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Antibacterial properties of propolis and products containing propolis from Brazil

H Menezes*, M Bacci Jr, SD Oliveira, FC Pagnocca

Centro de Estudos de Insetos Sociais, Instituto de Biociências, Unesp, Rio Claro, São Paulo, Brazil

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Summary — Crude propolis and commercial products containing propolis, such as ethanolic extracts, tablets, capsules and powders acquired in São Paulo City (Brazil) were analyzed. The resins of the solid products were extracted with ethanol and found to be present at various concentrations, independently of the propolis concentration specified on the label of the commercial products. The in vitro activity of these resins against *S aureus*, *B cereus* and *B subtilis* was also determined. The results showed that the antibacterial activity rather than the propolis concentration itself should be considered for quality control and that some resins are likely to display a species-specific action.

propolis / resin / *Apis mellifera* / quality control / antibacterial activity / *Staphylococcus aureus* / *Bacillus cereus* / *Bacillus subtilis*

INTRODUCTION

Propolis (bee glue) has been used in folk medicine for centuries (Ghisalberti, 1979). This is supported by recent findings about its biological properties such as antibacterial (Grange and Davey, 1990), antiviral (Amoros et al, 1992) or anti-protozoan (Starzyk et al, 1977) activity, inhibition of tumor processes (Frenkel et al, 1993), anti-inflammatory action (Dobowolski et al, 1991; Volpert and Elstner, 1996), induction of both bone (Stojko et al, 1979) and cartilaginous tissue regeneration (Scheller et al, 1977), free radical scavenging (Scheller et al, 1989; Volpert and Elstner, 1993), and allergenic effect (Hausen et al, 1987a, b; Ginanneschi et al, 1989). The biological properties and chemical composition of propolis have been recently reviewed (Marcucci, 1995).

The characterization of these biological properties together with the current trend towards utilizing natural products have resulted in an increased demand for propo-

* Corresponding author: IB-Unesp, av 24 "A" n° 1515, PO Box 199, Rio Claro, SP, Brazil, CEP: 13506-900.
Fax: (55) 195 340009.

lis and products containing propolis, such as ethanol extracts, tablets, capsules, sprays, or powders.

The expansion of the propolis industry requires standardization of these products, and should take into account regional vegetation diversity (Vanhaelen and Vanhaelen-Fastré, 1979), the harvesting activity of distinct races of honeybees (Singh, 1972), and establish the efficacy of the products. In addition, such a standardization needs to be simple and inexpensive to be accessible to manufacturers.

In this context, the objective of the present study was the evaluation of the in vitro antibacterial activity of the different formulations of propolis. Samples were bought in pharmacies, drugstores and from beekeepers.

MATERIALS AND METHODS

Propolis and commercial products containing propolis

Three groups of products were studied in this work: ten different commercial ethanolic extracts referred to as CE1 to CE10; commercial preparations of tablets, capsules and powders containing propolis; two other ethanolic extracts prepared in our laboratory using crude propolis from one site reforested with *Pinus* sp and another reforested with *Eucalyptus* sp. All these propolis samples were from *A mellifera* and the extracts were prepared with 95% pa ethanol (Merck).

Determination of resin concentration in commercial alcoholic extracts

Aliquots of 5.0 mL of each extract were transferred to previously weighted flasks and evaporated at 50 °C until constant weight was reached.

Determination of resin content in crude propolis and in solid commercial product

One hundred grams of crude propolis of *Pinus* sp or *Eucalyptus* sp were mixed with 200 mL of 95% ethanol and maintained in a rotatory water bath shaker during 1 week at 25 °C. The ethanolic extracts were then filtered in Whatmann no 1 filter paper and designated as E-*Pinus* or E-*Eucalyptus*. The resin extraction procedure for the solid commercial products was similar but using 200 g of every product/200 mL of ethanol. These extracts were designated as E-tablet, E-capsule and E-powder. The resin content of these five extracts was determinated by the same procedure as that used with the commercial alcoholic extract. After determinating the resin content, all 15 samples were diluted with 95% ethanol to 10 mg/mL (stock-solution) which was used for the antibacterial activity assay. The resin content (% resin) was expressed in grams of resin per 100 mL.

Antibacterial activity determination

This determination was adapted from Waksman and Reilly (1945). The propolis sensitive bacteria (Grange and Davey, 1990) *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* CCT 1436, and *Bacillus subtilis* CCT 2471 were cultivated (24 h, 35 °C) on Nutrient Agar medium (Difco) and then suspended (one unit on the MacFarland scale) in sterile saline 0.85%. The suspensions (0,1 mL) were sprayed with a cotton swab onto the surface of solid media in Petri dishes. The culture medium had been previously prepared by mixing 9.0 mL of melted (47 °C) NA medium with 1 mL of 95% ethanol (control) or with 1 mL of dilutions of resin stock solutions. The results were expressed in 'limit concentration' (LC), ie, the minimum concentration of resin (μg of resin per mL of culture medium) which completely inhibited bacterial growth. The LC values represented the average of at least two assays.

