Antibacterial properties of propolis (bee glue)

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Summary
Propolis (bee glue) was found to have antibacterial activity against a range of commonly encountered cocci and Gram-positive rods, including the human tubercle bacillus, but only limited activity against Gram-negative bacilli. These findings confirm previous reports of antimicrobial properties of this material, possibly attributable to its high flavonoid content.

Introduction

The therapeutic potential of honey has recently been reviewed by Zumla and Lulat [1]. Other bee products, royal jelly and propolis, have also been widely used in 'folklore medicine' for centuries. Propolis is a hard, resinous material derived by bees from plant juices and used to seal openings in the hives. It contains pollen, resins and waxes and large amounts of flavonoids which are benzo-γ-pyrone derivatives found in all photosynthesizing cells. Flavonoids have many biological effects in animal systems but have received relatively little attention from pharmacologists[2].

We are currently undertaking a screening study of a large number of plants and plant-derived materials in a search for possible new antimicrobial agents, particularly for use against methicillin resistant *Staphylococcus aureus* (MRSA). In this paper we report our findings with propolis and review the literature, mostly from Eastern Europe, on the antimicrobial and other properties of this substance and of its therapeutic applications.

Materials and methods

An ethanolic extract of propolis was obtained from Boiron et Cie, Lyon, France. On evaporation, 1 ml of this extract yielded 60 mg of solid resinous material.

Twenty-one bacterial strains were received from the Bacteriology Department of the Brompton Hospital and from the Public Health Laboratory, Dulwich: *Staphylococcus aureus* 6 strains (including the Oxford reference strain and 3 MRSAs), *Staph. epidermis* 2 strains, *Enterococcus* spp. 2 strains, *Branhamella catarrhalis* 1 strain, *Corynebacterium* sp. 1 strain, *Bacillus cereus* 2 strains, *Pseudomonas aeruginosa* 3 strains, *Escherichia coli* 2 strains, *Klebsiella pneumoniae* 1 strain and *Mycobacterium tuberculosis* 1 strain (the H37Rv reference strain).

Screening was performed by making a 1 : 20 dilution of the ethanolic extract of propolis in blood-agar base (Difco): 1 ml of propolis was added to 19 ml of molten medium at 45 °C, mixed and poured into a petri dish. After cooling and drying, the plates were inoculated with bacterial suspensions (approx. 1 mg wet weight in 1 ml of nutrient broth) with a Denley applicator. Control studies showed that the method of preparation allowed most of the ethanol to evaporate and that residual amounts, if any, did not inhibit bacterial growth. The minimal bactericidal concentrations (MBC) of propolis were estimated by making doubling dilutions from 1 : 20 in nutrient broth and inoculating each tube with one drop of a bacterial suspension (approx. 1 mg wet weight in 10 ml of nutrient broth). After 14 h, loopfuls of medium were taken from each tube and streaked on propolis-free agar medium to check for bacterial growth.

The MBC of propolis for *Mycobacterium tuberculosis* was determined by removing all ethanol (to which the tubercle bacillus is very sensitive) by evaporation and resuspending the residue in Middlebrook-Dubos 7H9 broth containing 0.05% Tween 80. This was used to make doubling dilutions from 1 : 20 in Middlebrook-Dubos 7H11 agar slopes which were inoculated with one drop of a 1 mg/ml suspension of the strain. The slopes were observed for bacterial growth for up to one month.

Results

In screening studies at a dilution of 1 : 20 (ie 3 mg of solid material per ml) in nutrient agar, the preparation of propolis completely inhibited the growth of *Staphylococcus aureus* (including the MRSA strains), *Staph. epidermidis*, *Enterococcus* spp., *Corynebacterium* spp., *Branhamella catarrhalis* and *Bacillus cereus*. It partially inhibited growth of *Pseudomonas aeruginosa* and *Escherichia coli* but had no effect on *Klebsiella pneumoniae*. Thus it appeared to have a preferential inhibitory effect on cocci and Gram-positive rods. Tube dilution studies showed that it was bactericidal for *B. cereus* and the Gram-positive cocci at dilutions of 1 : 160 to 1 : 320, and that growth of the H37Rv reference strain of *Mycobacterium tuberculosis* was totally inhibited at 1 : 320 and partially inhibited at 1 : 640.

Discussion

Unbeknown to us at the time of our studies, the antimicrobial properties of propolis have been well documented in a series of publications from Eastern Europe. Thus it has been shown previously that propolis is more active on Gram-positive than on Gram-negative bacteria[3]. On the other hand, *Listeria monocytogenes* is resistant to propolis which has therefore been used to develop a selective medium for this bacterium[4]. Alcoholic extracts of propolis are active against a wide range of dermatophytes at concentrations of 0.25 to 2%[5], antiviral properties have also been described[6] and the protozoa *Toxoplasma gondii* and *Trichomonas vaginalis* were killed within 24 h when incubated with 150 μg/ml of propolis[7].

The nature of the antimicrobial components of propolis has not been elucidated although there is

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evidence that they are to be found amongst the flavonoids and various esters of caffeic acid[8]. Caffeic acid phenethyl ester (CAPE) extracted from propolis has also been shown to be toxic for a range of tumour-derived cell lines[9]. A component active against *Bacillus subtilis* has been identified as 3,5,7-trihydroxyflavone (galangin)[10]. On the other hand, it has been suggested that the killing of staphylococci is the result of the combined action of several components, none of which alone are effective[11]. Bioautograms, ie chromatograms overlaid with bacteria or fungi in agar media, have revealed that propolis contains more than one agent active against bacteria and *Candida albicans*[12]. The mode of action likewise requires clarification: an unidentified water-soluble, u.v. absorbing component of propolis has been shown to inhibit bacterial DNA-dependant RNA polymerases[13]. In addition, synergy between propolis and a range of antibiotics has been demonstrated in several studies[14]. In our studies with the Oxford strain of *Staph. aureus*, we have demonstrated synergy between propolis and an ethanolic extract of *Aralia racemosa*, another plant with antistaphylococcal activity.

Honey has been used as a dressing to promote wound healing[1]. Likewise, ethanol extracts of propolis have been shown to promote the regeneration of bone[15], cartilage[16] and dental pulp[17]. This may also be a property of the flavonoids which have been shown to be anti-inflammatory and able to stimulate the formation of collagen[2].

Extracts of propolis are non toxic in experimental animals[18]. Aqueous solutions (0.5-1%) have been administered to human beings as aerosols for the apparently successful treatment of acute and chronic respiratory disease[19] and have been used as eye-drops[20]. A 10% alcoholic solution has been used for disinfection of hands in dental surgical practice[21].

It appears likely that the beneficial effects of propolis and honey are the result of their flavonoid content and both these natural compounds, and purified flavenoids, appear to be worthy of further appraisals of their therapeutic efficacy.

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