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Review

Brazilian stingless bee propolis and geopropolis: promising sources of biologically active compounds

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ABSTRACT

Stingless bee products such as honey, pollen, propolis, and geopropolis have been used for centuries in traditional medicine for the treatment of several illnesses. Investigation of the biological activity of stingless bee products, especially propolis and geopropolis, has revealed promising therapeutic properties. About 20% of total Neotropical stingless bees can be found in Brazil. Despite the species diversity, studies on their biological activity are scarce. The present review focuses on the antioxidant and antimicrobial activities of propolis and geopropolis from Brazilian stingless bees. In addition, the toxicity of these natural products was addressed. In order to provide new evidences for the toxic potential of propolis and geopropolis components, an *in silico* analysis was performed using the ADMET Predictor™ software. We observed that most of studies evaluated only crude ethanol extracts of a limited number of stingless bees species. Propolis and geopropolis displayed antioxidant capacity and antimicrobial activity. Concerning the toxic potential, the extracts of stingless bees propolis and geopropolis were considered safe. Nonetheless, *in vitro* and *in vivo* toxicological studies are still necessary.

Introduction

Meliponines, also known as stingless bees (SLB), are the largest group of eusocial bees in the world. More than 600 species have been described and they are spread throughout the tropical and subtropical areas of the globe. They are found in South America, Central America, south of North America, Africa, Southeast Asia and in Northern Oceania (Hrncir et al., 2016). More than 200 species in 29 genera are distributed throughout Brazil. According to Pedro et al. (2014), about 89 species are endemic in Brazil, which corresponds to approximately 20% of the total number of Neotropical stingless bees. Among the genera with the highest number of known species are: *Plebeia*, *Trigona*, *Melipona*, *Scaptotrigona* and *Trigonisca*.

The SLB belong to the family Apidae, subfamily Meliponinae, and they differ from honeybees (*Apis mellifera*, Apidae) in many aspects, including colony size, nesting biology, brood comb disposure, bee queen production, stocking strategy, and bee recruitment mechanisms (Hrncir et al., 2016). However, a marked difference between SLB and honeybees is the morphological aspect related to the sting. This is a defense structure found in females of the *Apis mellifera* species. On the other hand, the females of meliponines have no sting or present an atrophied form of it. The subfamily Meliponinae developed other defense methods such as a strong bite. In addition, some SLB present mandibular glands which are able to produce formic acid, increasing the pain of the bite (Landim, 2009; Michener, 2013). Fig. 1 shows some species from subfamily Meliponinae.

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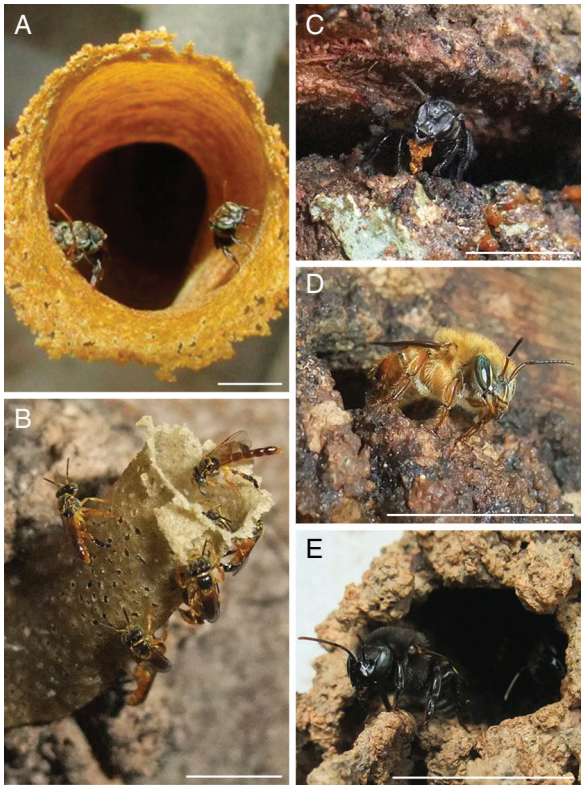


Fig. 1. Brazilian stingless bee. (A) *Nannotrigona testaceicornis*; (B) *Tetragonisca angustula*; (C) *Scaptotrigona* sp.; (D) *Melipona rufiventris*; (E) *Melipona quadrifasciata*.

