OP-017 [Apitherapy]
Antibacterial activity of honey in wound and oral environments: the latest in vitro findings

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BACKGROUND/PURPOSE: Antibacterial activity of honey has been considered as a major factor determining wound healing of acute and chronic wounds. Indeed, several antibacterial compounds have been identified and characterized including hydrogen peroxide (H2O2), defensin-1 and methylglyoxal (MGO). However, antibacterial efficacy of honey in wound and mouth environment remains unexplored. Therefore, the aim of the study was to characterize the in vitro antibacterial effect of different types of honey in wound-like environment (artificial wound fluid) and oral-like environment (artificial human saliva).

PROBLEM DESCRIPTION: Wound and oral environments represent favorable environments for different bacteria including pathogens producing a broad spectrum of proteolytic enzymes. Overall antibacterial efficacy of bee-derived proteinous honey components (e.g., glucose oxidase, defensin-1) in these hostile environments might be reduced due to their cleavage by bacterial/human proteolytic enzymes.

METHODOLOGY: Various types of honey including manuka honey with different MGO grading and medical-grade honeys were used in the study. Antibacterial activity of honey was determined by a broth dilution assay against selected Gram-positive and Gram-negative bacterial isolates in the absent/present of human artificial wound fluid and saliva. The GOX activity and H2O2 concentration in honey solutions were determined with a Megazyme GOX assay kit. The protein profile of honey samples was evaluated by SDS-PAGE and GOX enzyme was determined by immunoblotting.

RESULTS and CONCLUSION: The most effective honey against all tested bacteria was manuka honey and its medical product and their efficacy was enhanced with increasing amount of MGO. Artificial saliva did not affect the antibacterial activity of all honeys against oral bacteria. However, it significantly reduced the antibacterial activity of honey samples except manuka honey against non-oral pathogens. Furthermore, a significant reduction of GOX activity and a subsequent decrease in H2O2 content were observed in non-manuka honeys. The composition of the wound exudate significantly affected the antibacterial and bactericidal activity of the tested honeys, mainly against Staphylococcus aureus. Incubation of honey samples with artificial wound exudate did not cause degradation, or proteolytic cleavage of the protein content, including the GOX enzyme. In conclusion, the antibacterial effect of different honeys varied and is significantly affected by mouth and wound environments.

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Antibacterial activity of honey in wound and oral environments: the latest *in vitro* finding

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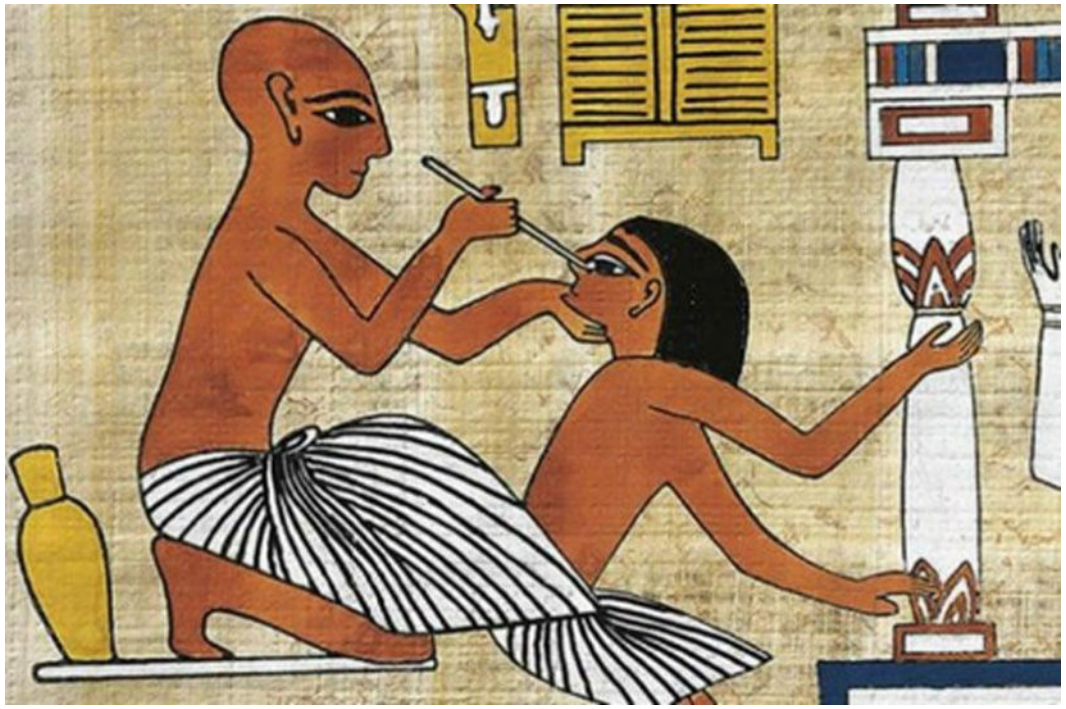
Honey health-promoting properties



Ancient Egyptian Medical Text

1550-1536 BC

Medical-grade honeys (medical device class IIa)

2000 - 2024

Selection of medical-grade honey source is based on honey antibacterial activity

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Honey antibacterial activity – not legislative parameter

