CHEMICAL AND BIOLOGICAL PROPERTIES OF PROPOLIS FROM THE WESTERN COUNTRIES OF THE MEDITERRANEAN BASIN AND PORTUGAL

MARIA GRAÇA MIGUEL

Universidade do Algarve, IBB-Centro de Biotecnologia Vegetal, Faculdade de Ciências e Tecnologia, Edif. 8, Campus de Gambelas, 8005-139 Faro, Portugal. Email: mgmiguel@ualg.pt

Received: 30 Mar 2013, Revised and Accepted: 12 May 2013

ABSTRACT

The chemical composition of propolis from the western countries of the Mediterranean basin and Portugal as well as their biological attributes were reviewed. In the vast majority of these countries, extracts of propolis were predominantly constituted by phenols, with the exception of those from Malta and Sicily in which diterpenes in relative high amounts were identified. In one sample from Algeria a diterpene was also found in high concentration, nevertheless it was not determined. The identification of the components constituting the balsam oils were also performed but only in two countries, Portugal and Algeria, albeit with substantially different results. The biological properties reported for propolis for the western Mediterranean basin and Portugal included antimicrobial, antioxidant, antiproliferative, anti-inflammatory, neuroprotective, and protection against the side effects of some medicines (vinblastine, cyclophosphamide, paracetamol) or γ-radiation. These properties were almost always attributed to phenol acids and flavonoids. Only in very few cases diterpenes were considered responsible for such beneficial properties, particularly antibacterial activity in samples from Malta.

Keywords: Propolis, Diterpene, Phenol, Biological properties, Mediterranean, Portugal.

INTRODUCTION

Propolis is a complex mixture composed of beeswax (30%), resins and plant balsams (50%), essential oils (10%), pollen (5%) and some organic and mineral compounds (5%) [1,2]. It is a natural substance collected by honeybees (Apis mellifera) from buds and exudates of certain trees and plants. Such substance is used by bees to seal holes in their honeycombs, smooth out the internal walls, protect against their enemies and to prevent the decomposition of living things that have been killed by them after an invasion of the hive and, at the same time, eliminating a potential source of microbial infections [3–5].

The capacity of honeybee to embalm intruders was followed by ancient Egyptians who embalmed their deads, due to the anti-patentative properties of propolis. The antimicrobial and anti-septic attributes of propolis were also known by Greek and Roman physicians such as Aristoteles, Dioscorides, Pliny and Galen. They used it as an anti-septic and healing product in wound treatment for topical therapy of cutaneous and mucosal wounds [1, 6]. Arab physicians continue to use propolis as a remedy. Incas also employed propolis as an anti-pyretic agent, and propolis was an official drug described in the London pharmacopoeias of the 17th century. The antibacterial activity of propolis made this product very popular in Europe between the 17th and 20th centuries [6].

In the Second World War, propolis was used by soviet clinicians for tuberculosis treatment. In the Balkan states it was largely used to treat wounds and burns, sore throat and stomach ulcer [2]. So, in addition to the antimicrobial activity of propolis, other biological properties have been attributed to this natural product and reported in review articles: hepatoprotective, antitumor, antioxidant, anti-inflammatory, anti-diabetic, antitubercular, against allergy, rhinitis, and asthma [1, 7–12].

Recently, propolis has been used for cold syndrome (upper respiratory tract infections, common cold, flu-like infections); as dermatological preparations in wound healing, treatment of burns, acne, herpes simplex and genitalis, and neurodermatitis; as mouthwashes and toothpastes to prevent caries and treat gingivitis and stomatitis; in cosmetics; and in health foods and beverages not only to improve health and prevent diseases, but also as an ingredient in many dietary supplements and nutraceuticals [2, 6, 13–15].

Although all of those properties and applications, propolis may not be entirely innocent because human allergies have been reported such as contact dermatitis or oral mucositis, with the risk of cross-sensitization with other natural products such as the balsam of Peru or essential oil [6, 15, 17].