The ecological relevance of stingless bees is undeniable since these insects are natural pollinators of native plants of different biomes. The species *Melipona subnitida*, for example, is endemic to the Brazilian Northeast, more specifically to the semi-arid region, where it is one of the most important pollinators of the Caatinga biome (Felipe Neto, 2015). Moreover, SLB also display an important socioeconomic role. Their performance as pollinators is not restricted to the natural flora; and they have been used to pollinate diverse species of cultivable plants (Villas-Bôas, 2012). The honey collected from SLB constitutes an important product commonly commercialized in some regions of Brazil due to its flavor and medicinal attributes. In addition, other products can be obtained from meliponiculture, such as bee colonies, pollen, cerumen and propolis (Jaffé et al., 2015). Propolis and geopropolis obtained from various SLB species have gained much attention from researchers around the world due to their pharmacological potential. Some of these biological properties of SLB propolis and geopropolis are discussed below.

Propolis, popularly known as bee glue, is a viscous bee product made by mixing insect secretions (saliva and wax) with plant resins. It is an important material related to the successful construction of the nest and the health of the colony (Araújo et al., 2016a). Propolis is used to seal the beehive, preventing air and undesired visitors to enter. Moreover, the antimicrobial properties of propolis provide a chemical defense against microbial action for the bees themselves and their honey (Campos et al., 2015). *Apis mellifera* bees and SLB are able to produce propolis. However, some meliponines mix the propolis with an extra material: clay or soil. The result of this mixture is a less malleable resinous material when compared to propolis. Geopropolis, as the soil-enriched resin is known, differs from propolis samples due to its mineral and soil content and the absence of plant trichomes (Barth and Luz, 2003). Despite differences in composition, geopropolis displays similar functions to the hive (de Souza et al., 2018).

Different biological activities of SLB propolis and geopropolis ((geo)propolis) have been investigated worldwide, including antioxidant (Cao et al., 2017; Ferreira et al., 2018), anti-inflammatory (Santos et al., 2017a; Guzmán-Gutiérrez et al., 2018), anticancer (Kustiawan et al., 2015; Bartolomeu et al., 2016), and antimicrobial activities (Molnár et al., 2017; Santos et al., 2017a,b,c). It is worth mentioning that the biological activities of geopropolis produced by SLB have been attributed to their phytochemical composition. The influence of the inorganic content (minerals, soil/clay particles) or even organic material associated to geopropolis, such as native microbiota or decomposing organisms, has not been addressed in the literature. This review focuses on the chemical profile and biological effects (antioxidant capacity, antimicrobial, and toxic potentials) of (geo)propolis produced by SLB native to Brazil. Moreover, major (geo)propolis components pointed here were subjected to toxicological analysis *in silico* in order to provide additional evidences of their safe use.

Methodology

Study design

This study consists in a narrative review. Literature data were prospected freely with no time limiting during the search due to the few number of studies evaluating the biological activity of Brazilian SLB (geo)propolis. Web of Science, PubMed and Scielo databases were used as search tools. The following search terms were used alone or in combination: Brazilian stingless bee, propolis, geopropolis, chemical composition antioxidant capacity, antimicrobial activity, antifungal activity, antivirus activity, antiprotozoal activity, cytotoxic effects, and toxicity *in vivo*.

In silico *analysis*

Main compounds of Brazilian stingless bee (geo)propolis prospected here were subjected to *in silico* toxicity analysis using the ADMET Predictor™ (Simulation Plus, Lancaster, CA) software. Toxicity endpoints evaluated were: skin sensitization, cardiotoxicity, acute toxicity, reproductive toxicity, hepatotoxicity, mutagenicity and carcinogenicity. Hepatotoxicity parameters were specifically studied using relevant biomarkers such as alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamil transferase (GGT) and lactate dehydrogenase (LDH). In addition, mutagenicity was evaluated in five individual strains of *Salmonella typhimurium* with or without metabolic activation and/or *Escherichia coli*.

Chemical composition of SLB (geo)propolis

An overview of relevant studies has shown that not all trees resins attract stingless bees. However, the terpenoids, mainly the mono and sesquiterpenoids in these resins are important for these bees. Despite the few studies with stingless bees until now, these chemical compounds, mentioned above, are considered the principal volatile constituents of (geo)propolis produced by SLB. In addition, diterpenoids, triterpenoids and phenolic compounds, mainly flavonoids, are found in different genera of stingless bees.

