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Original article

# The beeswax processing by-product: a potential antibacterial ingredient for food and nutraceutical applications

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**Summary** The purification of raw beeswax by melting produces a semi-solid beeswax by-product (BBR) composed by honey, resins and other constituents that is usually considered as a waste. In this article, the chemical characterisation of BBR is reported, with the aim to valorise this by-product following the principles of the circular economy. Carbohydrates, hydrocarbons and minerals were among the main constituents. Flavonoids and phenolic acids represent 1.5% of the BBR, and their qualitative profile resembles the propolis. To assess its potential usefulness, the BBR was tested against gram-positive and gram-negative bacteria of clinical interest, and results were compared with the raw propolis. *Klebsiella pneumoniae* and *Salmonella enterica* were inhibited at concentrations ≥ 0.001 mg $mL^{-1}$, while *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus* from 0.01 mg $mL^{-1}$. Only BBR was active on *Pseudomonas aeruginosa*. Below the concentration of 1 mg $mL^{-1}$, no significant toxicity on Caco2 cells was observed. These results indicate that the BBR presents a polyphenol composition similar to propolis and a significant antibacterial activity. Thus, on the basis of these results, we suggest that the BBR can represent a sustainable alternative to propolis as food preservative or nutraceutical.

**Keywords** Antibacterial activity, beeswax by-product, food preservative, nutraceutical, polyphenols, propolis.

## Introduction

Honey is the main product obtained from the hives, particularly from the sugar-rich floral nectar extracted from plants (Baglio, 2018). Depending on the beekeeper/industry goal, other bee products can be obtained, such as pollen, propolis, royal jelly and beeswax. All these substances are well known for their benefits on human health and are currently used according to their specific properties (Pasupuleti *et al.*, 2017). Propolis, albeit not produced by all bees, is considered one of the bee-products with the highest economic value. It is collected in the hive as a complex matrix of different chemical compounds, comprising resins and vegetable balm (70%), waxes (10–87%) and volatile compounds, usually around 1% (v/w) (Salatino & Salatino, 2021). Terpenes, flavonoids and lipids such as glycerides and phytosterols are also included (Šturm & Ulrih, 2020), and their presence is linked to the vegetative growth of the plants that produce them. Propolis is known since ancient times as a natural remedy for several diseases, particularly for the treatment of mild upper-respiratory tract infections (Kuropatnicki *et al.*, 2013). Thanks expecially to its content in bioactive flavonoids, propopolis is effective as antibacterial, antiviral, anti-inflammatory and antioxidant agent (Pahlavani *et al.*, 2020; dos Santos *et al.*, 2021; Nichitoi *et al.*, 2021). Also, the other components such as phytosterols can contribute to these properties (He *et al.*, 2022). The same compounds have been also associated to other beneficial effects of propolis on preventing chronic and systemic diseases such as diabetes, cardiovascular diseases, chronic kidney disease and cancers, and for this reason it is largely

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used as ingredient of food supplements. Thanks to its antimicrobial properties, propolis has been proposed also as a natural food preservative (Seibert *et al*., 2019). The global commercial market of propolis was valued at 607.10 million US dollars in 2020, and it is expected to grow at an annual rate of 5.48% in 2021–2026 (Zulhendri *et al*., 2022).

Beeswax is a mixture of hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohols, diesters and other substances produced endogenously by specialised organs in adult bees (Fratini *et al*., 2016). It is obtained by purifying raw beeswax in a heated tank, called melter. Heating allows the separation of wax from a residue (beeswax by-product residue, BBR) composed of a blend of honey and other constituents as propolis and wood, that stratifies at the bottom of the collection tank. At the end of the process, the apparently low-value deposit represents a potential source of nutrients such as carbohydrates and fatty acids, and other valuable compounds such as polyphenols. This work has been aimed at the valorisation of the BBR in the perspective of further improving profitability and sustainability of the honey/wax production chain following the principles of the circular economy. To the best of our knowledge, by-products from beeswax processing have been scarcely considered up to now. Giampieri *et al*. (2018a, 2018b) showed that a sediment separated from wax during the recycling process of the honeycombs and another one obtained from its decantation may represent valuable sources of nutrients such as fibre, proteins, carbohydrates and fats, and contain significant amounts of polyphenols. These residues can have a potential usefulness as anti-proliferative agents, considering their toxicity on HepG2 cells (Giampieri *et al*., 2018a, 2018b). In another work, the same authors demonstrated that the residue from the recycling process of the honeycombs exerts antioxidant effects in a different cell model (human dermal fibroblast cells), by improving mitochondria functionality and wound healing capacities (Giampieri *et al*., 2018a, 2018b).

