

ANNALES  
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA  
LUBLIN — POLONIA

VOL. XXXI, 11

SECTIO C

1976

Instytut Biologii UMCS  
Zakład Fizjologii Roślin

Zbigniew JÓŹWIK,  
Alicja BARANIECKA-WŁOSZYCKA

**The Effect of Propolis on *Mycobacterium* sp.**

Wpływ wyciągów z kitu pszczelego na *Mycobacterium* sp.

Влияние вытяжки из пчелиного клея на *Mycobacterium* sp.

Propolis has been used as a remedy for a very long time. It possesses strong bactericidal and bacteriostatic properties. The chemical composition of propolis has not been accurately investigated yet. Two separate substances are distinguished in propolis, i.e. the so-called propolis proper constituting a mixture of wax, pollen, resins and essential oils, and propolis balsam containing volatile oils, resins, tannins and also wax whose composition cannot be identified with beeswax (16).

Many authors made attempts to study the influence of propolis on various genera of bacteria (6, 9, 11, 12). It follows from Lindenfelser's studies (11) that alcohol extracts from propolis inhibit the growth of saprophytic acidoresistant bacilli. Studies on the influence of extracts from propolis on pathogenic bacilli were carried out among others by Karimova, Radionova (8) and Kivałkina (9) who demonstrated bacteriostatic action of propolis in egg media and observed that propolis considerably weakens or even inhibits the growth of bacilli previously kept in extracts from propolis. It is also interesting that the Soviet authors report that propolis collected in 15 different places in their country showed antibacterial properties only in 4 cases (8).

Propolis is used in therapy in the Soviet Union as ointment, in the form of solutions in milk and alcohol solutions. These preparations have been used in treatment of the skin with various kinds of exanthems, mycosis as well as in treatment of frostbites.

They were also used in staphylococci and streptococci infections. Propolis butter and milk were also used in treatment of human tuberculosis (2, 13, 14).

The reports presented above of therapeutic action of propolis, particularly against tuberculous bacilli have encouraged us to study the effectiveness of propolis extracts from propolis collected in Poland on the growth of saprophytic acidoresistant bacilli as well as typical pathogenic bacilli.

### MATERIAL

The authors used propolis gathered in Warsaw in spring and autumn as well as propolis gathered in the Lublin district in various seasons of the year. Antibacterial action of extracts from propolis was studied on pathogenic strains of *Mycobacterium* obtained from the Phthisical Institute in Warsaw: *Mycobacterium tuberculosis* v. *hominis* H<sub>37</sub>R<sub>v</sub>, *Mycobacterium tuberculosis* v. *bovis* AN<sub>5</sub>-Puławy, and *Mycobacterium tuberculosis* v. *avium* Pt-Cracow. Saprophytic acid-fast bacilli: *Mycobacterium* 607, *Mycobacterium* 279, *Mycobacterium phlei* and *Mycobacterium smegmatis* used in the experiments — came from the Strain Museum of the Department of Plant Physiology, Mariae Curie-Skłodowska University, Lublin. The studies with the use of pathogenic bacilli were carried out on Löwenstein-Jensen medium, in the case of saprophytic acid-fast bacilli on nutrient agar and Sauton's medium.

### METHODS

1. Preparation of alcohol extract from propolis. 100 g of propolis was added to 100 ml of 25% ethylene alcohol, shaken for 1 h and left for the next day. After 24 hrs propolis dissolved in alcohol and was shaken again for 1 hr. The solution was filtrated through Schott G-2 and G-5 filters. The extract obtained was kept in a dark bottle tightly closed and stored in a refrigerator (4°C).

2. Preparation of aqueous extract from propolis. 100 g of propolis was added to 100 ml of distilled water and heated at 60°C for 1 h. Then the solution was filtrated through Schott G-2 and G-5 filters. The extract was stored in a dark bottle tightly closed and placed in a refrigerator (4°C).

3. Preparation of moist bulk of bacilli. Pathogenic bacilli were multiplied on Löwenstein-Jensen medium at 37°C for 7 weeks. Then the bacterial suspension (1 mg/ml) was homogenized in bacterial mortar and diluted in physiological saline up to 0.001 mg/ml.