The compounds present in propolis resin have three sources: plant exudate collected by bees, secreted substances from bee metabolism and other materials introduced during propolis elaboration [3]. More than 300 compounds such as phenolic acids, terpenes, cinnamic acid, caffeic acid, several esters and flavonoids have been detected in propolis being their structures strongly dependent on the collection location, time and plant source [18–21]. In spite of the chemical differences found in propolis, some studies have revealed that they exhibit similar biological properties [1, 3].

Spain, France, Italy, Malta, Morocco, Algeria and Tunisia belong to the western Mediterranean basin. In the present work, the chemical characteristics of propolis from this area as well as their biological properties are reviewed. Propolis from Portugal is also reported.

Chemical Composition

Malta

Several studies have demonstrated the existence of a Mediterranean propolis type, which is characterized by relative high amounts of diterpenes [22–30]. For example, the silylated ethanol extracts of propolis from Malta analysed by gas chromatography coupled to mass spectrometry (GC-MS) revealed to be rich in sugars and diterpene compounds. The most abundant were diterpene acids: isocoumaric, comminic, pimaric and imbricatoic acids together with toarol and 13-epitotorulosal [27]. Ferutinin, teferin, 2-acetoxy-6-p-methoxybenzoyl-jaeschkeanidiol and 2-acetoxy-6-p-hydroxybenzoyl-jaeschkeanidiol were mono- and sesquiterpenyesters also detected in propolis samples of Malta. Later on, the authors concluded that the diterpene profile of Maltese propolis was similar to the profile of the resin of Cupressus sempervirens. Therefore, the authors concluded that cypress is the major plant source of propolis [28].

Italy

Hydro-alkoholic extracts of propolis from Sicily (Italy) analysed by GC-MS after silylation had also predominantly diterpenic acids, such as communnic acid, isocoumaric acid and acetylisocoumaric acid [23, 24]. However, other authors analysing propolis samples from central Italy concluded that the resin fraction was rich in caffeic acid, p-coumaric acid, ferulic acid, quercetin, apigenin, kaempferol, chrysin, caffeic acid phenyl ester, pinocembrin, galangin, benzyl salicylate and benzyl cinnamate. According to these results, the
authors concluded that all samples assayed from central Italy are of poplar origin [31]. Other studies also demonstrated the presence of apigenin, chrysin, galangin, naringenin, queretin, kaempferol, pinobanksin, pinocembrin, pinositol, caffeic acid, ferulic acid, p-coumaric acid, caffeic acid phenethyl ester, caffeic acid cinnamyl ester and pinobanksin-3-D-aceetae in ethanolic extracts of propolis from the province of Bologna (Italy) when analysed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) [14]. According to the authors, such chemical composition is characteristic in propolis from that zone of Italy, which led them to conclude that bud exudates of the Populus species are the main source of propolis of Bologna.

Portugal

For the first time, the phenolic characterization of northeast Portuguese propolis was performed. In this work, the electrospay mass spectrometry in the negative mode of hydro-alcoholic extracts revealed the presence not only of the typical phenolic acids and flavonoids found in propolis from temperate zones but also other components never reported in the literature, such as methylated and/or esterified or hydroxylated derivatives of poplar flavonoids, rare pinocembrin or pinobanksin derivatives containing basic structures of phenolic acids linked to the C-5, C-7 or C-3 of the flavonoid skeleton, which precise position is unknown, and a p-coumaric ester derivative dimmer [40]. Later on, the authors analysing hydro-alcoholic extracts of propolis of different continental regions of Portugal and islands by liquid chromatography with diode-array detection coupled to electrospray ionisation tandem mass spectrometry found variability on the phenolic profile [41]. Seventy six polyphenols were detected and two groups of propolis were established: the common temperate propolis with typical poplar phenolic compounds (flavonoids and their methylated/esterified forms, phenylpropanoid acids and their esters) and an uncommon propolis type with an unusual composition in quercetin and kaempferol glycosides. Even the samples included in the typical poplar phenolic compounds presented some distinct compounds such as kaempferide. The presence of this flavonol was detected in all samples of the central coast and in some samples from the north, Algarve and Madeira. In addition, some samples from central interior and Algarve had low levels of pinobanksin derivatives but contained a kaempferol dimethyl ether.

