ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/237509908

Antibacterial substances in honey

Article · January 1997

CITATIONS
28

READS
1,263

1 author:

Stefan Bogdanov
Bee Product Science
126 PUBLICATIONS **10,440** CITATIONS

SEE PROFILE

All content following this page was uploaded by Stefan Bogdanov on 05 August 2015.

The user has requested enhancement of the downloaded file.

Antibacterial substances in honey

Stefan Bogdanov
Bee Product Science

Compiled after:

Bogdanov S. (1997) Nature and origin of the antibacterial substances in honeyLebensm.-Wiss. und Technol., 30 (7) 748-753 and

Bogdanov, S. (1984) Characterisation of antibacterial substances in honey. Lebensmittel-Wissenschaft und -Technologie, 17, 74-76.

There are two types of antibacterial agents in honey. The peroxide one is destructed when honey is heated or stored in the light. The other one is a non-peroxide one and is stable to heat and storage. The chemical properties of the non-peroxide activity are determined. Most of the non-peroxide antibacterial activity originates from the bee, but some of it comes from the honey source (nectar or honeydew).

INTRODUCTION

The antibacterial action of honey was reported for the first time in 1892 (1). The different aspects of the antibacterial properties of honey have been recently extensively reviewed (2). There are two sorts of antibacterial agents or so called „inhibines". One of them is heat- and light-sensitive and has its origin in the $H_2 0_2$, produced by honey glucose oxidase (3,4,5). Some workers believe that hydrogen peroxide is the main antibacterial agent (3,6,7). Other authors find that the non-peroxide activity is the more important one (8-13). The argument of the latter is, that in ripe honey the glucose oxidase is inactive and honey contains only a small peroxide amount, not sufficient to inhibit bacterial growth. However, when eaten or when it is diluted, peroxide can be produced for an antibacterial action. The non-peroxide antibacterial activity is insensitive to heat and light (8,9,13) and remains intact after storage of honey for longer periods (8,10). The main honey substances are sugars, which by their osmotic effect exert an antibacterial action (2). However, the antimicrobial tests used in different studies are carried out at concentrations where the sugars are not osmotically active. It has been claimed that honey contains lysozyme, a well known antibacterial agent (11). However, in another study no lysozyme activity was found (8). The antibacterial flavonoid pinocembrin is present in honey, but its concentration and contribution to honey's non-peroxide antibacterial activity is small (14). In New Zealand honeys, mainly manuka and viper's bugloss honey , several aromatic acids with antibacterial activity have been isolated (2,15). Another investigation claimed, that the low honey pH, besides the high honey osmomolarity was responsible for the antibacterial activity (16). Some workers have isolated volatile substances with antibacterial activity (17-18), but their quantitative contribution to the antibacterial action of honey was not examined. Other workers found non-peroxide activity of honey, extractable by organic solvents, but were not able to identify the chemical nature of the substances (12,19,20). A major part of the antibacterial activity has been postulated to have bee origin (10). However, in 2 unifloral New Zealand honeys the main antibacterial substances were shown to have a flower origin (2,15). The determination of antibacterial activity can be measured quantitatively and can be used as an additional quality criterion for honey (21). Thus the chemical identity, the quantitative contribution, and the origin of the different honey antimicrobial substances remain to a great extent unknown.

The purpose of the present study was to clarify these problems by using honey fractionation of the major antibacterial substances using an antibacterial test, reflecting only the non-peroxide part of antimicrobial activity (8). The test strains *Staphylococcus aureus* and *Micrococcus luteus* were

utilised in a quantitative turbidometric assay because they are known to be sensitive to the honey antibacterial substances and are widely used for testing antibacterial action.

MATERIALS AND METHODS

These are described in detail in previous publications (8, 24).

RESULTS AND DISCUSSION

Correlation between acidity, pH and antibacterial activity

Table 1 summarises the results of the different unifloral and polyfloral honeys for the following parameters: pH, free- and total acidity and inhibition of growth of *Staph.aureus*. The linear correlation analysis between pH, free and total acidity on one side and bacterial inhibition on the other yielded following results (n=81 cases), summarised in table 2: the bacterial inhibition correlates significantly with the free- and total acidity, but not with the honey pH. This is in accordance with the results of this paper, which the main part of the non-peroxide activity is found in the acid fraction (see below). As the acids have a bee origin (23), these results can be interpreted, that a part of the antibacterial activity has a bee origin.