4. Cylindric method.

### EXPERIMENTS

#### 1. THE EFFECT OF INCUBATING PATHOGENIC TUBERCLE BACILLI IN PROPOLIS EXTRACTS

The suspension of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Mycobacterium tuberculosis* AN<sub>5</sub> and *Mycobacterium tuberculosis* Pt-Cracow (concentration of 0.01 mg of moist bacilli bulk in 1 ml) was incubated for 2, 6,

12, and 24 hours in aqueous and alcohol solutions of propolis. Propolis preparations were prepared from the basic solution (extracts from 100 g of propolis dissolved in 100 ml of alcohol or water) at dilutions 1:2, 1:5, 1:10 and 1:50. To 9 ml of particular dilutions of propolis was added 1 ml of bacilli suspension i.e. 0.001 mg of the moist bacteria bulk. As the control were used bacilli kept in distilled water and also in 25% ethylene alcohol for 2, 6, 12 and 24 hrs.

After 2, 6, 12 and 24 hour contact of the bacilli with the extracts from propolis, 0.1 ml of the liquid containing 0.0001 mg of moist bacilli was taken from each combination of the experiments. This suspension was inoculated on Löwenstein-Jensen slants and then incubated at 37°C. It appeared that a two-hour incubation of the strain in 1:50 water or alcohol solution of propolis was enough to inhibit its growth. In the case of AN<sub>5</sub> and Pt-Cracow strain no growth inhibition of these bacteria was observed. The results were checked after 6 weeks in comparison with the control (Table 1).

After 9 weeks of incubation the H<sub>37</sub>R<sub>v</sub> strain treated with propolis diluted at 1:5, 1:10 and 1:50 for 2, 4 and 6 hrs respectively showed some growth. After 10 weeks of incubation the H<sub>37</sub>R<sub>v</sub> strain grew in all the combinations of the experiment.

Table 1. The effect of extracts from propolis on pathogenic bacilli

Propolis solutions	<i>Mycobacterium tuberculosis</i> H <sub>37</sub> R <sub>v</sub>					<i>Mycobacterium tuberculosis</i> AN <sub>5</sub> and Pt-Cracow				
	Contact time in hrs				control	Contact time in hrs				control
	2	6	12	24		2	6	12	24	
1:2	—	—	—	—	+	+	+	+	+	+
1:5	—	—	—	—	+	+	+	+	+	+
1:10	—	—	—	—	+	+	+	+	+	+
1:50	—	—	—	—	+	+	+	+	+	+

— lack of bacteria growth, + growth of bacteria.

Table 2. The effect of extracts from propolis on the growth of pathogenic bacilli (water and alcohol extracts added directly to the medium)

% of propolis	Alcohol extract			Water extract			Control
	H <sub>37</sub> R <sub>v</sub>	AN <sub>5</sub>	Pt-Cracow	H <sub>37</sub> R <sub>v</sub>	AN <sub>5</sub>	Pt-Cracow	
1	—	—	—	+	+	+	+
5	—	—	—	+	+	+	+
10	—	—	—	—	—	—	+
30	—	—	—	—	—	—	+
50	—	—	—	—	—	—	+

— lack of bacteria growth, + growth of bacteria.



5 cylinders were previously mounted. Each well obtained after the removing of the cylinder was filled with 0.5 ml of aqueous or alcohol solution of propolis at concentrations 5, 10, 25, 50, 100 and 250 ml/mg. The control wells were filled with 0.5 ml of distilled water or with 0.5 ml of 25% ethyl alcohol. The results were checked after 4 days. The inhibition zones of bacteria growth were measured. Inhibition of bacteria growth was already obtained at the concentration of 10 mg/ml of propolis for all strains used in the experiments. The results are summarized in Table 3.

