THE ACTION OF PROPOLIS ON CERTAIN MICROORGANISMS ISOLATED FROM VARIOUS MEDIUMS

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The biological and pharmacological characteristics of propolis, a substance elaborated by *Apis mellifera L.* from resins of a vegetable origin, have been the object of numerous research studies up to the present.

The antimicrobial activity of this beehive product was tested, especially on the bacteria and the fungi which are pathogenous for man and for animals, and rarely, on microorganisms coming from other mediums (PEPELJNJAK et al., 1982; MILENA et al., 1989; BRUMFITT et al., 1990; BRUNELLI et al., 1990; LA TORRE et al., 1990; LORI, 1990; DETOMA and OZINO, 1991; ABD-AL-FATTAH et al., 1993; FERNANDES Jr. et al., 1994). However, the results of these studies bring contradictory information on the sensitive species, as well as on the doses of propolis which are necessary to inhibit the microbial development.

Considering all these, researches were effected in order to obtain better knowledge on the action of this natural product on microorganisms of various origins. At the same time, a preliminary study was effected on the possible variability of the propolis action depending on its geographical origin.

Materials and Methods

The antimicrobial Activity of Propolis on Microorganisms of Various origins

For the experiments, we used propolis samples collected from apiaries situated in the hilly region near the city of Torino (Piedmont). Immediately after it was collected, the product was introduced in the freezer at –18 ^{O}C, for 24 hours. Then, it was broken into small pieces and dissolved in 96% ethilic alcohol and then, it was filtered. After the content of dry substance was determined, the solution obtained was diluted to a propolis concentration of 15% and was used to effect the programmed tests.

The investigated microorganisms – a total of 29 strains (Table 1) were grouped under the following categories: blastomycetes which are pathogenous for man, phytopathogenous fungi, bacteria and fungi which are pathogenous for insects, yeasts which are important for oenology, and fungi from the soil with a high saprophytic competitiveness. These microorganisms belonged to the following species: *Candida krusei* (Castellani) Berkhout; *C. parapsilosis* (Ashford) Langeron and Talice; *Cryptococcus albidus* (Saito) Skinner: *Trichosporon cutaneum* (de Beurm., Gourgerot and Vaucher) Ota; *Botrytis cinerea* Pers.;*Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav.; *Bacillus thuringiensis* Berliner, *Beauveria brongniartii* (Sacc.) Petch; *Saccharomyces cerevisiae* Meyen and Hansen. *Torulaspore delbrueckii* (Lindner) Lindner; *Zygosaccharomyces baili* (Lindner) Guillermond; *Cylindrocarpon magnusianum* Wollenw; *Verticillium bulbillosum* W. Gams and Malla.

The action of propolis on the selected strains was evaluated by means of the minimal concentration that inhibits the microbial development (MIC). For this purpose, propolis solutions in increasing concentrations (from 0.1 mg/ml to 45 mg/ml) were added to the following culture mediums, in Petri dishes: Lab-Lemco-agar (Oxid) for bacteria: solid Sabouraud medium with dextrone (Oxid) for blastomycetes; agar with malt extract (Oxid) for fungi. Three retorts were effected for each concentration.

Moreover, control dishes, with untreated medium, as well as other dishes, to which we added 96% ethylic alcohol, in an amount equal to the one used to prepare the propolis solution which had the minimal concentration necessary to inhibit the development of the studied strains, were prepared. This last group of dishes was prepared in order to verify if the solvent has any effect on microorganisms.

The three groups of dishes (with treated medium, with untreated medium and with alcohol addition) were seeded with each one of the microbe strains, by means of a microbial suspension, with a density of 2 x 10^6 cells/ml in the case of the bacteria and the yeasts, and of 2 x 10^6 conidia/ml in the case of the fungi. In the case of this last group of germs, the suspensions obtained from the sporulated mycelium were filtered beforehand, in order to remove as much of the remaining hyphae as possible.