The low honey pH, besides the osmotic effect of the sugars was postulated to be the main antibacterial factor of honey (16). However, there are quite a few honeys (honeydew, chestnut), having pH values of 5 and more, which also inhibit bacterial growth. We varied the pH of our test from 5 to 7 and found optimal bacterial growth at all conditions (see Methods). It can thus be concluded that the honey acids exert the main antibacterial action, while honey pH could additionally act as an antibacterial factor.

Antibacterial activity of honeys of different origin

If the antibacterial substances originate from plants, differences in the inhibitory capacity of the different unifloral honeys should be expected. In fig.2 the bacterial inhibition of 9 unifloral and 2 mixed (different blossom and honeydew origins) honeys are shown, using the average values in table 1. There were slight differences between the different honeys: rhododendron and eucalyptus honeys had the lowest, while honeydew and rape honeys had the highest activity.

However, there is a considerable variation in each honey type (see table 1), so that these differences were not statistically significant. Differences of antibacterial activity of unifloral honeys have been reported (2). However, a great variation in the activities of the unifloral honeys was found. Also, in the reported studies it is often not clear which part of the antibacterial activity is measured.

Antibacterial activity of sugar adulterated honeys

If the antibacterial activity originates from the bee, then one would expect that the sugar adulterated honey has the same antibacterial activity as the genuine honey, produced under the same conditions. In table 3 the quality criteria of two genuine honeydew honeys are compared with those of 2 sugar-fed honeys, produced at the same time in the same apiary. In the sugar adulterated honeys the adulteration indicators prolin and ash were about one third of the values of the control honeydew honeys, which means that there was a major portion of the sugar, fed to the bees. The non-peroxide antibacterial activity, but also the peroxide accumulation capacity in both adulterated honeys was about the same as that of the control honeys. Thus it is evident, that the greater part of both types of antibacterial activity of honeydew honeys has a bee origin. These results corroborate with the conclusions of another study in our laboratory, that there is a highly significant correlation between the diastase and the invertase activity, both originating from the bees and the bacterial inhibition (21).

Sugar feeding experiments of this type during the flow of different unifloral honey sources is necessary in order to quantify the relative amount of bee- and plant-derived antimicrobial activity.

Relative distribution of antimicrobial activity among different honey fractions

We fractionated 10 different honeys into 4 basic substance groups: volatile, non-volatile and non-polar, acidic and basic substances. The relative inhibition of each honey fraction was tested against *Staph.aureus* and *Micrococcus luteus*. The results are summarised in table 4. The acidic fraction had the greatest inhibitory activity, while the volatiles were the weakest bacterial inhibitors. The relative distribution of the antibacterial activity in the different fractions was about the same when both bacterial species were tested. On the average, the following relative distribution of antibacterial activity was observed: 44% acids, 24% bases, 21% non-polar, non-volatile and 11% volatiles.

If the differences between the distribution of activity among the different groups were tested by a t-test, only the difference between the volatile activity on one side and the acidic ($p=0.000$) and the basic fraction activity on ($p=0.05$) on the other proved to be significantly different. This is due to the variation of distribution among the fractions of the different honey types. In the manuka honey 90% of the activity was found in the acidic fraction, in the rape honey the major part of the activity was in the non-polar fraction and in one Swiss blossom honey the basic fraction had the highest activity.

Full reports of this work is published elsewhere (8, 24).

Effects of heat and storage

The experiments were carried out with light blossom- and dark honeydew honeys.

Heating of both honey types at 70^{o} C for 15 minutes had no or very little effect on the non-peroxide activity (table 5.). Under the same conditions the peroxide accumulation capacity of blossom honeys is severely damaged (4).

In a next experiment glass pots with blossom or honeydew honeys were stored in the light (day-light) or in the dark at room temperature (about 20-25^{o} C). The results are summarised in table 6 After 15 months there is a small drop of activity of about 20 % of the non-peroxide activity. The results were the same both for both light and dark honeys stored in the light or in the dark. Under the same storage conditions the peroxide accumulating capacity of honey is strongly reduced, especially when light blossom honeys are stored in the light (4).