In our work, the chemical characterisation of BBR was first carried out to determine its composition of nutrients such as carbohydrates, minerals, amino acids and fatty acids, and the content of phytosterols and phenolic compounds such as flavonoids and phenolic acids. Afterwards, the residue was tested to assess its antibacterial properties against commonly diffused pathogens, and results were compared to those obtained from raw propolis. The outcomes of this study are intended to promote the potential use of the BBR as a novel bioactive ingredient of food supplements and nutraceuticals, or alternatively as food preservative, in a similar way to propolis. If compared to this latter, the BBR would represent a sustainable and lower cost alternative.

## Material and methods

The procedures for the collection of the BBR, its exhaustive chemical characterisation and the assessment of its antibacterial properties and cytotoxicity are described in the Supporting Information.

## Results and discussion

### Chemical characterisation of the BBR

Figure S2 summarises the chemical composition of the whole BBR. The moisture content, assessed by lyophilisation, was 8%. The non-polar fraction of the BBR, extracted with hexane, represented the 44% of the whole residue. Among the non-polar constituents identified, linear hydrocarbons were the most abundant, as revealed by GC–MS analysis (12.8% of the BBR). Their qualitative profile showed a prevalence of odd numbered hydrocarbons in the range *n*-$C_{21}$-$C_{33}$, as already reported in other papers (Svečnjak *et al*., 2019). *n*-C27 (30% of whole alkanes), *n*-C29 (23%) and *n*-C33 (15%) were the most representative species (Fig. S3).

Volatile terpenes and phytosterols were identified as minor constituents of the BBR. Results of their characterisation are discussed in the Supporting Information.

*Free fatty acids profile*

The fatty acid profile of the BBR was obtained after the transesterification of free fatty acids carried out in excess of MeOH under acidic catalysis. The results of GC–MS analysis are reported in Table S3. The ratio between saturated and unsaturated acids (SFA/UFA) was close to that reported for honey and propolis (Jarukas *et al*., 2021), but a preponderance of saturated derivatives was noticed. Analysis proved that the SFA/UFA ratio was higher in the BBR than in raw propolis (4.98 vs. 2.97). This was a somewhat expected result due to the origin of BBR which was recovered from a wax-rich matrix, in which free medium- and long-chain saturated fatty acids were abundant (Svečnjak *et al*., 2019).

The most representative fatty acid in the BBR was palmitic acid (4.62%) that represented almost 70% of the whole fats composition of the residue. Among unsaturated fatty acids, oleic acid (0.78%) was the most abundant compound followed by (Z)-pentadec-10-enoic acid (0.24%). Other derivatives as *n*-6 linolenic acid were far less important (0.05%; Table S3). Overall, the amounts of *n*-6 and *n*-9 derivatives were higher in BBR compared with the raw propolis.

*Minerals*

Minerals in the BBR were quantified using atomic absorption spectrometry. Results are shown in

Table S4. The total amount of minerals accounted for 10.1% of the whole residue, with potassium (6.7%) as the most abundant one. This was consistent with literature results that report potassium and copper as the major minerals in honey and propolis, although their content can be highly variable and dependent on the geographical origin of raw bee products (Ahangari *et al*., 2018; Hodel *et al*., 2020). In their work on different residues from beeswax processing, Giampieri *et al*. (2018a, 2018b) indicated that they represent relevant sources of Ca (up to 4 mg $g^{-1}$), Fe (up to 1.1 mg $g^{-1}$), Mg (up to 1.6 mg $g^{-1}$) and K (up to 9.6 mg $g^{-1}$), although these amounts are significantly lower than those of BBR. Compared with this work, differences were observed also in the content of heavy metals. Pb reached amounts of almost 1 μg $g^{-1}$, while transition metals such as Co and Ni were more concentrated in BBR compared to the residue studied by the other authors (Giampieri *et al*., 2018a, 2018b), probably due to the different geographical origin of the bee products. Nevertheless, the content of these heavy metals in BBR was significantly lower compared to raw propolis (Table S4), suggesting that its use should not represent a toxicological risk.