The genus *Melipona* is the most studied in terms of Brazilian geopropolis. The publications of Bankova et al. (1998, 1999) described the results obtained by a GC–MS investigation of silylated ethanol extracts of two geopropolis samples, collected from different bee species *Melipona compressipes* (Piauí State) and *Melipona quadrifasciata anthidioides* (Paraná State, South Brazil). Both species have a complex chemical mixture of compounds with significant amounts

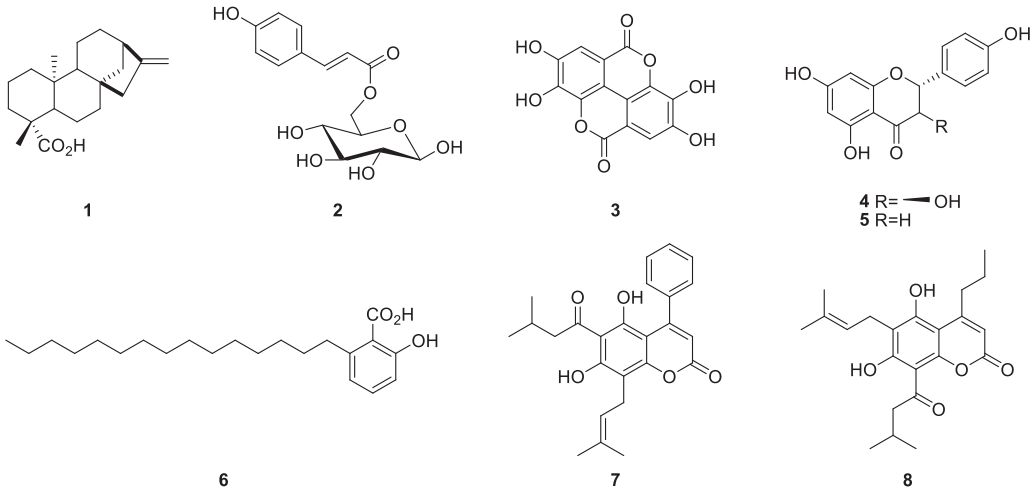
of fatty acids. Other constituents like phenolic compounds, terpenoids and sugars are found in both species of *Melipona*. However, flavonoids are present only in *M. compressipes*, and the diterpenoids are more strongly represented in *M. quadrifasciata* (Bankova et al., 1998). The major compounds in the essential oils of *M. compressipes* and *M. quadrifasciata* are ethylphenol and *p*-cimen-8-ol, respectively (Bankova et al., 1999). Studies with propolis from *M. quadrifasciata anthidioides* have shown three *ent*-kaurene diterpenoids, one of them, *ent*-kaur-16-en-19-oic acid (**1**) has moderate antibacterial activity (Velikova et al., 2000b). Two other works with this last bee species collected from the state of Mato Grosso do Sul (central-west Brazil) showed phytosterols, terpenes, phenolic compounds, and tocopherol after GC–MS and HPLC-DAD and HPLC-DAD-MS/MS analyses. The main compounds described by Santos et al. (2017b) were galloyl-hexoside derivatives and by Bonamigo et al. (2017a) are β-amyrin, β-amyrin acetate, tocopherol, cinnamic acid and apigenin.

The phytochemical approach to analyze geopropolis of *M. subnitida* from Paraiba state (north eastern Brazil) involves a partitioning process with solvents of increasing polarities. This process managed to separate flavonoids and phenylpropanoids from the ethyl acetate fraction by different column chromatography. Among the isolated substances, 6-*O*-*p*-coumaroyl-D-galactopyranose (**2**), a new phenylpropanoid was identified (Souza et al., 2013). Recently, de Souza et al. (2018) described a work with nine samples, collected in different months, of *M. subnitida* geopropolis analyzed by UPLC DADQTOF MS/MS. This analysis resulted in the characterization of 51 phenolic compounds, including ellagic acid (**3**), acyl hexosides, acyl galloyl hexosides and flavonoids. The authors did not mention the amount of each compound found but seven of them were identified by comparison to standard samples and two using NMR (de Souza et al., 2018).

Geopropolis of *Melipona interrupta* and *Melipona seminigra* (Amazon state, North Brazil) and *Melipona orbignyi* (Mato Grosso do Sul State) present phenolic and terpenoid compounds, respectively, that are commonly found in plant species (Silva et al., 2013a Campos et al., 2014). Santos et al. (2017a) described the chemical composition related to flavonoids, terpenoids and glycosylated phenolic acids of the hydroalcoholic extract of *M. orbignyi* geopropolis (Mato Grosso do Sul state) analyzed by HPLC-DAD-MS. Among the flavonoids, aromadendrin (**4**) and naringenin (**5**) have also been found in the geopropolis of *M. interrupta* and *M. seminigra*.

Analyses of the geopropolis extract of *M. fasciculata* by HPLC-DAD-ESI-MS/MS showed the efficacy of the technique to identify more polar substances. The chromatogram analysis of a 70% ethanol extract of this geopropolis collected from Maranhão state showed ellagic acid as the main substance of a complex mixture of tannins and other phenolic compounds (Dutra et al., 2014). The 70% ethanol extract of geopropolis from *M. fasciculata* (Maranhão, northeast Brazil) showed after silylation and GC–MS analysis constituents like carbohydrates, triterpenes, phenolics and sugar alcohols. The main components were the triterpene lupeol and the phenolic anacardic acid (**6**) (Araújo et al., 2015).