CONCLUSIONS

The non-peroxide antibacterial activity in honey was found to correlate significantly with the acid content of honey, but does not correlate with the honey pH.

There are differences in the activity of different unifloral honeys: rhododendron and eucalyptus honeys had the lowest, while honeydew and rape honeys had the highest activity. But due to the considerable variation of the antibacterial activity the differences were not statistically significant.

From experiments with sugar-adulterated honey it can be concluded, that the antibacterial activity of honeydew honeys was of bee origin.

By fractionation in different substance classes the following relative distribution of non-peroxide antibacterial activity was found:

acids > bases = non-polar, non-volatiles > volatiles.

This order was the same for *Staph.aureus and Micrococcus luteus* as test strains

The non-peroxide activity is only slightly affected by heat and by storage for 15 months in the light or in the dark.

After:

References

1. VAN KETEL, B.A., Festnummer der Berichten van den Niederlandsche Maatschappij, Bevordering der Pharmacie, 67/96, (1892)

2. MOLAN, P. The antimicrobial activity of honey, Bee world, **73**, 5-28, and 59-76 (1992)

3. WHITE, J.W. , SUBERS, M.H. AND SCHEPARTZ A.I. , The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in honey glucose-oxidase system. Biochimica Biophysica Acta **73**, 57-70, (1963),

4. WHITE, J.W. AND SUBERS, M.H. Studies of honey inhibine, 3. The effect of heat. Journal of Apicultural Research **3,** 454-450, (1964),

5. DUSTMANN, J.H. Ueber den Einfluss des Lichtes auf den Peroxid-Wert des Honigs. Zeitschrift für Lebensmittel Untersuchung und Forschung, **148**, 263-268, (1972)

6. DUSTMANN, J.H. Antibacterial effect of honey. Apiacta **14**, 7-11, (1979)

7. MORSE, R.A. The antibiotic properties of honey. Pan-Pacific Entomologist **62**, 370-37, (1986)

8. BOGDANOV, S. Characterisation of antibacterial substances in honey. Lebensmittel-Wissenschaft und -Technologie, **17**, 74-76, (1984)

9. GONNET, M. AND LAVIE, P. Influence du chaufage sur le facteur antibiotic du miel. Annales de l'Abeille (Paris) **3**, 349-364, (1960)

10. LAVIE, P. Propriétés antibactériennes et action physiologique des produits de la ruche et des abeilles in: Traité de Biologie de l'Abeille (R.Chauvin, editor) Masson & Cie pp.2-115, (1968)

11. MOHRIG, W. AND MESSNER, R., Lysozym als antibacterielles Agens im Honig und Bienengift. Acta Biologica Medica Germanica **21**, 85-95, (1968)

12. RADWAN S.S. , EL-ESSAWY A.A. AND SARHAN, M.M. Experimental evidence for the occurrence in honey of specific substances active against micro-organisms. Zentralblatt für Mikrobiologie **139**, 249-255, (1984)

13. ROTH, L.A., KWAN, S. AND SPORNS P. Use of a disc assay to detect oxytetracycline residues of honey. Journal of Food Protection **49**, 436-44, (1986)n

14. BOGDANOV S. Determination of pinocembrin in honey using HPLC. Journal of Apicultural Research, **28**, 55-57, (1989)

15. RUSSEL, K.M., MOLAN, P., WILKINS, A.L. AND HOLLAND, P.T. Idenification of some antibacterial constituents of New Zealand manuka honey. Journal of Agricultural and Food Chemistry, **38**, 10-13, (1988)

16. YATSUNAMI, K. AND ECHIGO, T. Antibacterial activity of honey and royal jelly. Honeybee Science **5**, 125-130, (1984)

17. TOTH, G., LEMBERKOVICS, E. AND KUTASI-SZABO The volatile components of some Hungarian honeys and their antimicrobial effects. American Bee Journal **127**, 496-497, (1987)

18. OBAEISEKI-EBOR, E.E., AFONOYA, T.C.A., ONYEKWELI, A.O. Preliminary report on the antimicrobial activity of honey destillate, Journal of Pharmacy and Pharmacology **35**, 748-749 (1983)