HONEY QUALITY
EUROPEAN UNION
STANDARDS

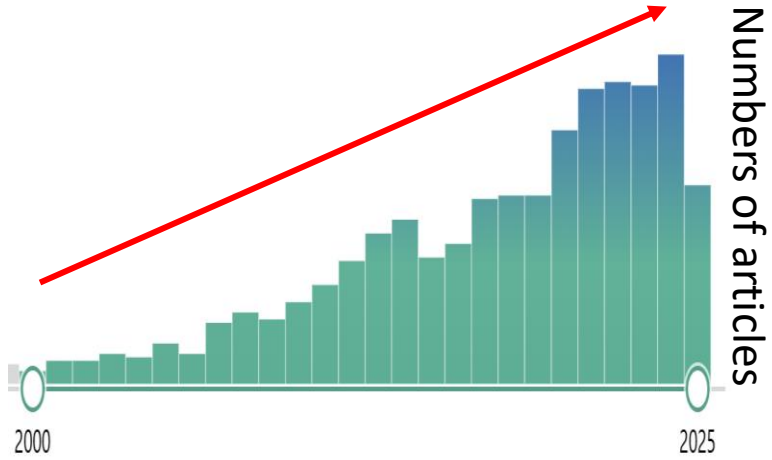
Composition criteria	Directive 2001/110 EU: Blossom honey General	Directive 2001/110 EU: Blossom honey Exceptions	Directive 2001/110 EU: Honeydew honey* General	Revised CODEX 2001
Moisture %	<20	*Calluna* and baker's honey <23Baker's honey from *Calluna* <25	<20	The same No indication for baker's honey
Fructose + glucose %	>60	–	>45	The same
Sucrose %	<5	*Robinia, Medicago, Banksia, Hedysarum, Eucalyptus, Eucryphia*spp, and *Citrus* <10*Lavandula & Borago* <15	<5	The same
Water-insoluble %	<0.1	Pressed honey <0.5	<0.1	The same
Electrical conductivity $mS.cm^{-1}$	<0.8	*Chestnut, Arbutus, Erica, Eucalyptus, Tilia, Calluna, Manuka* and *Melaleuca*	>0.8	The same
Free acid $meq.kg^{-1}$	<50	Baker's honey <80	<50	The same
Diastase activity DN**	>8	baker's honey and honey with low natural enzyme content: >3 when HMF is less than 15 $mg.kg^{-1}$	>8	Honeys with low natural enzyme content: > 3 DN
HMF $mg.kg^{-1}$**	<40	baker's honey Honeys of tropical climate and blends of these honey <80	<40	Honeys of tropical climate and blends: < 80

*Honeydew honey and blends of honeydew honey with blossom honey.
**Determined after processing and blending.

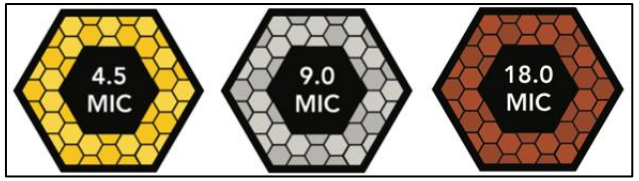
PubMed

keywords:

- antibacterial activity
- honey



Different grading system



TA10+, TA20+, TA30+

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Antibacterial activity of honey as suitable tool for assessment of honey quality

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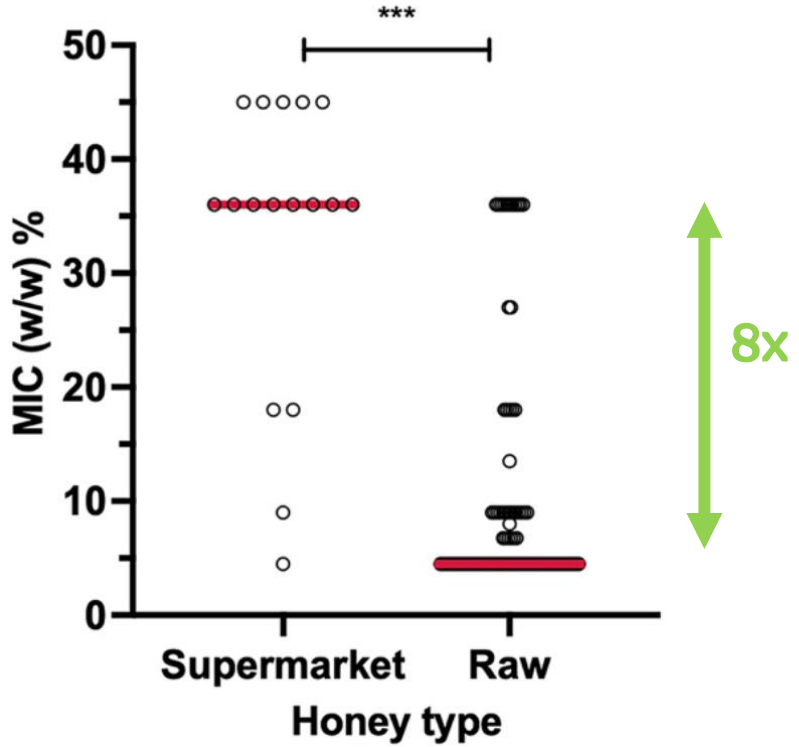
Antibacterial activity and bee-derived protein content of honey as important and suitable complementary tools for the assessment of honey quality

Marcela Bucekova [a], Jana Godocikova [a], Lenka Kohutova [b], Maksym Danchenko [b], Peter Barath [b], Juraj Majtan [a,c,*]

Article full pdf



Antibacterial activity of raw (n=92) and supermarket (n=17) honey samples against *Staphylococcus aureus*