Table 1

Microorganisms Used to Test the Activity of the Piedmont Propolis

Group	Genus and species	Strain	Origin	Source
Blastomycetes which are pathogenous for man	*Candida krusei*	Ck	Finger nail	BV
	Candida parapsilosis	Cp	Finger nail	BV
	Cryptococcus albidus	Ca	Leg epidermis	BV
	Trichosporon cutaneum	876-1	Hand epidermis	BV
	Trichosporon cutaneum	696-5	Toe nail	BV
Phytopathogenous fungi	*Botrytis cinerea*[1]	Gr 71/85	Vine	PV
	Botrytis cinerea[1]	Gr 74/89	Vine	PV
	Botrytis cinerea[1]	Gr 79/89	Vine	PV
	Botrytis cinerea[1]	Bs1	Vine	PV
	Botrytis cinerea[1]	Bs2	Vine	PV
	Botrytis cinerea[1]	Bs3	Vine	PV
	Botrytis cinerea[2]	Br1	Vine	PV
	Botrytis cinerea[2]	Br2	Vine	PV
	Botrytis cinerea[2]	Br3	Vine	PV
	Botrytis cinerea	B ot 1	Chrysanthemum	PV
	Botrytis cinerea	B ot 2	Air	BV
	Colletotrichum lindemuthianum	Coll	Beans	PV
Bacteria and mycetes which are pathogenous for insects Blastomycetes which are important for oenology	*Bacillus thuringiensis kurstaki*	K	Bioinsecticide	IC
	Bacillus thuringiensis tenebrionis	T	Bioinsecticide	SZ
	Beauveria brongniartii	B br 21	*Melolontha hippocastani*	BC
	Beauveria brongniartii	B br 92	*Melolontha melolontha*	MI
	Sacharomyces cerevisiae[3]	170	Passito di Caluso wine	MI
	Saccharomyces cerevisiae[4]	I 112	Chardonnay wine	MI
	Saccharomyces cerevisiae[4]	111	Gamay wine	MI
	Saccharomyces cerevisiae[4]	125	Trebbiano wine	MI
	Torulaspora delbrueckii[5]	118	Barbera wine	MI
	Zygosaccharomyces bailii[5]	123	Barbera wine	MI
Fungi from the soil	*Cylindrocarpon magnusianum*[6]	Cy ma	Beech roots	DB
	Verticillium bulbillosum[6]	Vb	Beech roots	DB

BC: Biol. Control Inst. Darmstad. Germany;
BV: Dip. Biol. veg., Torino University, Italy;
IC: Intrachem. Grassobbio, Bergamo, Italy;
MI: Di. VA. P.R.A., Micr., Ind. agr., Torino Univ., Italy;
PV: Di. Va. P.R.A., Veg. Pathol., Torino Univ., Italy;
SZ: Sandoz, Agrate Brianza, Milan, Italy;

[1] – Fungicide sensitive strains.
[2] – Fungicide-resistant strains.
[3] – Strains with high alcohologenous power.
[4] – Strains with medium alcohologenous power.
[5] – Strains with low alcohologenous power.
[6] – Fungi with high saprophytic competitiveness.

By means of a special sterile glass rod, 1 ml of microbial suspension is spread on the entire surface of the gelose in the dish. The seeded dishes are incubated for 24 hours at 37 ^{O}C for the bacteria, and for five days at 25 ^{O}C for fungi.

The Antimicrobial Activity of Propolis of Various Origins

In order to verify if there is a relationship between the antimicrobial activity of propolis and its origin, five samples of product were used: one from Italy (Calabria), two from different locations in China and two from different locations in Argentina.

For this series of experiments, we selected the *C. albidus* ca strain, which proved to be sensitive to the activity of Piedmont propolis.

The methods used in these experiments were identical to the ones described above. The Saubouraud – dextrone (Oxid) culture medium was mixed with increasing doses of propolis (from 0.1 mg/ml to 10 mg/ml).

Results

All the examined microorganisms proved to be sensitive to the Piedmont propolis samples, excepting the *C. krusei* Ck strain and the *C. paralopsis* Cp. Strain, which had a good evolution, even when maximal doses (45 mg/ml) of propolis were used. All the strains in the dishes containing control culture, as well as in those with an addition of 96% ethylic alcohol had a constant development.

The minimal concentrations necessary to inhibit the development of the propolis sensitive microorganisms are presented in Table 2. The values obtained allowed us to differentiate the strains and to determine the following groups: strains which are sensitive to propolis doses from 0.3 to 1.5 mg/ml; strains which are sensitive to propolis doses from 2.5 to 3.0 mg/ml; and strains which are sensitive to doses from 4.0 to 6.0 mg/ml.