19. LAVIE, P. Sur l'identification des substances antibacteriennes présentes dans le miel. Comptes Rendus Academie des Sciences Paris. **256**, 1858-1960, (1963)

20. SCHULER, R AND VOGEL, R. Wirkstoffe des Bienenhonigs. Arzneimittel Forschung **6**, 661-663, (1956)

21. BOGDANOV, S. , RIEDER, K. AND RÜEGG, M. Neue Qualitätskriterien bei Honiguntersuchungen. Apidologie, **18**, 267-278 (1987)

22. Swiss Food Manual, Chapter 23 A, Honey, Bern, Eidgenössische Druck und Materialzentralle (1995)

23. ECHIGO, T. , TAKENAKA, T. Production of organic acids in honeys by honeybees. Journal of the Agricultural Chemical Society of Japan, **48**, 225-230 (1974)

24. BOGDANOV, S. Nature and origin of the antibacterial substances in honey. Lebensmittelwissenschaft und Technologie, **30,** 748-753 (1997)

Table 1 pH, Acidity and inhibition of growth of Staph.aureus in different honeys

Honey	n	pH		free acid		total acid		% inhibition	
		$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
acacia	7	3,9	0,3	1,14	0,33	1,97	0,30	57	31
blossom	30	4,1	0,5	1,44	0,61	2,27	0,92	56	22
chestnut	7	5,4	0,6	0,58	0,30	1,01	0,43	56	26
dandelion	2	4,4	0,1	0,65	0,08	0,89	0,11	66	5
eucalyptus	4	4,4	0,5	1,10	0,44	1,78	0,54	40	8
lavender	5	3,4	0,2	2,18	0,13	3,80	0,72	64	9
orange	3	3,8	0,1	0,99	0,27	1,71	0,40	47	8
rape	7	3,9	0,1	0,93	0,37	2,01	0,92	74	18
rhododendron	3	3,7	0,1	0,86	0,24	1,51	0,48	37	8
sunflower	4	3,7	0,1	1,49	0,18	2,51	0,38	58	26
honeydew	10	4,4	0,3	2,24	0,71	2,96	1,09	67	19

Mean values ($\bar{x}$), standard deviation (s_x) for n= number of the unifloral honey samples

Table 2 Correlation between antibacterial activity, pH and acidity

parameter	pH vs. inhibition	free acidity vs inhibition	total acidity vs inhibition
r	0.06	0.35	0.31
P	0.58	0.001	0.005

r - coefficient of correlation,

P - Probability

Parameters were calculated for n = 82 honeys of different origin (see table 1)

Table 3 Antibacterial activity in honeys produced under sugar feeding

Honey	% inhibition Staph.aur.	$H_2 0_2$ µg/g/h	prolin mg/kg	ash %
honeydew 1	91	56	1670	0.45
honeydew 1+sugar feeding	81	56	760	0.12
honeydew 2	95	77	1200	0.42
honeydew 2+sugar feeding	96	49	480	0.15
mean honeydew*	93	66	1430	0.43
mean honey dew + sugar-feeding	91	52	620	0.13

***** mean value of honeydew honeys 1 and 2

Table 4 Relative distribution of antibacterial activity in different honey fractions

honey	% antibacterial activity in different fractions*							
	acidic		basic		non-polar		volatile	
	St.	*Mic.*	*St.*	*Mic.*	*St.*	*Mic.*	*St.*	*Mic.*
Manuka N.Z.	100	75	0	10	0	5	0	10
Sunflower It	58	46	13	15	16	25	13	15
Rape CH	25	40	7	33	63	22	5	5
Lavender Fr	25	27	34	30	23	29	18	14
Mountain CH	24	25	60	25	8	25	8	24
Blossom S. America	62	73	13	20	9	7	16	0
Honeydew CH	45	46	26	15	26	15	2	24
Honeydew CH	32	31	37	31	19	31	12	6
Honeydew CH	43	26	22	26	19	26	15	23
Honeydew Europe	43	32	25	31	26	37	6	0
average	46	42	24	24	21	22	10	12
standard deviation	23	18	17	8	17	10	6	9
minimum	24	25	0	10	0	5	0	0
maximum	100	75	60	33	63	37	18	24

* - values of individual honeys

St - Staphylococcus aureus

Mic - Micrococcus luteus

Table 5 Effect of heat on non-peroxide activity

honey	n	bacterial inhibiton % of initial
blossom (light)	3	86 ± 4
honeydew (dark)	4	94 ± 1

Fresh honeys of floral or honeydew origin were heated for 15 minutes at 70° C.

