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# Beeswax: A minireview of its antimicrobial activity and its application in medicine

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ABSTRACT

Beeswax is the substance that forms the structure of a honeycomb; the bees secrete wax to build the honeycombs where to store honey. Thanks to its rich hydrophobic protective properties, the beeswax is in fact present within cosmetics and body products. Also, beeswax is used in the food industry: as a film to wrap cheese for maturing or as a food additive (E901) to give shine to the products. Exactly as the honey which it contains, beeswax is also characterized by several therapeutic properties of great interest to us; it is thought to be particularly effective in healing bruises, inflammation and burns. Recently, the interest of researchers has moved even on antimicrobial properties of beeswax although there are still few studies in the literature focused only on the action of beeswax. The few studies showed an antimicrobic effectiveness of beeswax against overall *Staphylococcus aureus*, *Salmonella enterica*, *Candida albicans* and *Aspergillus niger*; these inhibitory effects are enhanced synergistically with other natural products such as honey or olive oil. This minireview aims to be a collection of major scientific works that have considered the antimicrobial activity of beeswax alone or in combination with other natural products in recent years.

## 1. Introduction

In nature, there are some insects that produce wax, but some Apoidea [1], especially bees, produce wax more appreciated and used by man. The most used wax, beeswax, is produced by species *Apis mellifera* and *Apis cerana*, which are the most bred by humans and, therefore, it provides easier access to this bee product that has a wide spectrum of uses.

Beeswax is a complex product secreted in liquid form by special wax glands in the abdomen of younger worker bees (aged between 12 and 18 days, that is to say at the end of the period in which the bees act as nurses) [2]. In contact with the air, it solidifies in scales (that the bees model with jaws to build the honeycombs, adding pollen and propolis) [3,4].

When secreted by the bee, the pure beeswax is almost white; only after contact with honey and pollen it assumes a variably intense yellowish color and turns brown after about four years, because it contains the cocoon [5]. It resists the action of acids and gastric juices of honeybees and is insoluble in water and cold alcohol; it dissolves partially in boiling alcohol, and completely in chloroform, in carbon disulfide, and in the essence of hot turpentine [4,6]. When the wax is treated with boiling alcohol the part that melts is formed by cerotic acid, free or mixed with small amounts of melissic acid, while the one that does not dissolve is formed by ether-melisil palmitic mixed with small amounts of ethers compounds of palmitic and stearic acid. Its density at 15 °C is about 0.960 kg/m$^3$ to 0.970 kg/m$^3$ and it melts at temperatures between 63.5 °C and 64.5 °C [3,5,6]. If subjected to a dry distillation it turns into a buttery mass which is called wax oil [7].

### 1.1. Composition of beeswax

Beeswax is a complex mixture (more than 300 components) of hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohol, diesters and exogenous substances [8,9].

The beeswax composition is: hydrocarbons (12%–16%) with a predominant chain length of C27–C33, mainly heptacosane, nonacosane, hentriacontane, pentacosane and tricosane [10]; free fatty acids (12%–14%), with a chain length of C24–C32 [11]; free fatty alcohols (ca. 1%) of C28–C35 [5]; linear wax monoesters and hydroxymonoesters (35%–45%) with chain lengths generally of C40–C48, derived fundamentally from

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palmitic, 15-hydroxypalmitic and oleic acids [11]; complex wax esters (15%–27%) containing 15-hydroxypalmitic acid or diols, which through their hydroxyl group, are linked to another fatty-acid molecule [7]; exogenous substances that are mainly residues of propolis, pollen, small pieces of floral component factors and pollution [5]. The composition of the beeswax may vary between and among the different families and different breeds of bees, because it is probable that wax production is closely related to bee genetics and diet [12].

### 1.2. Pharmaceutical and traditional use of beeswax

"Pharmaceutical" use of beeswax dates back to ancient Egypt: as reported by the Ebers Papyrus (1550 B.C.), beeswax was the main ingredient in many recipes for the preparation of ointments and creams used to help pull plugs, to treat burns and wounds and to soothe joint pain. The "father of medicine", Hippocrates, recommended the use of beeswax in case of purulent tonsillitis. In ancient Rome, many doctors of the time used to apply a cream known as "cold cream", which contained olive oil, beeswax and rose water for the treatment of burns, wounds, cuts, bruises and fractures [2]. Beeswax was one of the components of the first cosmetic cream, which was created by Galen, the great Greek physician, in 150 B.C., composed of beeswax and olive oil, with emulsion of water (or rose water) [13]. Beeswax plays an important role also in Ayurvedic medicine, the ancient and traditional Indian medicine, with the name of Madhuchishtha [14]. In Western countries, the search for natural products to be used together with drugs or, even, to replace them has lead to a "rediscovery" of Ayurvedic medicine.

The Madhuchishtha (beeswax) is used for the care of wounds from abrasion or even from burns with topical application [15]; it has been shown to be particularly effective in the treatment of heel cracking [16]. Some reports highlight the use of Madhuchishtha in combination with other natural products or mixtures, like Madhu (Honey) or Guda (Jaggery) or Taila (Oil) [17]. Today beeswax is widely studied and used for human medicine.