RESULTS

Table I shows the propolis concentration specified on the label of the commercial products by manufacturers, and the resin concentration of these products determined in the present investigation. The ethanolic extracts from *Pinus* (E-*Pinus*) or *Eucalyptus* (E-*Eucalyptus*) crude propolis, prepared to contain 33% (w/v) of propolis were found to contain resin at 13.4% and 12.8%, respec-

tively. Similar analysis showed that commercial products contained a minimum of 1.5 to 14.2 maximum of % (w/v) resin.

Results of antibacterial activity of the different resins (table II) showed that *B cereus* was always the most sensitive organism, followed by *B subtilis*, and *S aureus*. The LC values obtained with these organisms characterized the low activity of CE10, tablet, capsule and powder resins when compared to the higher activity of resins from CE1, CE7, and E-*Eucalyptus* and E-*Pinus*. In addition, resins from capsuled and powdered propolis were effective against *B cereus*, but presented low activity against *B subtilis* and *S aureus*.

DISCUSSION

The products analyzed in the current investigation showed different resin concentrations (table I), regardless of the propolis concentration specified on their labels. This finding probably reflects variable crude propolis composition including wax, wood fragments, debris, and water (Ackermann, 1991).

The results of antibacterial activity showed differences in the quality of the resins investigated. For instance, CE10 was found to have a high resin content (12.3 g %) (table I), but presented a high LC value for all microorganisms tested (table II). Other evidence showing that resin source and probably their composition can, at least for antibacterial purposes, be more important than the concentration itself, was obtained from the comparison of LC from *E-Eucalyptus* and *E-Pinus*. The antibacterial activity against *S aureus* and *B subtilis* of *E-Eucalyptus* was sligthly higher than *E-Pinus*. Since the only difference between them is the plant species, we think that the

Table I. Propolis and resin concentration of analysed products.

Sample	*Propolis (%) specified by manufacturer*	*Original resin concentration (%)*
E-*Pinus*	33*	13.4
E-*Eucalyptus*	33*	12.8
CE1	33	14.2
CE2	40	12.2
CE3	10	3.3
CE4	20	4.4
CE5	15	4.9
CE6	20	6.5
CE7	25	8.2
CE8	15	4.3
CE9	30	9.8
CE10	20	12.3
E-Tablet	NS	1.5
E-Capsule	NS	8.2
E-Powder	NS	7.7

E: ethanolic extract; CE: commercial ethanolic extract; NS: propolis concentrations not specified by the manufacturer; *: prepared in our laboratory.

Table II. Limit concentration (LC), in $\mu g/mL^{-1}$, of resins extracted from propolis or propolis-containing commercial products.

Sample	*S aureus*	*B cereus*	*B subtilis*
E-*Pinus*	100	50	90
E-*Eucalyptus*	80	50	80
CE1	80	30	70
CE2	100	50	80
CE3	100	40	70
CE4	100	60	80
CE5	130	60	100
CE6	100	40	70
CE7	80	30	50
CE8	150	50	110
CE9	100	30	60
CE10	600	200	500
E-Tablet	700	200	500
E-Capsule	200	50	140
E-Powder	300	50	140

E: ethanolic extract; CE: commercial ethanolic extract.

different chemical composition of their resin is responsible for the observed effects. Some *Eucalyptus* species are known for the antibacterial activity of some compounds in their essential oils (Haiji and Fkih-Tetouani, 1993). In addition, inadequate storage could also result in a propolis with high resin content, but low antibacterial activity.

Inactivation of antibacterial compounds during the manufacturing process and utilization of low quality propolis are some of the possible reasons for the low antibacterial activity of the capsule, tablet and powder but our data is too limited to form a definitive conclusion. Since we had no information on the industrial procedures, nor on the phytogeographic origin of the propolis contained in the commercial products, we were unable to determine which of these possibilities was the most significant. On the other hand, the high activity of powdered propolis resin against only *B cereus* (table II) indicates that this resin may have species-specific action.

The present findings indicate that the antibacterial activity, and perhaps other biological properties of propolis, could not be correlated with their resin concentration but mostly with their chemical composition, which can be variable according to the collection site (Tomas-Barberán et al, 1993) and the quality of the resin. Therefore the methodology used was effective in the evaluation of the quality of antibacterial activity from both propolis and commercial products containing propolis.

Resin quality and resin concentration can be used to have access to the in vitro activity of a given commercial product. This and the in vivo activity may be considered to establish the posology of each product. Currently resin quality and dosage are not specified in commercial products containing propolis from Brazil.

