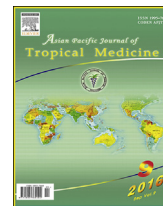




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Beeswax: A minireview of its antimicrobial activity and its application in medicine

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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 antimicrobial 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-melissil palmitic mixed with small amounts of ethers compounds of palmitic and stearic acid. Its density at 15 °C is about 0.960 kg/m³ to 0.970 kg/m³ 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 led 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 microorganisms (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 WEI 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	WEI
<i>Listeria monocytogenes</i> CCM 4699	0.33 ± 0.58	2.67 ± 2.31	0.33 ± 0.57	4.33 ± 3.79
<i>P. aeruginosa</i> CCM 1960	1.67 ± 1.54	2.33 ± 2.08	1.67 ± 1.53	2.67 ± 2.31
<i>S. aureus</i> CCM 3953	2.00 ± 0.00	1.67 ± 1.53	1.67 ± 2.08	1.00 ± 1.00
<i>S. enterica</i> CCM 4420	2.67 ± 0.58	2.67 ± 0.58	2.17 ± 1.89	3.67 ± 0.58
<i>E. coli</i> CCM 3988	1.50 ± 1.32	4.67 ± 2.52	1.67 ± 1.53	4.67 ± 0.58
<i>Aspergillus fumigatus</i>	2.33 ± 0.58	2.67 ± 1.15	2.00 ± 2.00	2.50 ± 1.32
<i>Aspergillus flavus</i>	0.67 ± 1.15	1.67 ± 0.58	1.67 ± 0.58	2.00 ± 1.00
<i>A. niger</i>	2.33 ± 0.58	3.00 ± 0.00	3.00 ± 1.73	4.00 ± 1.73
<i>Candida krusei</i>	2.00 ± 1.00	1.83 ± 1.76	2.50 ± 2.18	4.00 ± 3.46
<i>C. albicans</i>	2.33 ± 2.08	3.67 ± 1.15	2.67 ± 1.15	3.33 ± 0.58
<i>C. glabrata</i>	2.00 ± 1.73	2.67 ± 2.08	2.67 ± 1.53	4.83 ± 1.26
<i>Candida parapsilosis</i>	2.00 ± 1.00	2.67 ± 1.15	1.33 ± 1.15	3.00 ± 3.61
<i>C. tropicalis</i>	3.00 ± 1.00	4.67 ± 0.58	2.00 ± 0.00	3.67 ± 0.58
<i>Geotrichum candidum</i>	2.33 ± 0.58	2.67 ± 2.31	2.33 ± 1.53	4.17 ± 2.47
<i>Rhodotorula mucilaginosa</i>	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; WEI: 70% ethanol extract.

After the first incubation, the two microorganisms were incubated separately in different cultured media to test the efficiency of the various components. As shown in Table 2, *S. aureus* was not able to grow in media added with honey and agar, with the mixture and with agar and mix (both at 66% versus 50%); a slight growth was also shown in the media with agar and olive oil, while moderate and heavy growth was seen in other media. With regard to *C. albicans* and *S. aureus* (Table 2), growth was inhibited in media with agar – honey, with the mixture of honey, beeswax and olive oil (1:1:1) and with agar – mixture 66%, while in other media was noted a growth ranging from moderate to heavy [20].

The antifungal activity of the mixture of honey, beeswax and olive oil was also tested *in vivo* in patients with pityriasis versicolor, tinea cruris, tinea corporis and tinea faciei [21]. Pityriasis versicolor is a superficial skin infection caused by four of the eleven species of *Malassezia* (*Malassezia furfur*, *Malassezia sympodialis*, *Malassezia globular* and *Malassezia obtusa*), characterized by changes in skin pigmentation, with irregular and well demarcated patches and may be related to pregnancy, disease like diabetes, state of chronic malnutrition or inefficient personal hygiene; tinea cruris is a fungal infection of the groin area caused by *Trichophyton rubrum* and *Epidermophyton floccosum* that affects mainly males; tinea corporis is a form of superficial mycosis by *Trichophyton rubrum*, *Microsporum canis* and *Trichophyton mentagrophytes* that primarily affects the face and arms and spreads by contact with pets [22]; tinea faciei is a very rare infection that affects the face whose causative agents are *Trichophyton rubrum* and *Trichophyton tonsurans* [22,23].

The mixture was applied topically in 37 patients (14 suffering from pityriasis versicolor, 8 from tinea corporis, 14 from tinea cruris, and 1 from tinea faciei) every 8 h for 4 weeks [21]. For patients with pityriasis versicolor at the beginning of the therapy the average score \pm S.D. was (7.10 ± 1.35) while at the end of the treatment (fourth week) was (1.00 ± 1.20) , showing clinical sign in 12 out of 14 patients. The average score for patients with tinea corporis from (8.70 ± 2.40) is decreased to (1.30 ± 1.50) with clinical sign in 6/8 of patients, while the average score for patients with tinea cruris from (8.50 ± 2.40) is decreased to (1.00 ± 1.30) with clinical sign in 11/14 of 14 patients [21].