## 2. Antimicrobial activity of crude beeswax

During recent years, the antimicrobial activity of natural products and especially products of the hive is gaining importance and unlike other bee products, beeswax has been only recently studied. Crude beeswax showed antibacterial activity against several bacterial strains and against the *Candida albicans* (*C. albicans*) yeast [18]. The sample of beeswax was effective against both Gram-positive bacteria, in particular *S. aureus* ATCC25923 (*S. aureus* ATCC25923) (7 mm), *Streptococcus epidermidis* ATCC12228 (6.5 mm) and *Streptococcus pyogenes* ATCC19615 (6.5 mm), and against Gram-negative bacteria, in particular *Bacillus subtilis* ATCC27853 (*B. subtilis* ATCC27853) (7 mm), *Pseudomonas aeruginosa* ATCC27853 (4 mm), *Escherichia coli* ATCC25922 (*E. coli* ATCC25922), and a particular inhibitory effect was found against *C. albicans* NCTC2708 (20 mm); no effect was found instead against *Salmonella typhimurium* ATCC14028 and *Proteus mirabilis* ATCC14153 [18].

Considerable interest was aroused by beeswax methanol and ethanol extracts [19]. Beeswax was extracted with four different solvents: methanol aqueous medium at concentrations of 99.9% and 70%, respectively denominated Meh and Mel; ethanol aqueous medium at concentrations of 96% and 70%, respectively denominated Eh and El.

The results, expressed in millimeters by measuring the zone of inhibition obtained from the effect of the extracts on the tested microrganisms (Table 1) were very successful. As shown in Table 1, the most sensitive strains with WMeh were *S. enterica* CCM4420 and *Candida tropicalis* (*C. tropicalis*), while, for WMel the most sensitive strains were *E. coli* CCM3988, *C. albicans*, *C. tropicalis* and *Aspergillus niger* (*A. niger*). With Weh the strains with a higher zone of inhibition were *A. niger*, *C. albicans* and *Candida glabrata* (*C. glabrata*), and with WEl they were found to be *Listeria monocytogenes* CCM4699, *E. coli* CCM3988 and *C. glabrata* [19].

The reasons for the different antimicrobial actions of beeswax extracted with methanol and beeswax extracted with ethanol are still unknown, but it is assumed that they were derived from the different extraction method that inhibits or blocks certain molecules [19]. This research has considered the antimicrobial activity of beeswax alone.

## 3. Antimicrobial activity of beeswax in synergy with other natural products

Instead, a little more has been studied and tested regarding the antimicrobial action of beeswax in synergy with the other products of the hive and other natural products. Honey, beeswax and olive oil mixture (1:1:1, v/v) are useful to inhibit the growth of *S. aureus* and *C. albicans*, isolated by human patients [20]. After being incubated at 37 °C, the bacteria (for 24 h) and the yeast (for 48 h), a zone of inhibition of 4 mm and 3.5 mm was measured for *S. aureus* and *C. albicans*, respectively [20].

**Table 1**
Diameter in mm of inhibition zone of inhibition arising from beeswax against pathogenic bacteria, fungi and yeasts [19].

| Bacterial strains | WMeh | WMel | WEh | WEl |
|---|---|---|---|---|
| *Listeria monocytogenes* CCM 4699 | 0.33 ± 0.58 | 2.67 ± 2.31 | 0.33 ± 0.57 | 4.33 ± 3.79 |
| *P. aeruginosa* CCM 1960 | 1.67 ± 1.54 | 2.33 ± 2.08 | 1.67 ± 1.53 | 2.67 ± 2.31 |
| *S. aureus* CCM 3953 | 2.00 ± 0.00 | 1.67 ± 1.53 | 1.67 ± 2.08 | 1.00 ± 1.00 |
| *S. enterica* CCM 4420 | 2.67 ± 0.58 | 2.67 ± 0.58 | 2.17 ± 1.89 | 3.67 ± 0.58 |
| *E. coli* CCM 3988 | 1.50 ± 1.32 | 4.67 ± 2.52 | 1.67 ± 1.53 | 4.67 ± 0.58 |
| *Aspergillus fumigatus* | 2.33 ± 0.58 | 2.67 ± 1.15 | 2.00 ± 2.00 | 2.50 ± 1.32 |
| *Aspergillus flavus* | 0.67 ± 1.15 | 1.67 ± 0.58 | 1.67 ± 0.58 | 2.00 ± 1.00 |
| *A. niger* | 2.33 ± 0.58 | 3.00 ± 0.00 | 3.00 ± 1.73 | 4.00 ± 1.73 |
| *Candida krusei* | 2.00 ± 1.00 | 1.83 ± 1.76 | 2.50 ± 2.18 | 4.00 ± 3.46 |
| *C. albicans* | 2.33 ± 2.08 | 3.67 ± 1.15 | 2.67 ± 1.15 | 3.33 ± 0.58 |
| *C. glabrata* | 2.00 ± 1.73 | 2.67 ± 2.08 | 2.67 ± 1.53 | 4.83 ± 1.26 |
| *Candida parapsilosis* | 2.00 ± 1.00 | 2.67 ± 1.15 | 1.33 ± 1.15 | 3.00 ± 3.61 |
| *C. tropicalis* | 3.00 ± 1.00 | 4.67 ± 0.58 | 2.00 ± 0.00 | 3.67 ± 0.58 |
| *Geotrichum candidum* | 2.33 ± 0.58 | 2.67 ± 2.31 | 2.33 ± 1.53 | 4.17 ± 2.47 |
| *Rhodotorula mucilaginosa* | 2.33 ± 0.58 | 2.50 ± 1.32 | 1.67 ± 0.58 | 2.33 ± 0.58 |

WMeh: 99.9% metanolic extract; WMel. 70% methanol extract; WEh: 96% of ethanol extract; Wel: 70% ethanol extract.