The volatile profile of 70 propolis samples collected in three regions of Algarve (southern Portugal) at three different periods was evaluated by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS). All of them were characterized by the presence of viridiflorol, n-tricosane, n-nonenadecane, and the oxygen-containing diterpenes labd-7-en-15-ol, labd-8-en-15-ol and lab-8(17)-en-15-ol [42]. In this way, and for the first time diterpenic compounds were also reported in the essential oils of propolis from Algarve.

Biological Properties

The therapeutic properties of propolis are many times attributed to the presence of polyphenols [1]. Nevertheless a great heterogeneity has been found in its chemical composition and, as reported above, diterpenes may also occur in propolis from countries of the Mediterranean basin. Consequently, diverse components, and not only the phenophenols, may have biological attributes. In fact, there are already some studies reporting biological properties of propolis attributed to diterpenes and not only to phenols. For example, diterpene-rich propolis from Brazil was already reported as possessing antimicrobial, antiviral and cytotoxic activities as well as hepatoprotective effects [43-47].

In the Mediterranean basin there are also some studies reporting the biological properties of propolis attributed to diterpenes and their derivatives, particularly in countries of the eastern Mediterranean. For example, Popova et al. [25] demonstrated that the diterpene totarol and totarone isolated from propolis from Crete had the strongest activity against all microorganisms tested: two Gram-positive bacteria Staphylococcus aureus (ATCC 25923) and Staphylococcus epidermidis (ATCC 12228); four Gram-negative bacteria Escherichia coli (ATCC 25922), Enterobacter cloacae (ATCC 13047), Klebsiella pneumoniae (ATCC 13883) and Pseudomonas aeruginosa (ATCC 227853); yeast Candida albicans (ATCC 10231); oral pathogens Gram-positive bacteria Streptococcus mutans and Streptococcus viridans; whereas 3,4-seco-cycloarten-4(28),24-diene-12-hydroxy-3-oic acid and cycloarten-3,7,12-dihydroxy-24-en-28-oic acid were more effective against Candida tropicalis (ATCC 13001) and Candida glabrata (ATCC 28838).
The diterpene manool, isolated from Greek propolis, was the most active compound against the proliferation of HT-29 human colon adenocarcinoma cells, with the advantage of not affecting the normal human cells [43].

However, great part of the studies regarding biological and/or therapeutic properties of propolis in countries from the western Mediterranean is attributed to phenols and not to diterpenes, as this revision seems to conclude.

Anti-proliferative

The in vitro antitumor potential of ethanolic and ethyl acetate extracts of Moroccan propolis on three mammalian tumor cell lines BSR (hamster renal adenocarcinoma), Hep-2 (human laryngeal carcinoma) and P815 (murine mastocytoma) revealed cytotoxic activity in dose-dependent manner. However, such activity depended on the extracts' chemical composition and the target tumor cells. In vivo, propolis ethanolic extract significantly reduced the volume of P815 tumor-bearing mice (DBA/2/P815) after oral route administration [39]. In spite of these cytotoxic properties, both ethanolic and ethyl acetate extracts of Moroccan propolis only had minimal cytotoxicity against normal human peripheral blood mononuclear cells (PBMC). With such results, the authors concluded that the extracts only had small side effects [39]. The best activities found in ethanolic extracts led authors to attribute to polyphenols, and particularly flavonoids, such properties. Nevertheless, the authors also consider that is very important to determine which compounds are really responsible for in vitro and in vivo anticancer properties as well as the molecular mechanisms involved in this process.

The extracts from Bornes (in the northeast of Portugal) and Fundão (in the centre of Portugal) also possessed antiproliferative activity on primary cultured cancerous renal cells in a concentration-dependent manner but being weakly toxic to human normal renal cells [40]. According to the authors, these results are very important in cancer prevention and therapy due to the fact that the main target of propolis samples is cancerous cells [40]. These authors proposed caffeic acid phenethyl ester as possible responsible by such antiproliferative activity.