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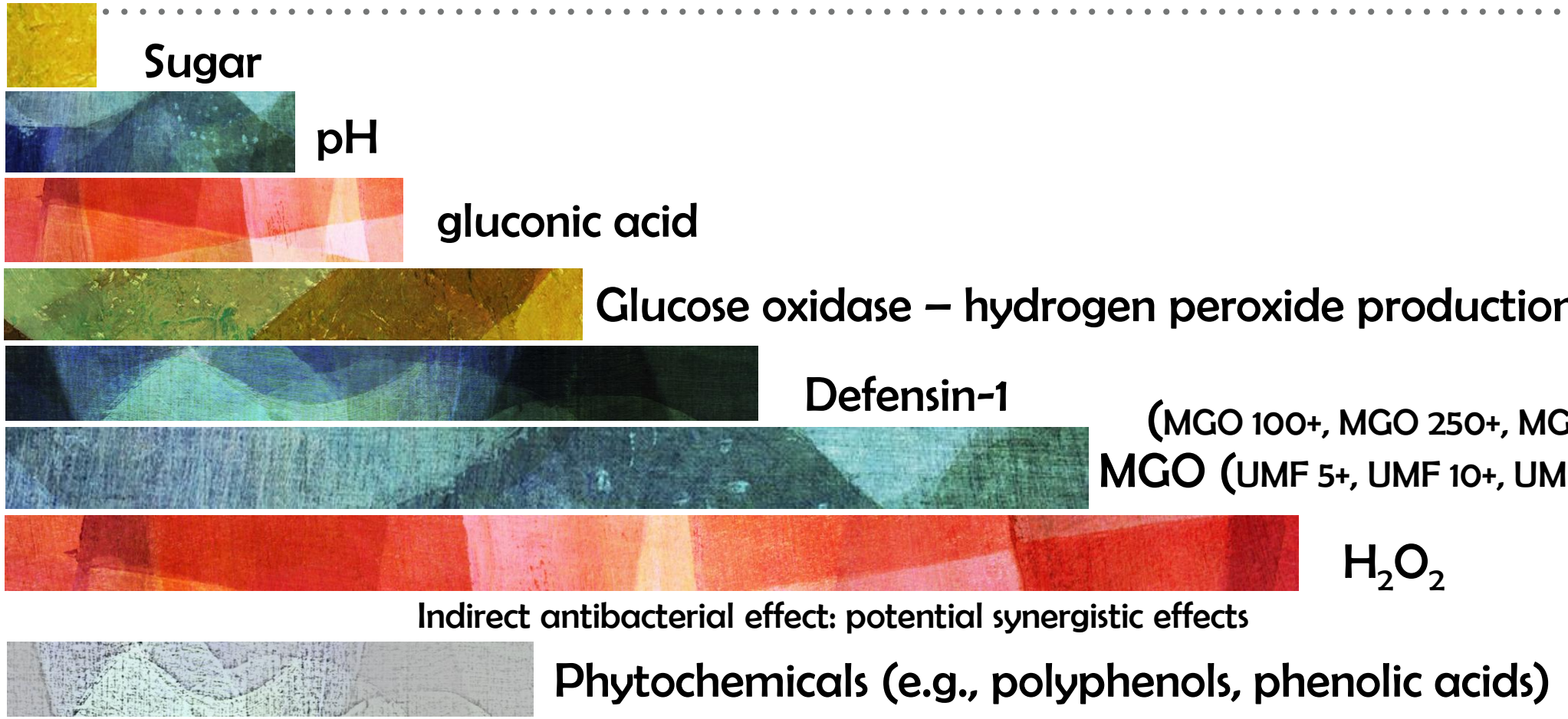
Antibacterial activity of honey

Antibacterial activity is the main and the most studied and characterised biological activity of honey



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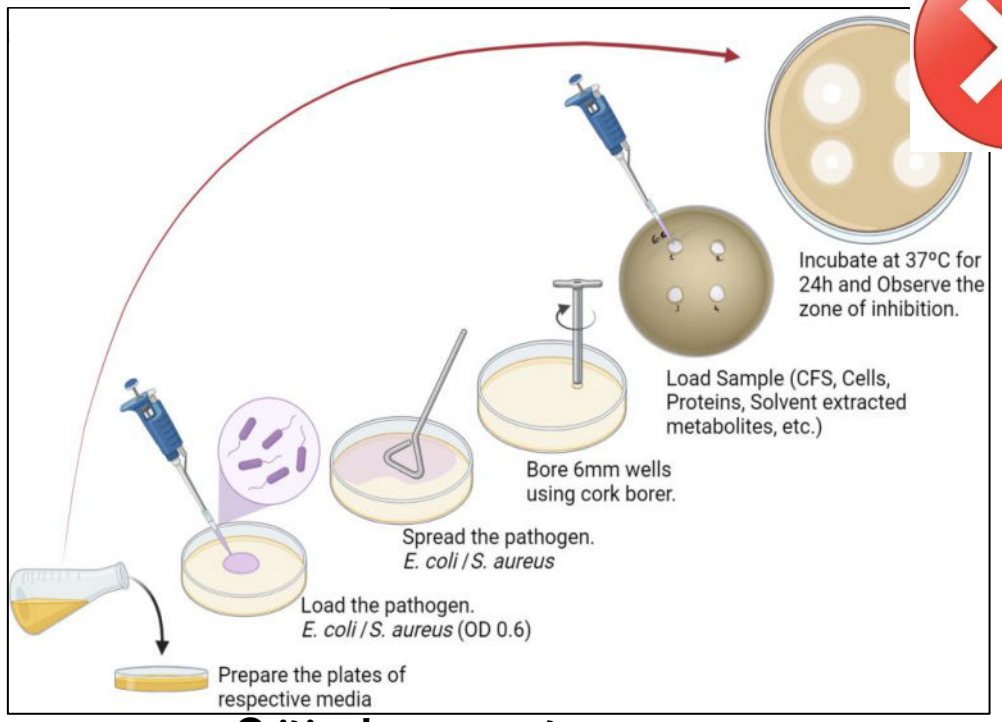
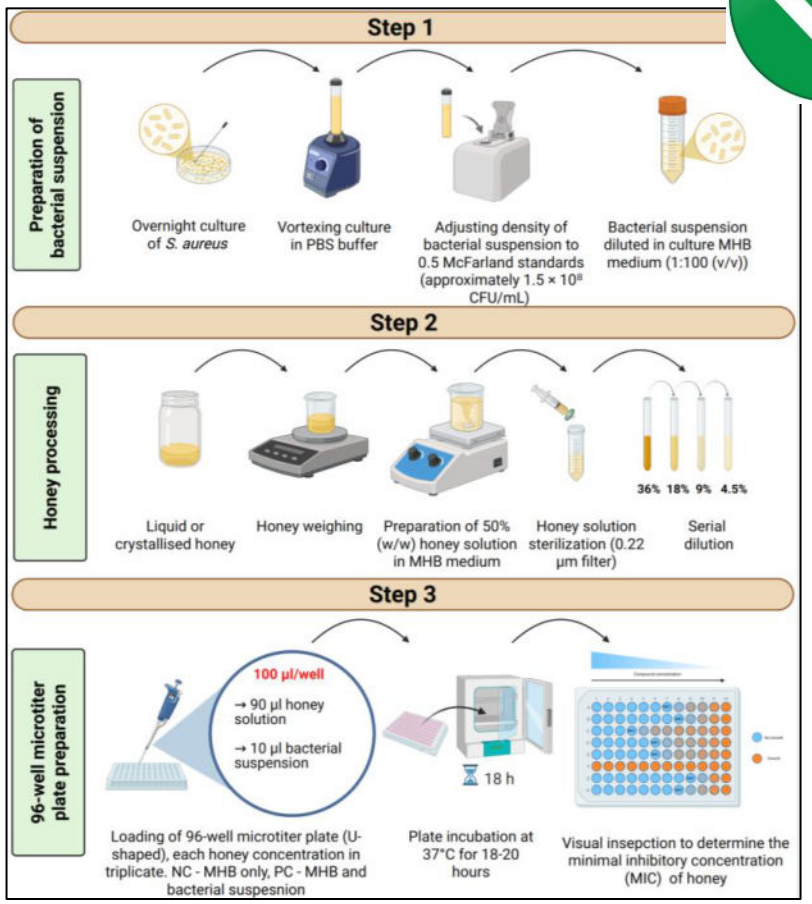
Antibacterial activity of honey – major concern I

