rinaldi, M.G. Antifungal drugs: Mechanisms of action, drug resistance, susceptibility testing and assays of activity in biological fluids. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Lorian, V., Ed.; Williams and Wilkins: Baltimore, MD, USA, 1986; pp. 223–281. ISBN 0-683-05167-9.
- 22. Juliano, C.; Demurtas, C.; Piu, L. *In vitro* study on the anticandidal activity of *Melaleuca alternifolia* (tea tree) essential oil combined with chitosan. *Flavour Fragr. J.* **2008**, *23*, 227–231. [CrossRef]
- 23. European Pharmacopoeia Commission. *European Pharmacopoeia*, 7th ed.; European Directorate for the Quality of Medicines & Healthcare (EDQM): Strasbourg, France, 2011.
- 24. Kalapos, M.P. Methylglyoxal in living organisms. Chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* **1999**, *110*, 145–175. [CrossRef]
- 25. Committee on Toxicity of Chemical in Food, Consumer Products and the Environment. Statement on Methylglyoxal. 2009. Available online: https://cot.food.gov.uk/committee/committee-on-toxicity/cotstatements/cotstatements/cotstatements2009/cot200904 (accessed on 23 September 2018).
- 26. IARC. Coffee, tea, mate, methylxanthines and methylglyoxal. IARC Working Group on the evaluation of carcinogenic risks to human. *IARC Monogr. Eval. Carcinog. Risks Hum.* **1991**, *51*, 1–513.
- 27. Aranaz, I.; Acosta, N.; Civera, C.; Elorza, B.; Mingo, J.; Castro, C.; de Los Llanos Gandía, M.; Caballero, A.H. Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives. *Polymers* **2018**, *10*, 213. [CrossRef]



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