Extracts of Tunisian propolis also had antiproliferative activity [49]. Cancer cells Hep-2 were the most sensitive to bioactive compounds of Tunisian extracts of propolis. Such compounds were not determined by the authors; nevertheless they suggest some groups of compounds as being responsible by the activities found in these samples: flavonoids, acid cinnamic derivatives and some diterpenoids [46].

Radioprotective and prevention of toxic effects of medicines

Propolis extracts have also been reported as useful on the prevention of toxic effects of some medicines or acting as radioprotective [50, 51]. For example, Algerian propolis in which flavones and flavonol predominated revealed to protect against the powerful side effects of vinblastine (lipid peroxide, a downfall of hepatic glutathione and severe leucopenia and thrombopenia), cyclophosphamide (severe leucopenia and thrombopenia), and paracetamol in rats when submitted to these drugs [50]. According to these authors, the correction of the aplasic, leucopenia and thrombopenia induced by vinblastine and cyclophosphamide, as well as the re-establishment of peroxide and glutathione rates is attributed to flavonoids, particularly diosmine and quercetin, which seem to act by activating the turnover of glutathione and enzymes stimulating glutathione-5-transferases. Such turnover would allow the scavenging of the reactive metabolites of those drugs.

Ethanolic extracts of propolis from Spain (Lleida) had radioprotective effect, reducing significantly the radiation-induced chromosomal damage in human cells exposed in vitro to γ-rays. The authors submitted cultured lymphocytes to increasing concentration of ethanolic extracts of propolis and then exposed them to 2-Gy γ-rays. Such working conditions permitted to find a decrease in the frequency of chromosome aberrations treated with propolis, a protection against the formation of dicentrics, with a maximum protection at 120 µg/L of extract [51]. According to these authors, the radioprotective effect of propolis can be attributed to the flavonoids due to their scavenger ability against free radicals.

The benefits of flavonoids in Algerian propolis were also reported as preventing doxorubicin cardiotoxicity and nephrotoxicity [32, 52, 53]. These authors found that the pre-treatment of rats with extracts of propolis would reduce both mitochondrial malonaldehyde (MDA) formation and production of superoxide anion with the consequent diminution of peroxidative damage in the heart mitochondria and kidney induced by doxorubicin and a significant increase of glutathione levels as well [32, 52, 53]. These results suggest protective effects of Algerian propolis against doxorubicin-induced oxidative stresses, meaning therefore the antioxidant ability of propolis extracts. This antioxidant capacity was also reported by Rebiai et al. [54] when measuring the capacity of propolis for scavenging free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and the reducing power capacity [54].

Antioxidant and anti-inflammatory activities

The in vitro antioxidant capacity of propolis from Italy was reported by Papotti et al. [14]. The methods used were the same reported for Rebiai et al. [54] for Algerian propolis. In Italian samples, the capacity for preventing lipid peroxidation measured through the TBARS (thiobarbituric acid reactive substances) was also checked. Samples mainly constituted by flavonoids possessed antioxidant ability. This capacity depended on the harvesting method employed for extracting propolis as well as the solvent used for isolating the bioactive compounds [14].

Ethanolic extracts of propolis from Venetia also presented high antioxidant activity, expressed as the ability for preventing lipid peroxidation and according to the authors such property was mainly due to caffeic acid and its derivatives caffeic acid phenethyl ester and 1,1-dimethylhydrocinnamate. Chrysanthrin and pinocembrin present in relative high amounts in samples showed poor antioxidant characteristics [55]. These authors were able to correlate polyphenol composition of propolis extracts with antioxidant activity. They demonstrated the importance of the existence of two ortho-hydroxy groups in an aromatic ring such as in caffeic acid and derivatives on the antioxidant activity. The presence of only one hydroxyl group or two meta-hydroxyl groups in the aromatic ring (pinocembrin and chrysanthrin) contributed to a low inhibition of lipid peroxidation [55].