*Amino acids and carbohydrates*

The profile of free and total amino acids obtained after acidic hydrolysis of de-waxed BBR were evaluated by HPLC–MS. Results, shown in Table S5, indicate that in 100 g of the BBR the content of total amino acids after hydrolysis is 293.1 mg, while free amino acids are 60.15 mg. In comparison, 146.52 mg of total amino acids and 10.83 mg of free amino acids were detected in raw propolis.

Proline was the most abundant free amino acid in the BBR (29.44 mg/100 g), with an amount more than doubled compared to phenylalanine (13.85 mg/100 g), the second most abundant one. Alanine and glutamine were also detected in concentrations of 4.49 and 2.18 mg/100 g, respectively. Instead, sulphur-containing amino acids were not detected, and this was probably related to the low abundance of these constituents in starting material, and to thermal degradations occurring during the raw beeswax melting (especially for cysteine).

The findings were in reasonable agreement with the content of free amino acidic constituents reported for honey [proline and glutamine, followed by serine, phenylalanine and tyrosine (Kowalski *et al*., 2017; Biluca *et al*., 2019)] and propolis [leucine, proline, alanine, and valine (Eroglu *et al*., 2016)]. In the total amino acids profile of the BBR obtained after hydrolysis, proline was by far the most abundant one with a concentration of 127.83 mg/100 g of the BBR, followed by phenylalanine and tryptophan (Table S5). S-containing amino acids were not detected also in this case, probably due to the same reasons reported above.

Overall, results on the BBR were qualitatively comparable to those obtained from the analysis of raw propolis, and they partially reflected what previously reported in literature, except for valine which was absent in propolis. Interestingly, however, the content of both free and total amino acid contents in the BBR was significantly ($P < 0.05$) higher than that in propolis (i.e., 60.15 and 293.1 mg/100 g BBR, respectively; 10.83 and 146.52 mg/100 g propolis, respectively), while the ratio total/free amino acids was higher in propolis than in the BBR. Other significant differences were noticed on the presence of single amino acids, especially glycine and threonine that, together with valine, were detected only in the BBR.

The analysis of the carbohydrate content on BBR proved the occurrence of only glucose and fructose in quantities of 9.03 ± 0.02% and 14.87 ± 0.02% of the whole residue, respectively. This marked a difference with respect to honey not only for the total amount of carbohydrates which is the main fraction in honey up to ≈85% (Nguyen *et al*., 2019) but also in the profile of carbohydrates. Indeed, albeit fructose and glucose are the main constituents (≈40% and 35%, respectively) in honey, the latter also contains other carbohydrates such as maltose and sucrose within the amount of 5% (Nguyen *et al*., 2019).

*Phenolic compounds*

The analysis of phenolic compounds in the BBR showed the presence of flavonoids and phenolic acids. This matched previously reported data on both honey and propolis that indicated the prevalence of the same two families of derivatives (Pyrzynska & Biesaga, 2009; Osés *et al*., 2020).