Assay use to determine antibacterial activity of honey must be robust, quantitative, discriminatory and widely acceptable to both researchers and industry.

Well diffusion assay



Broth dilution assay



Critical parameters:

- culture medium
- bacterial test strains (*Staphylococcus aureus*)
- inoculum preparation and concentration
- choice of methodology

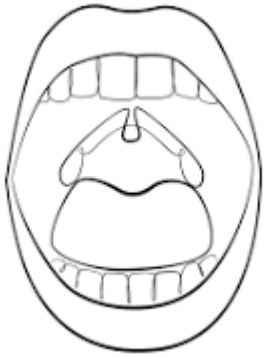
Antibacterial activity of honey – major concern II

The vast majority of published articles characterizing the *in vitro* antibacterial efficacy of different honey samples around the globe were conducted under laboratory control conditions without taking into account the real wound and oral environmental conditions.

Chronic wound environment

- ❑ Bacteria embedded in bacterial biofilm
- ❑ Presence of polybacterial infection
- ❑ Elevated level of bacterial and human proteolytic enzymes
- ❑ Presence of human catalase
- ❑ pH imbalance (pH 7.5 – 8.5)
- ❑ High exudation of wound fluid

Oral environment



- ❑ Bacteria embedded in bacterial biofilm
- ❑ Presence of polymicrobial biofilm
- ❑ Elevated level of bacterial and human proteolytic enzymes
- ❑ Presence of bacterial catalase
- ❑ pH imbalance (pH 7.5 – 8.5)
- ❑ Dilution effect (saliva flow: 0.5-2.0 ml/min)

Honey antibacterial activity under "real" wound/oral environment is largely unexplored

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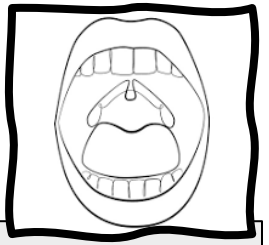
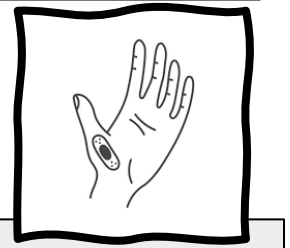
Aims of the study



- to determine the antibacterial efficacy of several honeys with different botanical origin, and manuka honeys with different content of methylglyoxal (MGO) against oral and non-oral bacterial pathogens in the presence of artificial human saliva
- to characterize the effect of artificial saliva on the enzymatic activity of bee-derived glucose oxidase (GOX) and production of H_2O_2



- to determine the antibacterial efficacy of different medical-grade honey products against wound bacterial pathogens in the presence of artificial wound fluid
- to characterize the effect of artificial wound fluid on the enzymatic activity of bee-derived glucose oxidase (GOX) and production of H_2O_2

Effect of artificial saliva on antibacterial activity of honey

Honey samples:

- three raw honey samples (honeydew, acacia, and linden honey) from *Apis mellifera* were obtained from local beekeepers from Slovakia
- commercially available manuka honey sample MGO 514+ and samples with MGO ratings of 100+, 250+, and 550+

Bacterial isolates:

- oral bacterial isolates *Streptococcus mutans, Streptococcus sobrinus, Rothia dentocariosa*
- non-oral *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis*

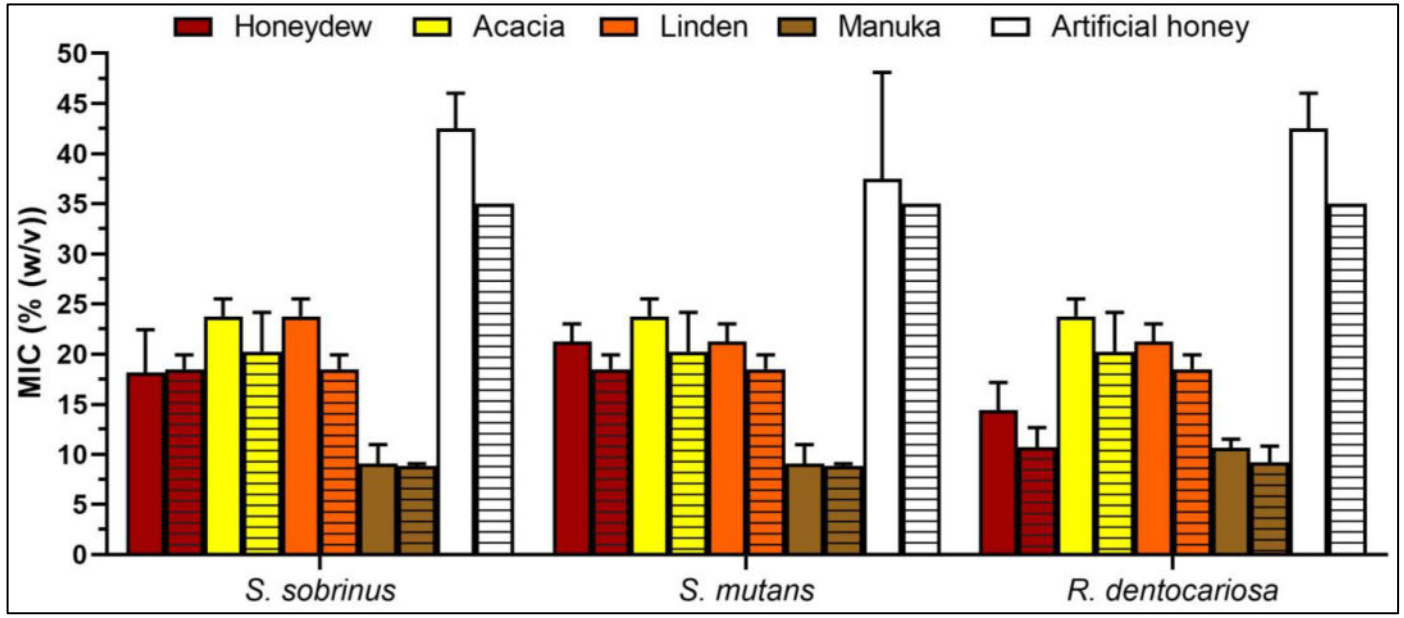
Artificial human saliva:

Components in Artficial Saliva		Concetration (g/L)
Sodium chloride	NaCl	1.594
Ammonium nitrate	NH_4NO_3	0.328
Potassium phosphate	KH_2PO_4	0.636
Potassium chloride	KCl	0.202
Potassium citrate	$K_3C_6H_5O_7 \cdot H_2O$	0.308
Uric acid sodium salt	$C_5H_3N_4O_3Na$	0.021
Urea	H_2NCONH_2	0.198
Lactic acid sodium salt	$C_3H_5O_3Na$	0.146
Porcine gastric Mucin Type II		1.35
D- (+) glucose		0.1
α-amylase		100,000 U
Lysozyme		750 U
Water	Four times distilled H_2O and 0.1 M NaOH used to achieve pH 6.8	

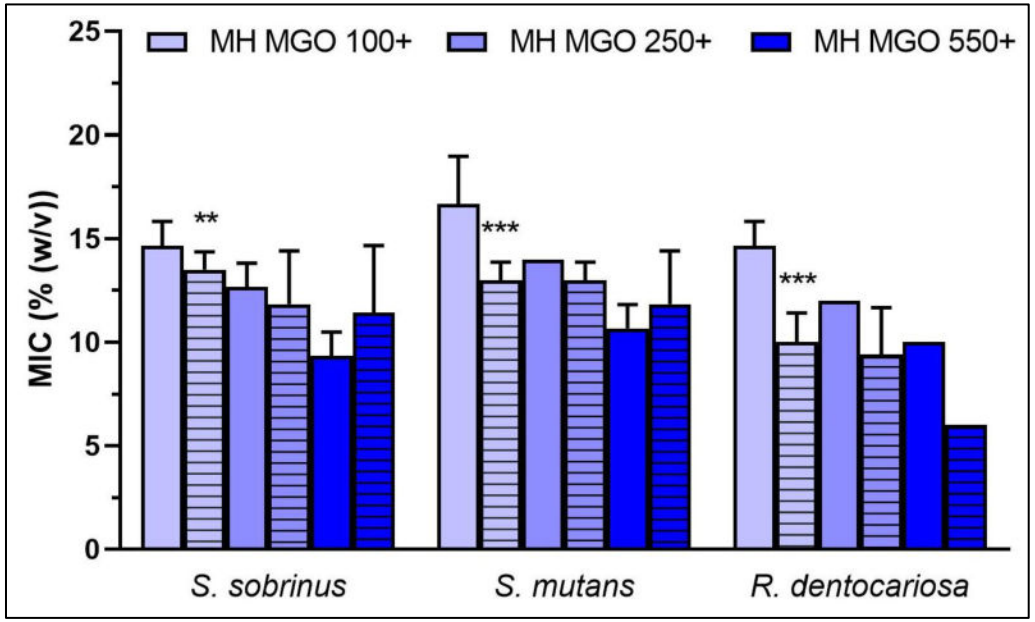
Voidarou et al (2021)