Propolis from Portugal has also been reported as possessing antioxidant activity along with antiproliferative and anti-inflammatory activities [48, 56-58]. Extracts of propolis from the northeast (Bornes) and centre of Portugal (Fundão) had capacity for scavenging free radicals and reductive power being such activity dose-dependent and well correlated with phenol content [56]. The antioxidant activity of hydro-alcoholic extracts of propolis from different places of Algarve (south of Portugal) was dependent on the collection zone and also closely correlated with the levels of total phenols, flavones and flavonols in samples [57]. The antioxidant potential of propolis was also reported by Valente et al. [48] but using human erythrocytes as a cellular model. Low concentrations of propolis extracts from two distinct zones (Bornes and Fundão) were able to inhibit the oxidative hemolysis induced by peroxyl radicals as well as the lipid peroxidation in human erythrocytes. The protection of erythrocyte membrane from hemolysis was time- and concentration dependent. The chemical composition of extracts from Fundão was not determined by the authors but they suggested that it should be similar to those from Bornes as already reported by Falco et al. [40]. In this way, flavones, flavonols, flavanones, dihydroflavonols, and substituted cinnamic acids and their esters should have some responsibility in the antioxidant properties.

The anti-inflammatory activity of propolis from Portugal was determined by measuring the effect of propolis samples on the activity of hyaluronidase. The authors have chosen this method because the degradation of hyaluronic acid by hyaluronidase enzyme cause bone loss, inflammation and pain, and therefore may constitute and indirect way to measure anti-inflammatory activity [58]. The results obtained by these authors revealed the capacity of propolis extracts for inhibiting hyaluronidase in a dose-dependent
manner and strongly dependent on the place where they were collected. Nevertheless, the authors found that the activity was not solely due to the phenols and/or flavonoids, suggesting that other components such as vitamins and proteins should also be involved in the hyaluronidase inhibition [58].

Staurosporine and hydrogen peroxide are two stress inducers which act under several pathways, including the production of reactive oxygen species (ROS) and the induction of apoptosis by activation of caspase-3. These two inducers when added to cultured cortical neurons increased intracellular ROS and caspase-3 activity, but the presence of ethanolic extracts of propolis decreased moderately ROS production stimulated by hydrogen peroxide and attenuated the activity of caspase-3 induced by staurosporine- or hydrogen peroxide [59]. In this way, ethanolic extracts of northeast Portuguese propolis were considered by these authors as possessing moderate neuroprotective effect against the cytotoxic effects of staurosporine and hydrogen peroxide on primary cortical neurons in concentrations lower than 10 µg/mL, because higher concentrations evidenced toxic effects in the cells [59]. The authors did not determine the chemical composition of propolis extracts. However and according to a study made by other authors with propolis of the same region [40], the authors suggested that the activity found may be due to the presence of pinocembrin, chrysin and pinobanksin-3-acetate, caffeic acid phenylethyl ester, caffeic acid cinnamyl ester, even in small amounts.

The evaluation of antioxidant activity of ethanolic and propylene glycol extracts of propolis from different location throughout the Basque country (northeastern Spain) were evaluated by some authors [60]. All Basque propolis samples showed considerable antioxidant activity, albeit ethanolic extracts had generally better activity. The authors also showed the contribution of total phenols and flavonoids in the ability of propolis for preventing oxidation [60].

Chicoric acid, a dicafeylex ester present in a butanolic extract of Algerian propolis has potent in vitro stromelysin-1 (matrix metalloproteinase-3-MMP-3) inhibitory activity. This enzyme is responsible for the cutaneous intrinsic and extrinsic aging by acting in collagenolytic and elastolytic cascades [34]. Due to the fact that oxidative stress is the leading cause of MMP upregulation during intrinsic and extrinsic aging, by triggering NF-κB and MAPKinase pathways, along with the fact that caffeic acid derivatives possess antioxidant activity, the authors analysed the ability of that fraction and both caffeic acid and chicoric acid methyl ester to suppress MMP-3 overexpression when fibroblasts were exposed to UVA radiation [34]. The authors found that such compounds were, in fact, able to suppress such MMP-3 overexpression.