Qualitative and quantitative results from the analysis of phenolic compounds in the BBR are reported in Table S6, and a representative chromatogram obtained by UPLC–QTOF is shown in Fig. 1. In both Fig. 1 and Table S6, a comparison between the qualitative and quantitative results obtained for BBR and raw propolis is reported. Although a higher number of flavonoids were identified with respect to phenolic acids, the total amount of the latter was almost four times higher than that of the former, i.e., 1161.47 mg/100 g vs. 283.11 mg/100 g, respectively. In raw propolis instead, the ratio between phenolic acids and flavonoids was reversed (5246.49 vs. 3057.69 mg/100 g, respectively). These results are in line with the data published by Giampieri *et al*. (2018b), which indicated a total phenol content of 1435.66 mg/100 g and a total flavonoid content of almost 296 mg/100 g in the residue obtained from the recycling process of honeycombs. Nevertheless, it should be noted that these amounts were calculated by using a different method

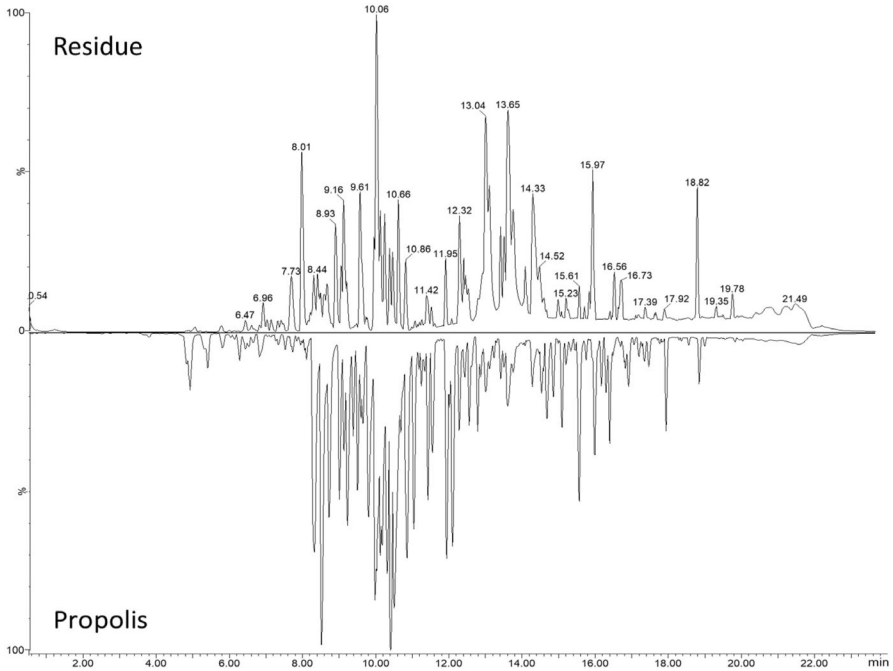


**Figure 1** Comparison between representative chromatograms obtained from the UPLC–QTOF [ESI(−)] analyses of BBR (upper panel) and raw propolis (lower panel). Identified metabolites, together with respective chromatographic and MS information, are reported in Table S6.

compared to our work (colorimetric assay), and this may explain the slight differences observed.

Most importantly, the typical phenolic markers of propolis were identified in the BBR, namely the flavonoids chrysin, galangin, quercetin, pinocembrin and pinobanksin 3-O-acetate, and the phenolic acids ferulic, p-coumaric and caffeic, together with the phenethyl (CAPE), isoprenyl and cinnamyl esters of the latter (Šuran *et al.*, 2021). These compounds have been previously indicated as responsible for the bioactivity of propolis (Osés *et al.*, 2020). Though, these were not the main phenolic constituents of BBR: in this sample, the most abundant phenolic acid and phenolic compound was acetyl-di-p-coumaroylglycerol (343.64 mg/100 g), followed by caffeic acid derivatives such as p-coumaroyl-caffeoyl-acetylglycerol (148.27 mg/100 g), acetyl-p-coumaroyl-caffeoylglycerol (139.65 mg/100 g) and acetyl-dicaffeoylglycerol (131.14 mg/100 g) (Table S6).