https://doi.org/10.3390/app11146318

Effect of artificial saliva on antibacterial activity of honey

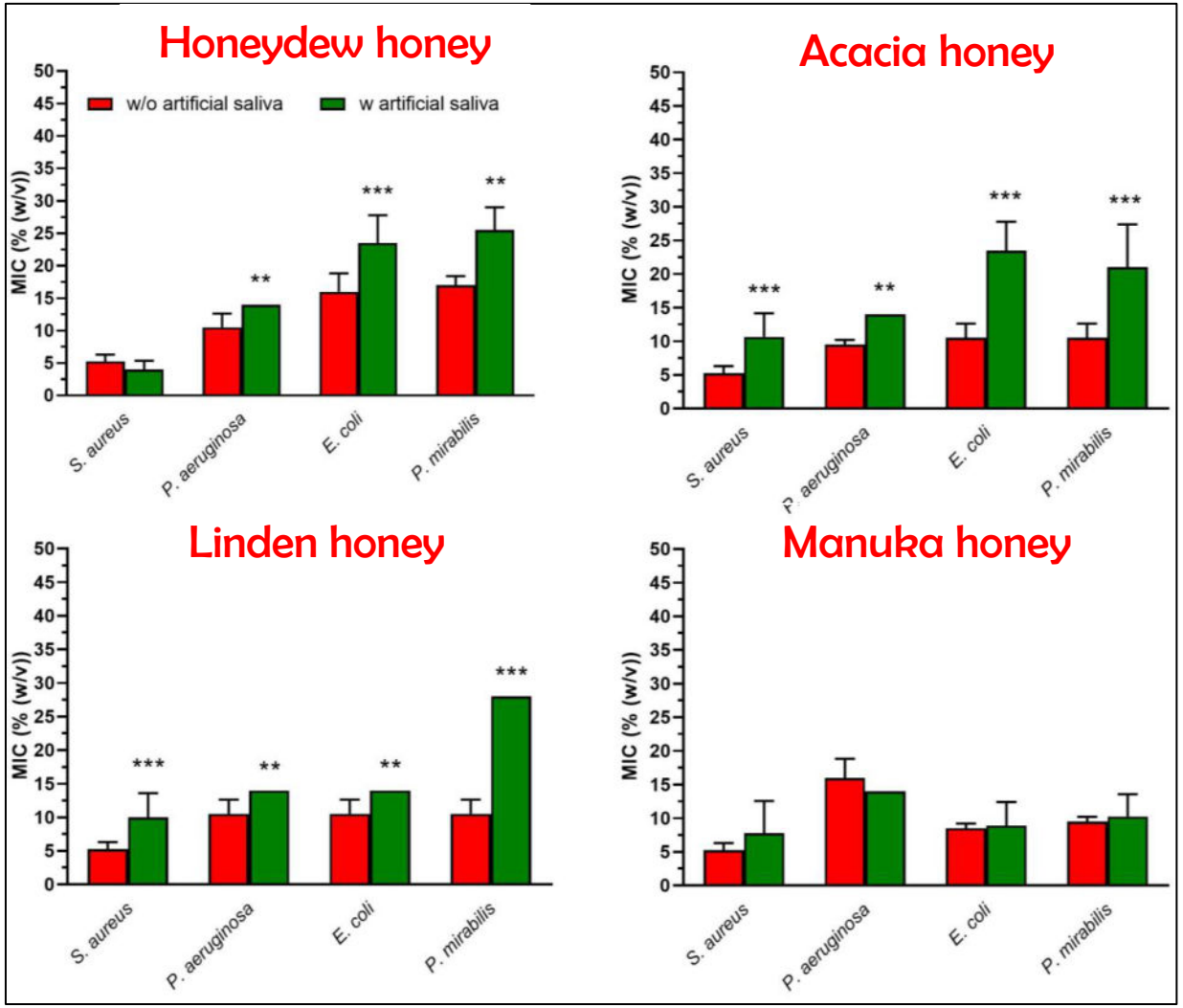


Antibacterial activity of honey samples and artificial honey express as MIC values of honey solutions against oral bacteria. The hatched horizontal rectangle corresponds to honey samples incubated with artificial saliva.

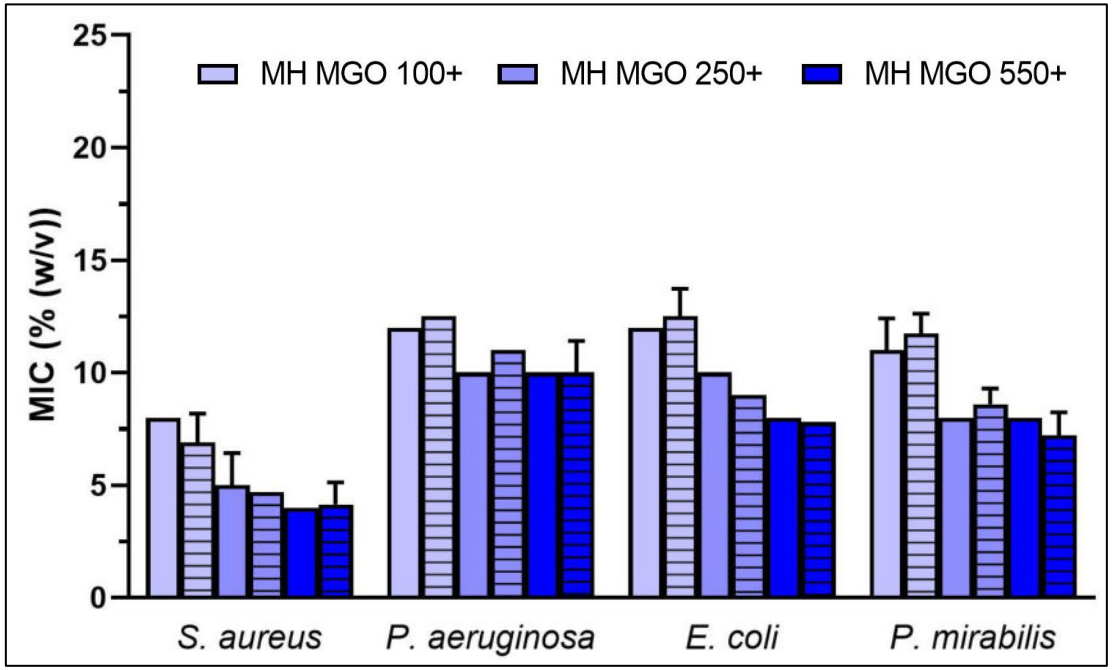


Antibacterial activity of manuka honey with different contents of methylglyoxal in the absence or presence of artificial human saliva against oral bacteria.The hatched horizontal rectangle corresponds to honey samples incubated with artificial saliva. ***$P < 0.001$, **$P < 0.01$.