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Résumé — Propriétés antibactériennes de la propolis et des produits renfermant de la propolis du Brésil. Des produits renfermant de la propolis disponibles dans le commerce au Brésil ont été analysés du point de vue de leur concentration en résine et de l'activité antibactérienne. Ils comprenaient dix extraits alcooliques (CE1 à CE10) et des produits du commerce sous forme solide. Les résines des produits solides ont été extraites à l'éthanol et nommées E-tablette, E-capsule et E-poudre selon leur formulation. On a fait, en outre, des extraits de deux propolis brutes, nommés E-*Pinus* et E-*Eucalyptus* (propolis provenant de sites reboisés respectivement en *Pinus* sp *et Eucalyptus* sp). La concentration en résine a varié de 1,5 à 14,2 % (tableau I), alors que les étiquettes des produits du commerce annonçaient une teneur comprise entre 10 et 33 %. La détermination de la « concentration limite » (LC), c'est-à-dire la concentration minimale en résine (µg de résine par mL du milieu de culture) qui inhibe complètement la croissance bactérienne, a servi à évaluer l'activité bactérienne (tableau II). Les valeurs de la LC ont montré que l'organisme le plus sensible était toujours *Bacillus cereus*, suivi par *B subtilis* et *Staphylococcus aureus*. Certains produits, comme CE10, ont présenté une concentration en résine élevée mais une faible activité bactérienne. Cela montre que des résines différentes ont des qualités différentes. La qualité de la résine dépend peut-être de son origine, puisque les deux préparations de propolis faites au laboratoire (E-*Pinus* et E-*Eucalyptus*) avaient une activité contre *S aureus* et *B subtilis* légèrement différente. La qualité de la résine peut être aussi influencée par le procédé de fabrication ou les conditions de stockage. La résine de la propolis en poudre avait une activité élevée contre *B cereus* uniquement, ce qui montre que son action est spécifique à l'espèce. La méthode présentée ici peut être utilisée pour évaluer la concentration en résine et l'activité antibactérienne in vitro des produits du commerce renfermant de la propolis. Il faudrait prendre en compte ces deux caractéristiques, ainsi que l'activité in vivo, pour établir la posologie de chacun de ces produits.

propolis / contrôle qualité / activité antibactérienne / *Staphylococcus aureus* / *bacillus subtilis* / *Bacillus cereus*

Zusammenfassung — Antibakterielle Wirkung von Propolis und von propolishaltigen Produkten in Brasilien. Der Harzgehalt und die antibakterielle Aktivität von propolishaltigen Handelsprodukten aus Brasilien wurden untersucht. Die Proben setzten sich aus 10 Alkolholextrakten (CE 1 bis CE 10) und aus festen Substanzen zusammen. Aus den festen Handelsprodukten wurde das Harz mit Ethanol extrahiert, die Produkte wurden entsprechend als E-Tablette, E-Kapsel und E-Pulver bezeichnet. Zusätzlich wurden noch 2 andere rohe Propolisproben extrahiert und als E-*Pinus* (Propolis aus einer mit *Pinus* spec aufgeforsteten Region) und E-*Eukalyptus* (Propolis aus einer mit *Eucaliptus* spec aufgeforsteten Region) bezeichnet. Die Harzkonzentration variierte in allen Aufarbeitungen von 1,5 - 14,2% (Tabelle I), obwohl auf den Etiketten der Handelsprodukte ein Harzgehalt von 10 - 33% angegeben war. Die Bewertung der bakteriziden Aktivität (Tabelle II) erfolgte durch die Bestimmung der „limit concentration" (LC), also der geringsten Konzentration (µg Harz per ml Kulturmedium), bei der eine totale Hemmung des Bakterienwachstums erfolgte. Die LC-Werte ergaben, daß *Bacillus cereus* immer der empfindlichste Organismus war, gefolgt von *B subtilis* und *Staphylococcus aureus*. Einige

der Produkte wie CE 10 hatte eine hohe Harzkonzentration, aber eine niedrige bakterizide Wirkung, wodurch eine unterschiedliche Qualität der verschiedenen Harze belegt wird. Die Harzqualität dürfte von seiner Herkunft abhängen, da die Propolis - Laborextrakte von E-*Pinus* und E-*Eucalytus* leicht unterschiedliche Aktivitäten gegenüber *S aureus* und *B subtilis* zeigten. Zusätzlich kann die Harzqualität auch durch den Herstellungsprozess oder die Art der Lagerung beeinflußt werden. Das Harz vom pulverisierten Propolis hatte nur gegen *B cereus* eine hohe Aktivität, demnach ist dieses Harz artspezifisch wirksam.Die hier beschriebene Methode ist geeignet, die Harzkonzentration und Aktivität von Harz in vitro bei Handelsprodukten mit Propolis zu überpüfen. In Verbindung mit der in vivo Aktivität sollten diese Versuche die Möglichkeit eröffnen, eine Dosiologie für jedes dieser Produkte festzusetzen.

Apis mellifera* / Propolis / Harz / Qualitätsprüfung /antibakterielle Wirkung / *Staphylococcus aureus* / *Bacillus cereus* / *Bacillus subtilis

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