## Antibacterial activity of the BBR

Propolis has been recognised as an agent to treat bacterial infections since ancient ages due to its bioactive property, which finds a number of applications even nowadays, in association also with antibiotics. Clinical uses of propolis range from the treatment of infections of the upper respiratory tract to those affecting the gastrointestinal system, thanks to its antimicrobial activity against several gram-positive and gram-negative bacteria (Rivera-Yañez *et al.*, 2021). Due to its efficacy as antibacterial against a broad range of species, propolis has also been proposed as a natural food preservative (Seibert *et al.*, 2019). The proven antibacterial activity of propolis has been attributed mainly to its high content in polyphenols, and more in detail, to the presence of specific flavonoids and phenolic acids in its composition. Among these, ferulic acid, caffeic acid, p-coumaric acid, CAPE, galangin, chrysin, pinocembrin and pinobanksin are those that have been more frequently associated to the observed bioactivities (Rivera-Yañez *et al.*, 2021). Considering that all these compounds were detected in the BBR, the antibacterial properties of the residue were tested *in vitro* against several bacteria of clinical interest, and they were compared to those exerted by raw propolis. Results are reported in Figs 2 and 3.

The anti-bacterial activity of BBR was comparable to that of propolis against the tested species. More specifically, among the gram-negative species, the antibacterial activity against *K. pneumonia* and *S. enterica* was still detectable at the lowest concentration tested (0.001 mg $mL^{-1}$), while *A. hydrophila*, *P. aeruginosa*, *H. influenzae* and *E. coli* were significantly inhibited at concentrations comprised between 0.01 and 0.1 mg $mL^{-1}$. Interestingly, *P. aeruginosa* was not

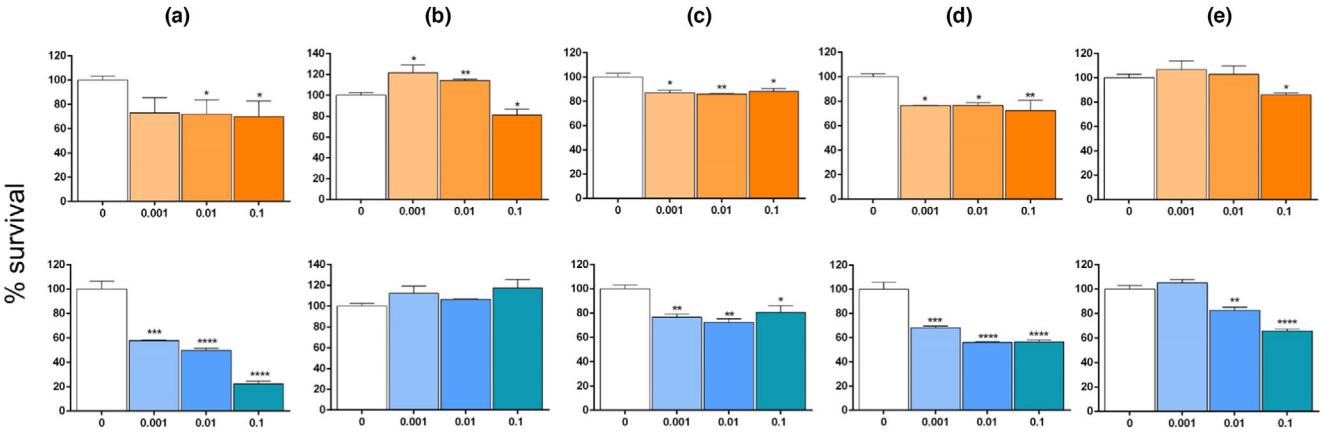


**Figure 2** Antibacterial activity of BBR (orange graphs) and raw propolis (blue graphs) against common gram-negative pathogens. a: *Aeromonas hydrophila*; b: *Pseudomonas aeruginosa*; c: *Klebsiella pneumonia*; d: *Salmonella*; e: *Escherichia coli*. *X*-axes report the concentration of tested samples in mg $mL^{-1}$. Asterisks indicate significant differences with controls (white bars). *: $P < 0.05$; **: < 0.01; ***: $P < 0.001$; ****: $P < 0.0001$.