Table 2

The Antimicrobial Activity of Piedmont Propolis, Expressed in Terms of Minimal Inhibitory Concentration (M.I.C.)

Group	Genus and species	Strain	M.I.C (mg/ml)
Blastomycetes which are pathogenous for man	*Candida krusei*	Ck	>45.0
	Candida parapsilosis	Cp	>45.0
	Cryptococcus albidus	Ca	3.0
	Trichosporon cutaneum	876-1	1.0
	Trichosporon cutaneum	696-5	0.6
Phytopathogenous fungi	*Botrytis cinerea*	Gr 71/85	4.5
	Botrytis cinerea	Gr 74/89	5.0
	Botrytis cinerea	Gr 79/89	4.5
	Botrytis cinerea	Bs1	6.0
	Botrytis cinerea	Bs2	5.0
	Botrytis cinerea	Bs3	5.5
	Botrytis cinerea	Br1	2.5
	Botrytis cinerea	Br2	4.0
	Botrytis cinerea	Br3	4.0
	Botrytis cinerea	B ot 1	5.0
	Botrytis cinerea	B ot 2	4.5
	Colletotrichum lindemuthianum	Coll	3.0
Entomopathogenous bacteria and mycetes	*Bacillus thuringiensis kurstaki*	K	0.3
	Bacillus thuringiensis tenebrionis	T	0.3
	Beauveria brongniartii	B br 21	1.5
	Beauveria brongniartii	B br 92	1.0
Blastomicetes which are important for oenology	*Sacharomyces cerevisiae*	170	4.5
	Saccharomyces cerevisiae	I 112	0.8
	Saccharomyces cerevisiae	111	0.3
	Saccharomyces cerevisiae	125	0.4
	Torulaspora delbrueckii	118	0.4
	Zygosaccharomyces bailii	123	0.3
Fungi from the soil	*Cylindrocarpon magnusianum*	Cy ma	1.5
	Verticillium bulbillosum	Vb	3.0

The entomopathogeneous microorganisms were the most sensitive; they were followed by the blastomycetes which are important for oenology, by the yeasts which are pathogenous for man, by the fungi from the soil and by the phytopathogenous mycetes (Fig. 1).

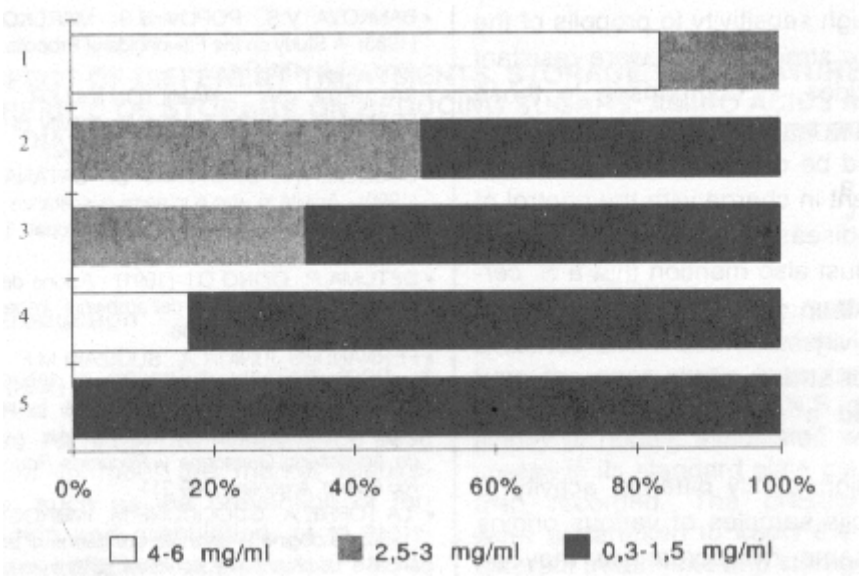

Fig. 1 – The Distribution of the Microorganism Groups Depending on Their Sensitivity to Propolis
1. Phytopathogenous fungi; 2. Fungi from the soil; 3. Blastomycetes which are pathogenous for man; 4. Blastomycetes which were extracted from wine; 5. Entomophagenous microorganisms

In the group of blastomycetes which are important for oenology, we noticed that the *S. cerevisiae* 170 strain, endowed with a high alcohologenous capacity, was much less sensitive (MIC = 4.5 mg/ml) than the other strains of the same species, which had a medium alcohologenous power (MIC = 0.3-0.8 mg/ml).