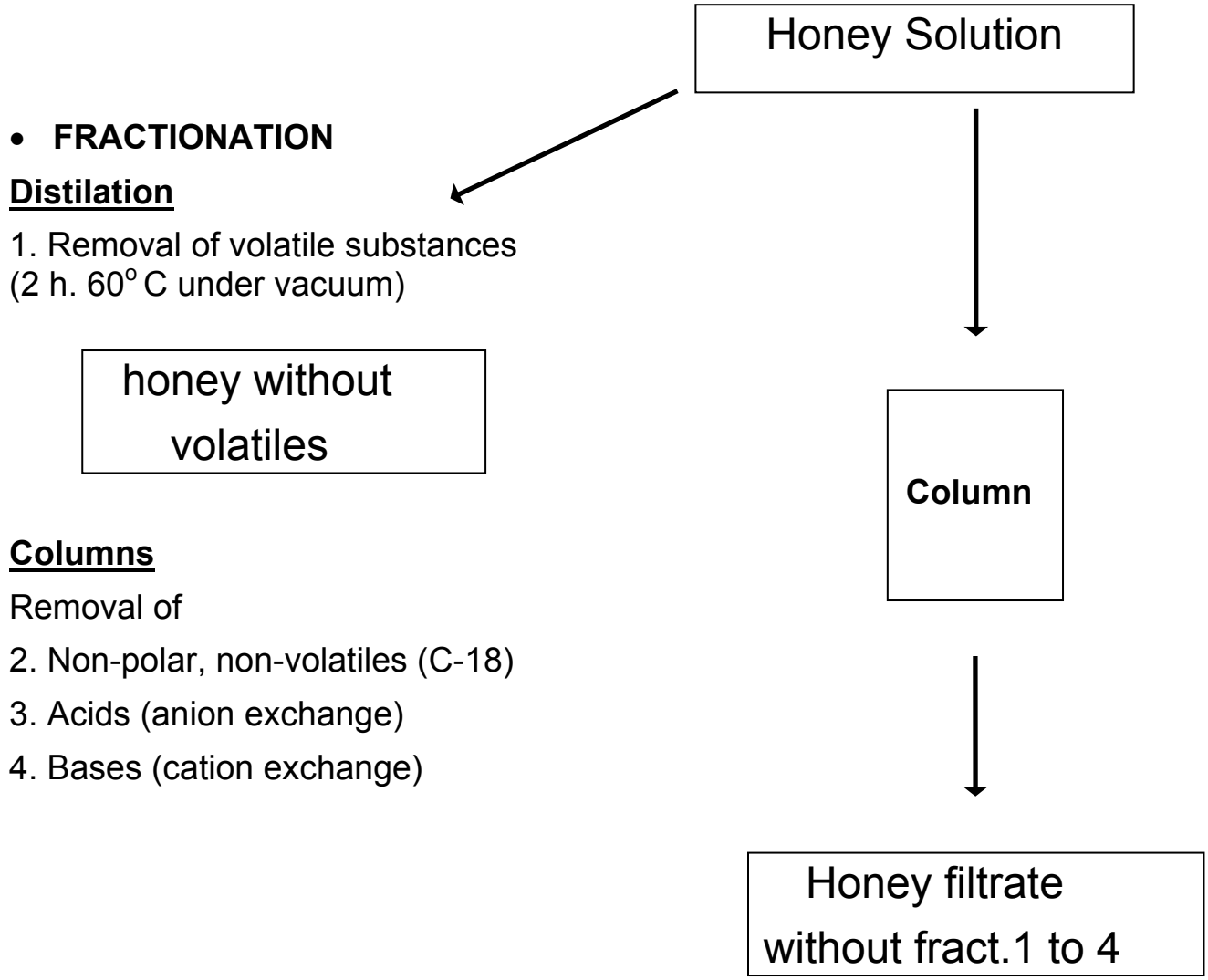
Values are means ± SEM and are expressed in % of the initial inhibition