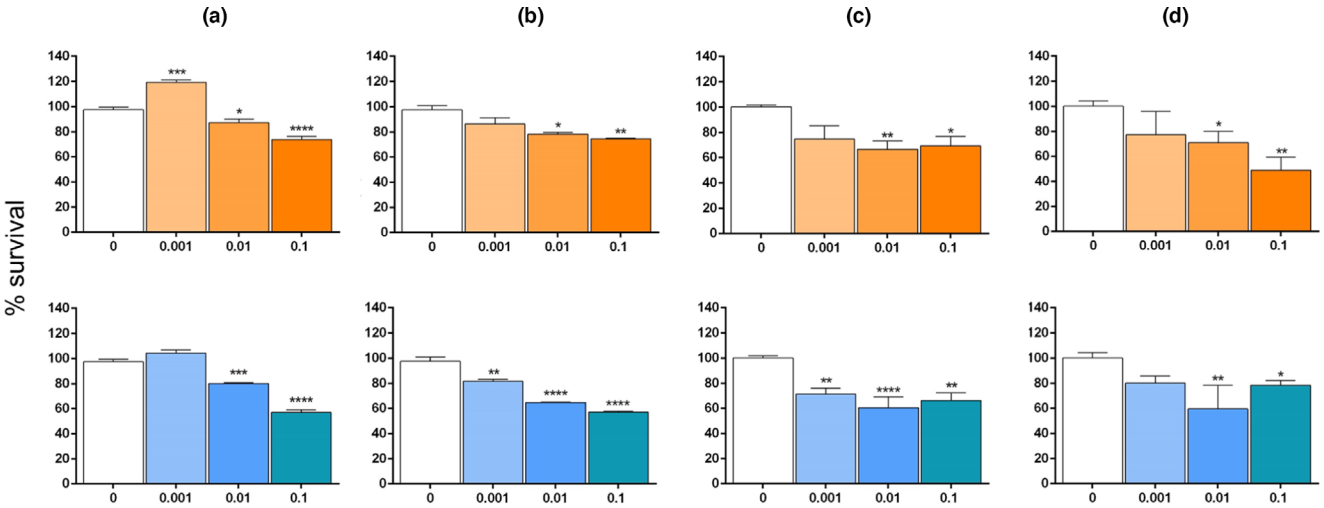


**Figure 3** Antibacterial activity of BBR (orange graphs) and raw propolis (blue graphs) against common gram-positive pathogens. a: *Enterococcus faecalis*; b: methicillin-resistant *Staphylococcus aureus* (MRSA); c: *Haemophilus influenzae*; d: *Streptococcus pyogenes*. *X*-axes report the concentration of tested samples in mg $mL^{-1}$. Asterisks indicate significant differences with controls (white bars). *: $P < 0.05$; **: < 0.01; ***: $P < 0.001$; ****: $P < 0.0001$.

inhibited by raw propolis at the concentrations tested, while a significant effect was observed for BBR at 0.1 mg $mL^{-1}$ (Fig. 2). For gram-positive species, a significant activity of BBR was observed only at concentrations > 0.01 mg $mL^{-1}$, but the effects on bacteria were dose-dependent (Fig. 3).

### Cytotoxicity of BBR on Caco2 cells

The cytotoxicity of BBR was evaluated on Caco2 cells. This cell model was considered because it is one of the most widely used to assess the interactions between exogenous compounds and the intestinal wall, especially in matter of toxicity, adhesion and absorption (Ding *et al*., 2021). Hence, it can be useful for a preliminary safety evaluation on compounds or matrices destined to oral consumption. On the other hand, the same cell model can be used to study the anti-proliferative properties of the same compounds, in case a high toxicity is observed.

The results on BBR indicated that the it can be suitable for *in vivo* administration (i.e., animal use), since the residue did not lead to significant toxic effects at the concentration tested (Fig. 4). It has to be