Within the group of the phytopathogenous fungi, the *B. cinerea* strains, which were resistent to fungicides, proved to be more sensitive to propolis, the difference being highly significant ($p<0.01$), in comparison to the strains which were sensitive to fungicides.

As regards the antimicrobial activity of the propolis samples of various origins on the *C. albidus* selected strain, we noticed that the sensitivity of the respective strain varied, depending on the propolis sample, between 0.5 and 4.5 mg active principle/ml (Fig. 2). The exception was one of the samples which came from Argentina and for which even the 10 mg/ml propolis concentration was insufficient in order to ihhibit the development of the respective blastomycete.

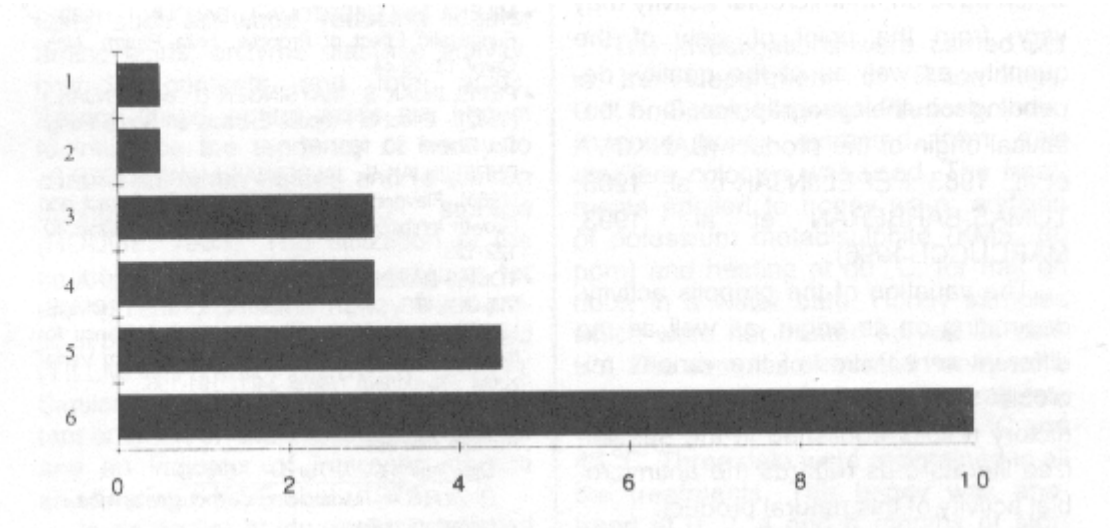

Fig. 2 – The Minimal Inhibitory Concentration (M.I.C.) of the propolis Samples of Various Origins. Tested on Cryptococcus albidus
1. Calabria; 2. China; 3. China; 4. Piedmont; 5. Argentina; 6. Argentina* (* M.I.C. > 10 mg/ml)

Conclusions

The experiments we effected showed that most of the microbial strains we used were sensitive to the Piedmont propolis.

We noticed that the antimicrobial activity of the product varies from one microorganism species to another, as well as from one strain to another, within the same species. The latter was confirmed mainly by the results obtained in the case of the *B. cinerea* phytopathogenous mycete and of the *S. cerevisiae* blastomycete, for which we had the opportunity to examine a sufficient number of strains.

The high sensitivity to propolis of the *B. cinerea* strains, which were resistant to fungicides, in comparison to those which were sensitive to the same products could be of certain interest for the department in charge with the control of the plant diseases.

We must also mention that a *S. cerevisiae* strain with a high alcohologenous activity was much less sensitive than other strains of the same species which had a medium alcohologenous activity.

The significantly different activity of the propolis samples of various origins on the same microorganism may be considered an indirect demonstration of the extremely varied chemical composition of the samples we used. It is well known that the propolis components which have an antimicrobial activity may vary, from the point of view of the quantity, as well as of the quality, depending on the geographical and botanical origin of the product (BANKOVA et al., 1983; PEPELJNJAK et al., 1985; TOMAS-BARBERAN et al., 1993; MARCUCCI, 1996).

The variation of the propolis activity, depending on its origin, as well as the different sensitives of the various microbial strains, fully confirm the contradictory results published in the specialized literature as regards the antimicrobial activity of this natural product.

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