#### RESULTS AND DISCUSSION

It follows from the studies of Karimova (6, 7, 8) and Kivałkina (9) that the action of propolis extracts on *Mycobacterium* depends on the period of its storing as well as on the localities of its collection. We agree with this opinion. It appeared that the propolis gathered in Warsaw did not show any antibacterial action either on saprophytic or pathogenic strains. The extracts from propolis gathered in the Lublin district, however, acted on *Mycobacterium*. In experiments with keeping pathogenic bacilli in propolis solutions, only the growth inhibition of the strain *M. tuberculosis v. hominis* H<sub>37</sub>R<sub>v</sub> was found, whereas bacilli of the avian type (Pt-Cracow) grew more abundantly after two hours of their contact with the extracts from propolis. The extracts from propolis stimulated the growth of this strain after a short time of keeping these bacteria in propolis extracts. No inhibitory effect of the propolis extracts on bacilli *v. bovis* (AN<sub>5</sub>) was found in these experiments. Karimova found in experiments on direct addition of propolis to the media that bacteriostatic action of propolis extracts occurred only at large concentrations of propolis. Joirish (4) states that addition of propolis to the media did not inhibit the growth of bacilli, on the contrary, it accelerated their growth.

In this investigation the authors observed the action of alcohol extracts on bacilli of all types already at low concentrations of propolis. Water extracts inhibited the growth of these bacteria only at the concentration of 10% in the medium. It seems that the differences between the data chiefly result from the fact that Joirish did not define the types of the bacilli he studied, and it could have also happened that the propolis samples used in the studies, were inactive to the bacilli. It is known that bacteriolytic properties of propolis depend on its chemical composition, the way of storing, the preparation and on the localities of its collection. Therefore, some samples of propolis were not active with regard to the strains used in the experiments.

As regards saprophytic acid-fast bacilli, the results obtained in this paper agree with those of other authors. Lindenfelster (10, 11) obtained growth inhibition of *M. phlei* and *M. smegmatis* using alcohol extracts from propolis. Similarly, in our studies it was shown that aqueous and alcohol extracts inhibit the growth of saprophytic acid-fast bacilli as well as that of the strains of *M. phlei* and *M. smegmatis*.

On the basis of the experiments carried out and the results obtained it can be concluded that *M. tuberculosis v. hominis* are more sensitive to propolis preparations than those of the bovine and avium types. The results obtained encourage us to further studies which would help to determine what component of propolis is responsible for its antibacterial action.

#### CONCLUSIONS

1. Water and alcohol extracts from propolis added directly to the medium inhibit the growth of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Mycobacterium* AN<sub>5</sub> and *Mycobacterium tuberculosis* Pt-Cracow.

2. A temporary treatment of H<sub>37</sub>R<sub>v</sub> with water and alcohol extracts inhibited bacterial growth. Incubating the strains AN<sub>5</sub> and Pt-Cracow in propolis solutions has had an inhibitory effect on the growth of these bacteria.

3. Extracts from propolis inhibit the growth of saprophytic acidoresistant bacilli.

4. The activity of propolis depends on the locality of its collection.

#### REFERENCES

1. Fredericq P.: Colicines. Ann. Rev. Microbiol. 2, 7 (1957).
2. Iirasek L., Lenger J.: Profesionalni ekzem wcelare po propolisu. Ceskosl. Dermatol. 2, 82—85 (1960).
3. Йориш Н. П.: Применение прополиса в лечебных целях. Пчеловодство 2, 56—57 (1959).
4. Йориш Н. П.: О применении продуктов пчеловодства с лечебной целью. Пчеловодство 12, 49—54 (1959).
5. Каримова З. Н.: Использование прополиса в медицине. Пчеловодство 9 (1960).
6. Каримова З. Н., Цевастьянова К. И., Савина К. А., Вайнер Л. М.: К вопросу о бактерицидном действии экстракта прополиса на некоторые патогенные микроорганизмы. Каз. Мед. Журнал 1, 71—73 (1960).
7. Каримова З. Н.: О лечебных свойствах прополиса. Пчеловодство 8, 32 (1961).