Effect of artificial saliva on antibacterial activity of honey

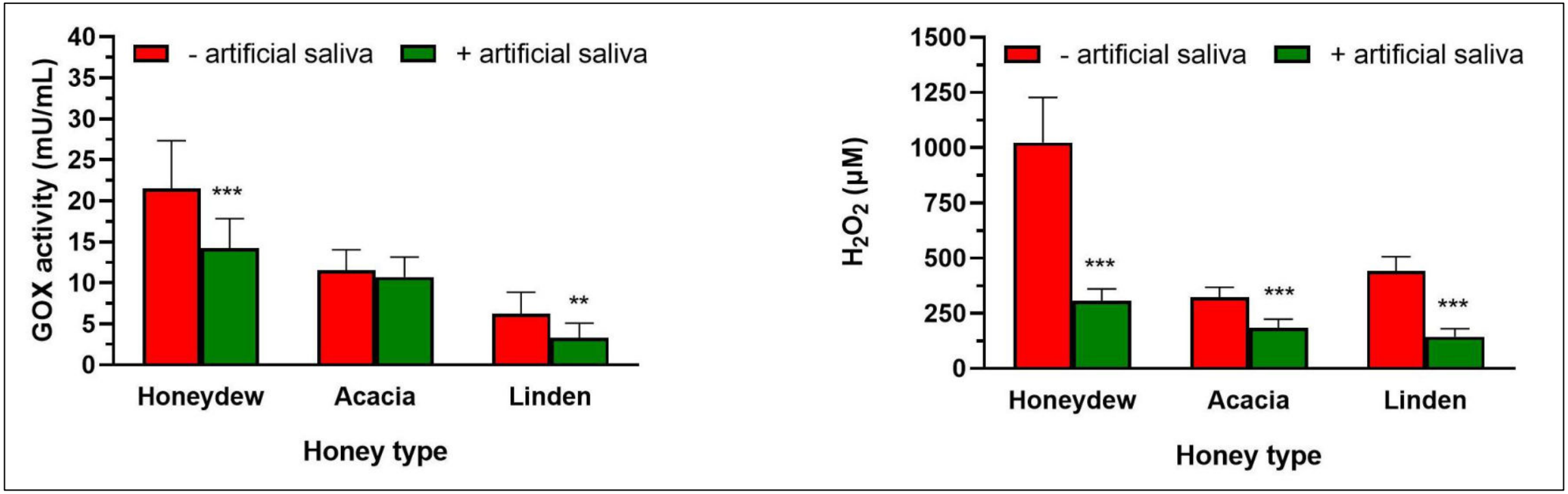


Antibacterial activity of honey samples of different botanical origins in the absence or presence of artificial human saliva against non-oral bacteria. ***P < 0.001, **P < 0.01.



Antibacterial activity of manuka honey with different contents of methylglyoxal in the absence or presence of artificial human saliva against non-oral bacteria. The hatched horizontal rectangle corresponds to honey samples incubated with artificial saliva.

Effect of artificial saliva on GOX activity and H_2O_2 production



Determination of glucose oxidase (GOX) enzymatic activity and hydrogen peroxide (H_2O_2) content in diluted honey samples after incubation with (+) or without (-) artificial human saliva. ***$P < 0.001$, **$P < 0.01$.

Effect of artificial wound fluid on antibacterial activity of honey

48%

100%

100%

Honey-based porduct samples:

- three medical-grade honey-based products (L-mesitran, Activon and Vivamel)

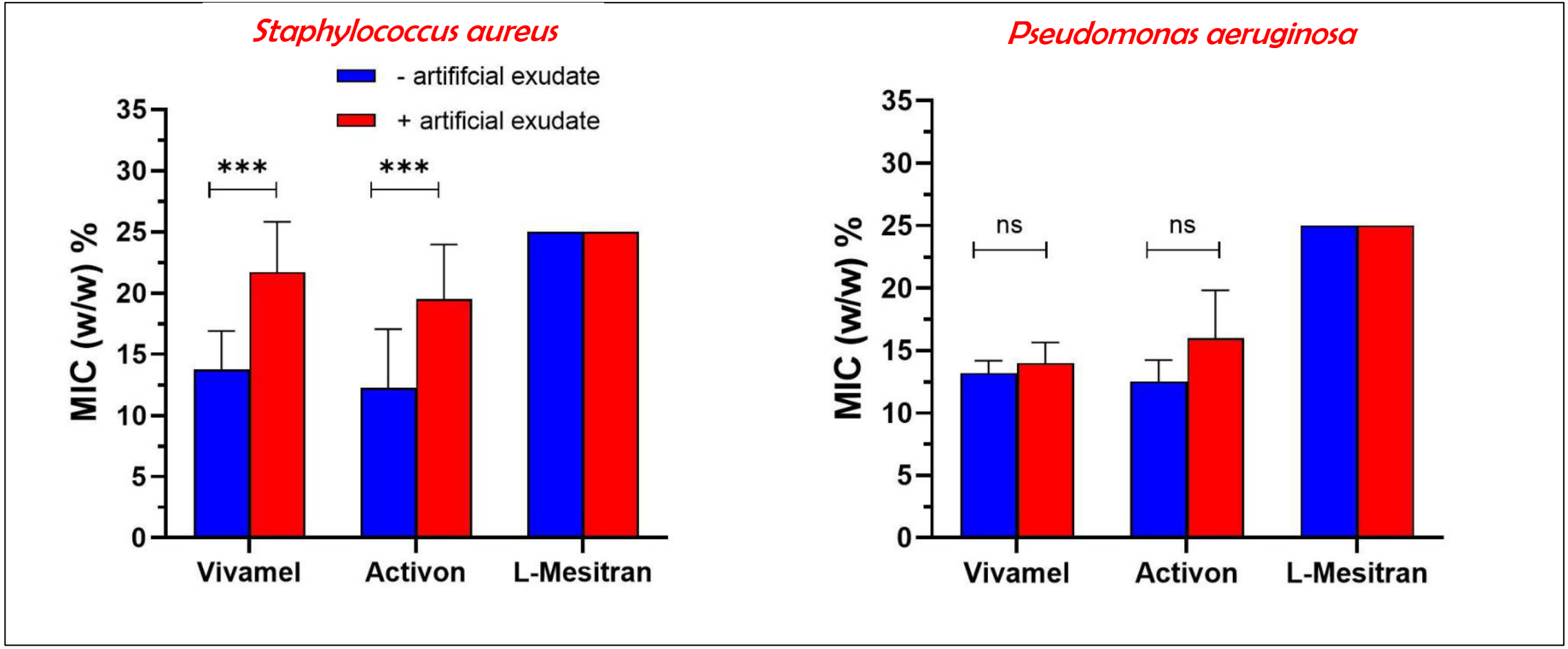
Bacterial isolates:

- *Staphylococcus aureus and Pseudomonas aeruginosa*

Artificial human wound fluid:

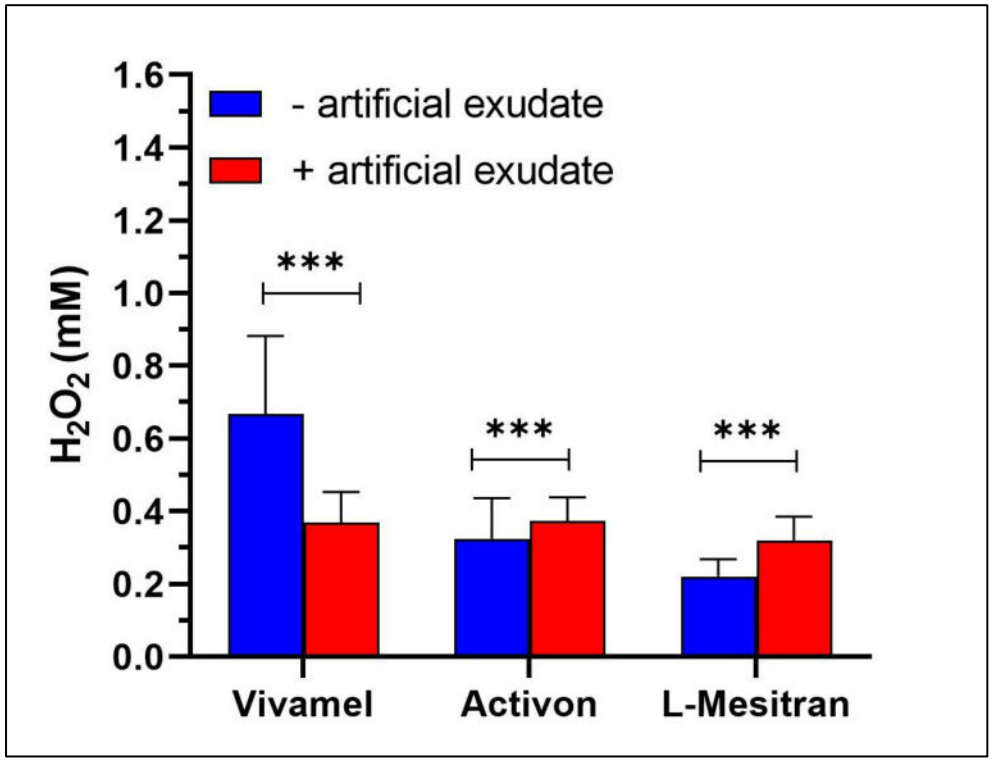
- Commercially available artificial wound fluid purchased from Biochemazone (Canada)

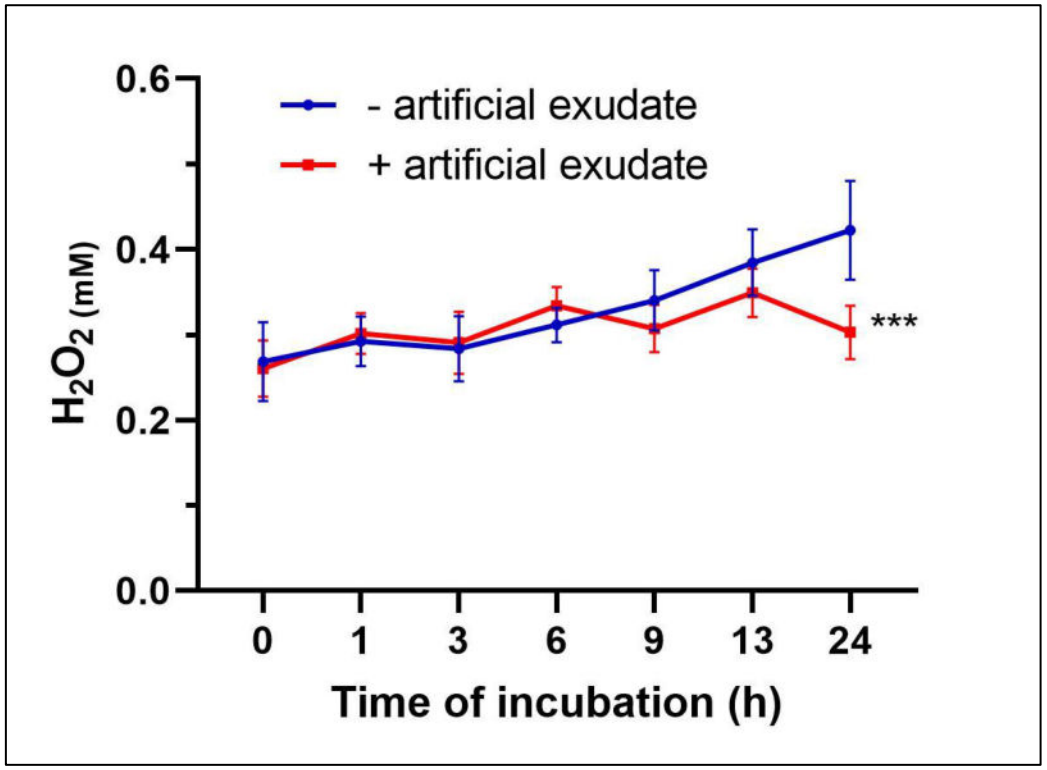
Effect of artificial wound fluid on antibacterial activity of honey



Antibacterial activity of medical-grade honey - based products in the absence or presence of artificial human wound exudate against wound bacteria. ***$P < 0.001$, **$P < 0.01$.

Effect of artificial wound fluid on H_2O_2 production





Kinetic of H_2O_2 production

Determination of hydrogen peroxide (H_2O_2) content in diluted medical-grade honey-based products after incubation with (+) or without (-) artificial wound fluid. ***$P < 0.001$.

Conclusions

- Non-manuka and manuka honey samples varied in their antibacterial effects against oral and non-oral bacteria, and the observed effects were negatively affected by human saliva in non-manuka honey samples
- The mechanism of honey action against typical oral bacteria seems to be H_2O_2-independent
- Wound fluid may have negative impact on antibacterial activity of medical-grade honeys

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