Table 6 Effect of storage on antibacterial activity

	% of initial non-peroxide activity		% of inititial peroxide activity	
Storage at	light	dark	light	dark
Blüten	76	86	19	48
Wald	78	80	63	70

Honeys were stored in the light and in the dark at room temperature (20-25° C) for 15 months

FRACTIONATION OF HONEY ANTIBACTERIAL SUBSTANCES



- **TEST LOSS OF ANTIMICROBIAL ACTIVITY OF HONEY SOLUTIONS AFTER REMOVAL OF THE DIFFERENT FRACTIONS AND COMPARE TO INITIAL ANTIBACTERIAL ACTIVITY**

Fig.1 Scheme of the fractionation and testing of the different antimicrobial fractions

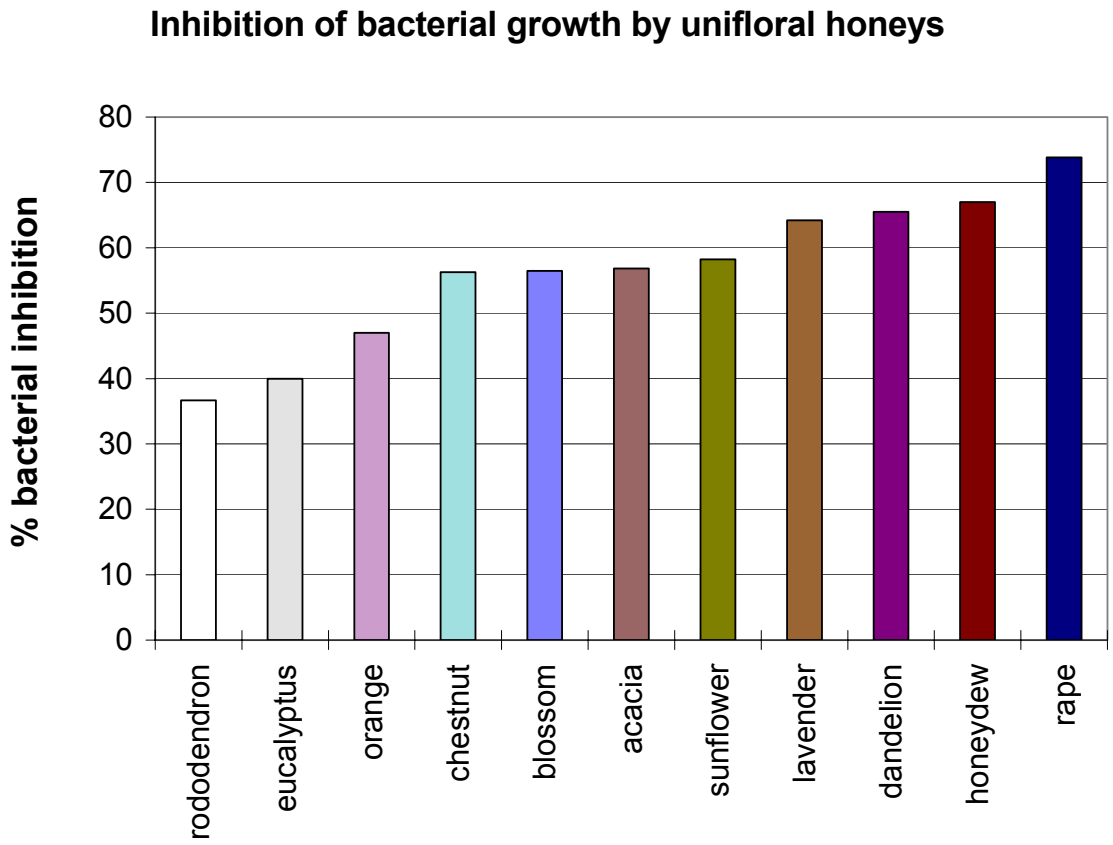


Fig.2 Non-peroxide activity of different honeys against *Staph.aureus*

View publication stats