**Antimicrobial activity**

Antimicrobial activity is another great attribute of propolis. The antimicrobial activity of Italian propolis has also been reported, namely against *Streptococcus pyogenes* and *Staphylococcus aureus* [61, 62]. Practically all clinical specimens of *S. aureus* (pharyngeal swab, expectoratium, bronchus-aspirate, urinoculture, peritoneal catheter infection, foot wound, sacral decubitus ulcers, leg ulcers, aerobic hemoculture, auricular pus) showed sensitivity to propolis from distinct regions of Piedmont, Italy (hilly zone and a mountain valley) [62]. The chemical composition of both propolis samples was not reported by the authors. However, the bacteriostatic and bacterical activities found for propolis of the same place in Italy against *Streptococcus pyogenes* strains, a microorganism responsible for several otorhinolaryngological infections, were attributed to the presence of pinocembrin and galangin [61].

Extracts of propolis collected at diverse places of Apulia, Italy had antifungal activities, mainly against dermatophytes and *Candida* species, although with different power. The authors attributed these differences to the origin of the propolis as well as to the solvent used for extraction [63]. The authors did not report the chemical composition of the extracts; however they described the flora of each place where propolis had been harvested. Propolis sample with the best activity was that collected in a place of Italy in which *Rosmarinus officinalis*, *Quercus* spp. and *Spartium junceum* predominated [63].

In Spain, some authors verified that ethanolic and propylene glycol extracts of propolis from different locations throughout the Basque country had antimicrobial activity [64]. These authors reported the antimicrobial activity of the same extracts of propolis which had antioxidant activity [61] and concluded that such samples were very active against Gram-positive bacteria and yeasts (*Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans* and *Saccharomyces cerevisae*) and moderately active against *Streptococcus pyogenes*. They also found a significant activity against the Gram-negative bacteria *Salmonella enterica*, whereas *Escherichia coli* was resistant to propolis samples. The authors also detected a dose-dependent activity against the microorganisms tested and a strong correlation between total phenolic content and the antimicrobial activities and, particularly, between flavonoids and activity [64].

Ethanolic extracts of Tunisian propolis was reported as being effective against oral pathogens including streptococci and enterococci. *S. pyogenes* was the most susceptible strain towards the ethanolic extract while *S. anginosus* was the most resistant species [49]. These authors also reported the effectiveness of the ethanolic extracts of propolis from Tunisia as strong anti-biofilms activity against the tested oral streptococci either by the inhibition or reduction of biofilms formation. The antimicrobial activity was also reported for propolis extracts from Malta. According to this study, the authors found that the extracts of the Maltese propolis samples were ineffective against *E. coli*, but effective against *S. aureus*. This activity was attributed to the presence of diterpenes in this sort of propolis from Malta. The activity of propolis against *C. albicans* was also reported in the same study; nevertheless attributed to the terpenyl hydroxybenzoates [27]. Antimicrobial activity due to the presence of diterpenic compounds, although not completely identified, was also reported in some samples from Algeria, particularly against bacteria [22].

The antimicrobial activity of the extracts from French propolis samples were also active against *Staphylococcus aureus* and *C. albicans* as reported for Maltese propolis, nevertheless they were also active against *E. coli*. This activity was attributed to benzyl caffeate which predominated in the extracts [37].

Antimicrobial activity was also reported for hydroalcoholic extracts of propolis from Portugal: Bragança, Coimbra and Beja [58], different places of Trás-os-Montes (Mirandela, Mogadouro, Nogueira and Vinhaia) [65] and Algervar [66]. All propolis samples from Bragança, Coimbra and Beja showed antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Candida albicans* either isolated from biological fluids or purchased from an authorized distributor, nevertheless depending on the origin of samples and the microorganism under study. For all microorganisms checked, samples from Beja were the least effective. *S. aureus* (Gram-positive) was the most sensitive to propolis effect in contrast to *C. albicans* (yeast). Generally, propolis samples were more effective against Gram-positive than against Gram-negative microorganisms [58]. According to these authors, the antimicrobial activity may be due to the presence of flavonoids in hydro-alcoholic extracts (rutin, quercetin and naringenin).