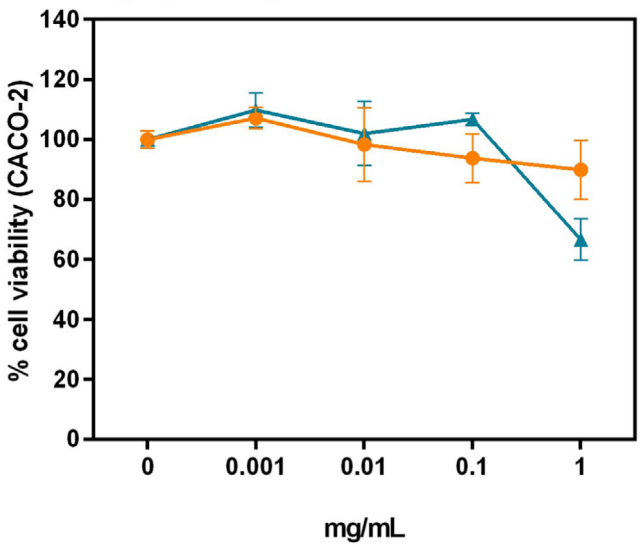


**Figure 4** Effect of BBR (orange line) and raw propolis (blue line) on the viability of Caco2 cells.

highlighted that a moderate toxicity was observed at the highest dose tested (1 mg $mL^{-1}$), although cell viability was reduced of almost 5%. In comparison, raw propolis at the same dose showed a significantly higher toxicity, being cell viability reduced by almost 30%.

To the best of our knowledge, the cytotoxicity of a residue from the beeswax processing was previously studied only by Giampieri *et al.* (2018a, 2018b). In their work, the authors showed that a residue obtained after the recycling process of the honeycombs, having a significant content in minerals and phytochemicals, could induce apoptosis in HepG2 cells. This effect was presumably due to a dose-dependent (0.25–1 mg $mL^{-1}$) induction of intracellular ROS production, caused by the interference of the residue with the mitochondrial function (Giampieri *et al.*, 2018a, 2018b). Overall, our results partially agree with these data, since a dose-dependent cytotoxicity was observed, although not significant at the doses tested. Nevertheless, the two cell models used were different, hence, further assays should be performed in other cell types to assess the anti-proliferative potential of BBR.

## Conclusion

To the best of our knowledge, these results represent the first comprehensive chemical characterisation of the BBR. As expected, the most abundant constituents are carbohydrates and waxes. These derive respectively from honey residues and beeswax that deposit on the bottom of the melter during the heat-induced separation of pure wax from honey and other impurities. Nevertheless, a relatively important contribution to the whole BBR composition is due to flavonoids and phenolic acids, whose qualitative profile resembles that of propolis. We suppose that these compounds are responsible for the antibacterial activity of BBR observed *in vitro*, on the basis of previously published data regarding their biological properties. Furthermore, it has to be noted that the BBR was not fully dispersed during sample preparation for bioactivity assays, and a white wax flocculate was formed. This is a further indication that at least the most apolar constituents such as hydrocarbons and waxes are not involved in the bioactivity of the BBR.

Overall, the results here presented indicate that the by-product from beeswax processing is a source of bioactive compounds and could be used as an alternative to propolis as nutraceutical ingredient, or as natural food preservative. The same residue could find other applications such as animal (e.g., bees) feeding thanks to the content in nutrients such as carbohydrates and fatty acids, or to increase the yield of wax production. Nevertheless, appropriate clean-up procedures should be developed, in order to selectively isolate compounds of interest depending on their physicochemical properties. To this aim, supercritical $CO_2$ extraction would be a feasible and sustainable approach to extract more lipophilic compounds such as waxes and fatty acids, and concentrate more polar ones such as carbohydrates, amino acids and polyphenols. All these routes represent possible strategies to valorise underutilised products of apiculture and increase the sustainability of the whole honey/wax production chain.

### Limitations of the study

Some limitations of this study should be highlighted. First of all, it was focused on a BBR obtained from bees' products of a limited geographical origin. Other authors have already pointed out how the chemical composition of bees' products depends on several parameters related to the geographical area of their production, most importantly climatic conditions and type of vegetation (plants, flowers, etc; Giampieri *et al.*, 2022). In the future, it will be important to study the reproducibility of our results, in order to assess the influence of such parameters on the composition and bioactivity of BBR. Moreover, here only the antibacterial activity of BBR was tested *in vitro*, while the efficacy *in vivo* but also its safety, allergy and toxicity correlated to its use were not assessed, hence, further animal studies and clinical trials will be required.