8. Каримова З. Н., Радионова Е.: Туберкулёз лёгких и прополис. Пчеловодство **1**, 36—37 (1963).
9. Кивалкина В. П.: Противмикробное действие прополиса. Пчеловодство **10**, 50—51 (1959).  
Dermatol. **2**, 82—85 (1960).
10. Lindenfelster L. A.: Antimicrobial Activity of Propolis. Ann. Bee J. **3**, 90—92 (1967).
11. Lindenfelster L. A.: Antimicrobial Activity of Propolis. Ann. Bee J. **4**, 130—131 (1967).
12. Lindenfelster L. A.: *In vivo* Activity of Propolis against *Bacillus larvac*. J. Invert. Pathol. **12**, 129—131 (1968).
13. Мухамедияров Г. З.: Лечение прополисом некоторых кожных заболеваний. Пчеловодство **12**, 52—53 (1959).
14. Тихонов В. Д., Бургов П.: Опыт лечения прополисовой мазью. Вестник Дерматол. Венерол. **1**, 76—78 (1960).
15. Виноградова Т. В., Зайцев Г. П.: Пчела и здоровье человека. Поселхозиздат. Москва 1966.
16. Wojtacki M.: Produkty pszczele i przetwory miodowe. PWRiL, Warszawa 1970.

## STRESZCZENIE

Zbadano wpływ ekstraktów propolisowych z kitu pszczelego, zebranego w Polsce, na wzrost saprofitycznych i patogennych prątków gruźlicy. Wyciągi wodne i alkoholowe z kitu pszczelego dodane bezpośrednio do podłoża hamują wzrost *Mycobacterium tuberculosis* v. *hominis* H<sub>37</sub>R<sub>v</sub>, *Mycobacterium tuberculosis* v. *bovis* AN<sub>5</sub> Puławy i *Mycobacterium tuberculosis* v. *avium* Pt-Kraków. W próbach z przetrzymywaniem prątków patogennych w roztworach alkoholowym i wodnym z kitu pszczelego zauważono zahamowanie wzrostu tylko szczepu typu ludzkiego H<sub>37</sub>R<sub>v</sub>. Prątki typu ptasiego Pt-Kraków rosły obficie już po 2 godz. kontaktu bakterii z ekstraktami z propolisu. Przetrzymywanie szczepu bydłowego AN<sub>5</sub> w roztworach z kitu nie wpływa hamująco na wzrost tych bakterii.

Wykazano, że wyciągi z kitu hamują wzrost saprofitycznych prątków kwaso-  
opornych. Aktywność przeciwpłątkowa preparatów z kitu pszczelego zależy od miejsc zbioru kitu. Kit zebrany w Warszawie nie był czynny w stosunku do prątków. Natomiast wyciągi propolisowe z kitu zebranego na terenie województwa lubelskiego działały na *Mycobacterium*.

## РЕЗЮМЕ

Изучалось влияние экстрактов прополиса из собранного в Польше пчелиного клея на рост сапрофитных и патогенных туберкулезных палочек. Оказалось, что водная и спиртовая вытяжки из пчелиного клея, подаваемые непосредственно в питательную среду, тормозят рост *Mycobacterium tuberculosis* v. *hominis* H<sub>37</sub>R<sub>v</sub>, *Mycobacterium tuberculosis* v. *bovis* AN<sub>5</sub> Puławy, *Mycobacterium* v. *avium* Pt-Kraków. При хранении патогенных палочек в спиртовом и водном растворах из пчелиного клея наблюдалось торможение роста только штамма

человеческого типа ( $H_{37}R_v$ ). Палочки птичьего типа (Pt-Краков) росли более обильно уже спустя 2 часа после контакта этих бактерий с экстрактами из прополиса. Хранение скотного штамма ( $AN_3$ ) в растворах из пчелиного клея на рост этих бактерий тормозяще не влияет. Установлено, что вытяжки из пчелиного клея тормозят рост кислотоустойчивых сапрофитных палочек. Противопалочная активность препаратов из пчелиного клея зависит от места его сбора. Клей, собранный в Варшаве, на палочки не действовал. В то же время вытяжки из прополиса из пчелиного клея, собранного на территории Люблинского воеводства, были активными по отношению к *Mycobacterium*.