The antimicrobial efficacy of the mixture of honey, beeswax and olive oil was also tested on strains of *C. albicans* isolated from four infants with diaper dermatitis. Patients who had candidiasis, showed severe erythema with pustules and/or

ulceration. Diaper dermatitis associated with *C. albicans* was treated topically with the mixture of honey, beeswax and olive oil for 7 days. After the treatment period, clinical improvements were observed. At the end of treatment 50% of infants with *C. albicans* completely or almost completely reduced the level of injury, while the remaining 50% still showed yeast infection and the level of lesions slightly decreased with the disappearance of ulcers and pustules [21].

Recently, the antimicrobial activity of propolis and beeswax in synergy (1:1, v/v) has also been investigated in order to inhibit the growth of *S. aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *B. subtilis* ATCC27853 and *C. albicans* NCTC270 [25].

Propolis is a resinous product that bees collect from the buds of trees and which is subsequently treated with the addition of beeswax, pollen and enzymes. For its insulating properties it is used by bees as a glue to seal the cell walls. For its antimicrobial, antiviral, antifungal and anti-inflammatory properties, its use in medicine dates back to ancient times [26,27].

S. aureus and *C. albicans* were the most sensitive microorganisms to the mixture with a zone of inhibition, respectively, 20 and 22 mm [25].

Staphylococcus epidermidis and *B. subtilis* have proved to be slightly more resistant to the mixture (13.5 mm and 10.5 mm), but still have confirmed the antibacterial action of propolis and wax together [28].

4. Other properties of beeswax

The use of mixture of honey, beeswax and olive oil has proved to be very effective for the treatment of atopic dermatitis, psoriasis and diaper dermatitis [20,28–30]. All patients subjected to therapy showed significant improvements after a month of local application to three times a day. Therapy with the mixture is found to be more effective than treatment with the conventional medicines used for the treatment of the diseases specified before. Significant effects regarding the application of the mixture of honey, beeswax and olive oil have also been demonstrated in patients with hemorrhoids and anal fissure [29]. A new mixture of honey, olive oil, propolis extract and beeswax (called HOPE by authors [29]) was used in treatment of chemotherapy-induced mucositis proving to be a very fast method for healing the patients with high degrees of mucositis [31]. *C. albicans* proliferation is often associated with diaper dermatitis, and the application of mixture of honey, olive oil and beeswax in some cases has the same effect of nystatin cream; thus the synergy of nystatin cream with the mixture could represent an effective therapeutic tool [32]. Moreover, the same mixture showed high effectiveness in treatment of canine deep second-degree burns, so it is possible an use in veterinary medicine [33]. A component of beeswax of major interest in medicine is D-002. D-002, a natural alcoholic mixture of high molecular weight which has been isolated and purified from the beeswax of *Apis mellifera* [34,35]. The D-002, in addition to having among its components a high amount of triacontanol (fatty alcohol which stimulates the growth of many plants), has anti-ulcer activity [34,36], anti-inflammatory activity [37,38], protective action for the gastrointestinal mucosa [39,40], effect in patients with non-alcoholic fatty liver disease [41] and osteoarthritis symptoms [42], and, in studies carried out on murine animal models, was found to be particularly effective on gastroesophageal reflux [43] and intestinal problems of acidity [38,44–47].

Table 2

Growth of *C. albicans* and *S. aureus* on different media [20].

Types of terrains	Growth of <i>C. albicans</i>	Growth of <i>S. aureus</i>
Nutrient agar – olive oil	+	+
Nutrient agar – beeswax	++	++
Nutrient agar – honey	–	–
Mixture honey, beeswax and olive oil (1:1:1)	–	–
Nutrient agar – mixture 66%	–	–
Nutrient agar – mixture 50%	++	–
Nutrient agar – mixture 33%	+++	++
Nutrient agar – mixture 25%	+++	+++
Nutrient agar – mixture 12,5%	+	+++
Nutrient agar (control)	++	+++

–: No growth; +: mild growth, ++: moderate growth, +++: heavy growth.

Beeswax is also often used in the food packaging to protect the cheese during the seasoning as a polishing agent E901 or as edible coverage mixed with the polylactic-acid PLA [48–52].

5. Conclusions

In conclusion it would be appropriate to carry out further studies on the antimicrobial activity of beeswax alone and in combination with other natural products and in particular with beehive products. It would also be interesting to investigate the antimicrobial activity of the individual substances that characterize the beeswax produced under different environmental and geographical conditions.

Moreover, although the information provided by the studies collected in this review are important and show the effectiveness of beeswax, it will be crucial in future investigations to develop the quantitative tests incorporating the use of the MIC (Minimum Inhibitory Concentration) and the FIC (Fractional Inhibitory Concentration) to determine quantitatively the real contribution of this product to the antimicrobial activity.

Furthermore, antibiotics, pesticides, herbicides, fungicides and acaricides are widely used in beekeeping. The residues of these substances, especially antibiotics, are a relevant risk for the quality of bee products and for human health and the accumulation of pesticides overall in beeswax is well documented as a result from environmental pollution [54–59].

No reference and/or information on this issue have been reported in the studies analyzed, but it is possible that the data can be skewed by the presence of antibiotic residues that continue to have a certain residual antibacterial and antifungal activity even against bacterial strains used in the laboratory.

For this reason in future investigations it will be necessary to employ pure and residue free beeswax with guaranteed chemical composition.

Conflict of interest statement

We declare that we have no conflict of interest.

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