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## Author contributions

**Gregorio Peron:** Data curation (equal); investigation (equal); methodology (equal); software (lead); writing – original draft (equal). **Nádia Alessandra Carmo dos Santos:** Investigation (equal); methodology (equal); writing – original draft (equal). **Irene Ferrarese:** Investigation (equal). **Filippo Rizzo:** Investigation (equal). **Giulia Bernabé:** Data curation (equal); investigation (equal); methodology (equal). **Michela Paccagnella:** Investigation (equal); methodology (equal). **Marina Panozzo:** Conceptualization (equal); writing – review and editing (equal). **Stefano Francescato:** Validation (equal); writing – review and editing (equal). **Ignazio Castagliuolo:** Data curation (equal); formal analysis (equal); supervision (equal). **Stefano Dall'Acqua:** Data curation (equal); formal analysis (equal); methodology (equal); resources (equal); supervision (equal). **Maurizio Selva:** Formal analysis (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (equal). **Alvise Perosa:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (equal).

## Ethical guidelines

Ethics approval was not required for this research.

## Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.16520.

## Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Fratini, F., Cilia, G., Turchi, B. & Felicioli, A. (2016). Beeswax: a minireview of its antimicrobial activity and its application in medicine. *Asian Pacific Journal of Tropical Medicine*, **9**, 839–843.

Giampieri, F., Gasparrini, M., Forbes-Hernández, T.Y. *et al*. (2018a). Beeswax by-products efficiently counteract the oxidative damage induced by an oxidant agent in human dermal fibroblasts. *International Journal of Molecular Sciences*, **19**, 2842–2855.

This reference was cited because, to the best of our knowledge, it is one of the few in literature dealing with the valorization of beeswax by-products. Here, only the antioxidant activity of such products was evaluated, differently to our work.

Giampieri, F., Quiles, J.L., Orantes-Bermejo, F.J. *et al*. (2018b). Are by-products from beeswax recycling process a new promising source of bioactive compounds with biomedical properties? *Food and Chemical Toxicology*, **112**, 126–133.

As stated for the other reference by Giampieri et. al, also this one was chosen and cited because, to the best of our knowledge, it is the only one reported in literature describing the chemical analysis of a beeswax processing residue. In our work, we moved a step forward, presenting a more exhaustive characterization of a similar residue.

Giampieri, F., Quiles, J.L., Cianciosi, D. *et al*. (2022). Bee products: an emblematic example of underutilized sources of bioactive compounds. *Journal of Agricultural and Food Chemistry*, **70**, 6833–6848.

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This reference reports results dealing with the phenolic composition of propolis and its antibacterial activity. These data were useful for the discussion of the antimicrobial activity of the residue considered in our study, as well as its phenolic composition.

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We cited this reference for the same reasons indicated above. Also, this work presents data about the phenolic composition of propolis and its antibacterial activity. These results were compared to those obtained in our study.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Scheme showing the different characterizations performed on the BBR.

**Figure S2.** Summary of the chemical composition of BBR.

**Figure S3.** GC–MS chromatogram of the non-polar fraction of BBR. The peaks are associated to linear hydrocarbons, whose identification is reported as $C_n$ ($n$ = number of carbon atoms in the molecule). IS: internal standard (squalene).

**Figure S4.** Representative chromatogram obtained from the HPLC–APCI–MS analysis of plant phytosterols in the BBR.

**Table S1.** Volatile compounds detected in the BBR.

**Table S2.** Results of quantitative and qualitative analysis of plant sterols in BBR and raw propolis.

**Table S3.** Fatty acid profiles of BBR and raw propolis. Both full names and abbreviations of each identified compound are reported in the table.

**Table S4.** Amount of minerals in BBR and raw propolis, expressed as mg $g^{-1}$. Results are reported as means ± SD of three replicates.

**Table S5.** Amino acid content of BBR and raw propolis. Amounts are reported as mg/100 g of sample.

**Table S6.** Qualitative and quantitative results from UPLC–QTOF and HPLC–$MS^n$ analyses of phenolic compounds in the BBR.