All samples from different places of Trás-os-Montes presented antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), but such effect was dependent on the dosage and on the origin of the product. At the same time, the authors verified that the reference strain was the most sensitive to the propolis action [65]. These authors did not identify the compounds constituting the extracts but they were able to establish a direct relationship between phenol and flavonoid content and antimicrobial activity.

Propolis samples from different places of Algarve showed anti-helminth activity, particularly those collected at springtime. Variations were detected depending on the location of harvesting. The authors did not present the chemical composition, reporting such diversity of results to plant source from which the product was done [66].
Allergic effects

Although the importance of some extract of caffeic acid antioxidants, they are also responsible for allergic reactions in sensitive individuals [67]. These derivatives along with benzyl salicylate and benzyl cinnamate are reported in lists of allergens in cosmetic and flavouring products [68, 69]. In this way, there are studies regarding the reduction of those allergens in hydro-ethanolic extracts of propolis [67]. These authors used a thixotropic wax-oil mixture constituted by corn oil and bee wax, which disperses well with the hydro-ethanolic solution after stirring. This procedure decreased the charge of allergens species in propolis from central Italy. After reduction of the allergens species in hydro-alcoholic extracts of Italian propolis, the residues of phenols and flavonoids were around one third of the initial sample, and consequently a diminution of the antioxidant activity was found, nevertheless this property was higher than expected [67].

According to some authors, other way to prevent allergic reactions is using aqueous extracts of propolis. This kind of formulations has low content of allergic species [67]. In addition, the irritating and drying effects of ethanol of hydro-alcoholic extracts of propolis on mucosal membranes are also lowered if replaced by water [70]. These authors even consider that this sort of formulation is the most adequate in paediatrics. The authors assayed a novel aqueous commercial formulation of a new hydrophilic propolis product from Italy (Acthecelated® Propolis, contained in ‘LeniGola PropoliEffect Spray Senza Alcohol’, Pharenbia, Milan, Italy) for its topical anti-inflammatory activity and compared with a hydrogliceric propolis spray solution and a hydro-ethanolic preparation. They found that such formulation provoked significant and dose-dependent inhibitions of the croton oil-induced ear oedema in mice, but the novel formulation was more active than the remaining samples and even that of the two reference commercial sprays Tantum® Verde and Froben®.

A random study population of 1044 patients made in Italy revealed that propolis along with herbs were the most natural products frequently used, mainly to stimulate the immune system and to cure respiratory, gastrointestinal or cardiovascular problems, as well as to treat anxiety/sleep disturbances [71]. Propolis was also reported as being one of the natural products used among Italian pregnant women, particularly in sore throat situations [72]. Propolis was frequently administered to relieve symptoms of respiratory pathologies, as for example common cold due to its antibacterial and anti-inflammatory attributes. Some side effects of these natural products were reported by the users and for propolis the most frequently described were gastrointestinal symptoms and allergic reactions [71]. These side effects of propolis are, therefore, very important to prevent because the majority of users purchased such product in a pharmacy or in a herbal store without the advice of a health care provider, being the decision practically based on personal judgement [71].

CONCLUSION

The chemical composition reported for propolis samples from the western countries of the Mediterranean basin and Portugal is heterogeneous. However, two main groups may be considered: one type characterized by relative high amounts of phenol acids and their derivatives and flavonoids of poplar-type; and another type in which diterpenes dominate. The biological properties of propolis of this region have been predominantly attributed to the presence of phenols. Only few studies have demonstrated the potentialities of diterpene-rich propolis, particularly as antimicrobials. Therefore, much more surveys on chemical and biological properties of propolis of that region are necessary. It becomes extremely important to confirm if in Morocco, Algeria, Tunisia, Spain, France and even Portugal, propolis exists in which diterpenes are present in considerable amounts. If such occurs, the identification of these components is primordial as well as the evaluation of their biological properties.

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