The bioguided fractionation of the ethanol extract from geopropolis of *M. scutellaris* (Bahia state, Northeast Brazil) carried out by Cunha et al. (2015) resulted in the isolation of cinnamic acid esters and coumarins. This bioguided work showed the characterization of two potentially active coumarins, mammeisin (**7**) and mammein (**8**), against colon cancer cell lines (Cunha et al., 2015). Torres et al. (2018) presented chemical characterization of an 80% ethanol extract from *M. quadrifasciata quadrifasciata* geopropolis (Rio Grande do Sul state, south Brazil) using UPLC-QTOf-MS analyses and standard samples. The authors characterized diterpenoids and flavonoids as the principal components of the extract.



1 2 3 4 5 6 7 8

The propolis produced by the stingless bee *Frieseomelitta longipes* collected in the city of Belém, state of Pará, presented in its chemical composition an expressive amount of terpenes, mainly monoterpenes and sesquiterpenes. This analysis was performed by GC–MS with the essential oil obtained from *F. longipes*, and β-caryophyllene (34.5%) was found to be the major compound. The polar extract obtained by maceration of this propolis with ethanol was analyzed by LC–ESI-MS/MS with interesting results concerning its chemical composition, since the polyprenylated benzophenones identified are uncommon in Brazil propolis (Souza et al., 2018b).

Reports of propolis produced by *Frieseomelitta* bees are scarce; however, an interesting study by Patricio et al. (2002) presented the chemical composition of the posterior tibia of foraging workers of three species of *Frieseomelitta*, *F. silvestrii*, *F. silvestrii languida* and *F. varia*. In that study, the GC–MS chromatogram analyses showed monoterpene, α-pinene, the sesquiterpenes β-caryophyllene, α-cubebene, α- and γ-muurolene, γ-cadinene, germacrene-D, and elemol and the diterpenes manool and totarol. These substances are collected by the bees to produce the propolis that will be deposited around the entrance of their nests.

The geopropolis produced by *Scaptotrigona postica* is used by natives of the state of Maranhão (Northeast Brazil) as an ointment in the treatment of tumors and wound healing (Coelho et al., 2015). Analyses of geopropolis from *S. postica* using HPLC-DAD-ESI-MS/MS indicated the presence of di-*C*-glycosides flavones, showing vicenin-2 as the major constituent together with pyrrolizidine alkaloids such as 7 (3-methoxy-2-methylbutyryl)-9-echimidinylretronecine and caffeoylquinic acid-*O*-arabinoside.

The results of the analysis by HPLC-DAD-MS/MS demonstrated a chromatographic profile consisting mainly of flavonols, such as quercetin methyl ethers, and methoxychalcones. In addition, the authors suggested that from the robust evidence that the source of the chemical constituents for the production of the *S. postica* geopropolis resin was the plant species *Mimosa tenuiflora*, popularly known as the "jurema-preta". The propolis of *Scaptotrigona depilis* bees analyzed by GC-MS and HPLC-DAD showed phytosterols, terpenes, phenolic compounds, and tocopherol (Bonamigo et al., 2017a). *Scaptotrigona bipunctata* bees produce a propolis whose chemical characterization performed by UPLC-ESI-QTOF/MS/MS analyses revealed the uncommon presence of piperidinic alkaloids together with *C*-glycoproteinide flavonoids (Cisilotto et al., 2017).

Freitas et al. (2008) reported the identification and isolation of cycloartane triterpenes and flavonoids as the main constituents from the nest of *Trigona spinipes*, a species commonly found in Brazil Northeast region. The authors suggested that one of the plant species that served as the source of the chemical constituents for the production of propolis by *T. spinipes* was *Eucalyptus citriodora*.

Sawaya et al. (2007) demonstrated that *Tetragonisca angustula* is a selective bee, compared to *A. mellifera*, in the choice of vegetal species as the source of the chemical constituents necessary for the production of propolis. In that study, in spite of the different geographical locations, Minas Gerais, Santa Catarina and Bahia states, and, equally important, the variety of plant species in these locations, *T. angustula* visited and collected preferably the exuded resin of *Schinus terebenthifolius*, a plant popularly known as "aroeira-vermelha". However, despite this preference, worker bees of *T. angustula* visited and collected resins from other plant species, for example *Euphorbia milii* and *Clusia fluminensis* (Gastauer et al., 2011). There are some interesting works analyzing the chemical composition of the geopropolis produced by *T. angustula*. Miorin et al. (2003) described a study using HPLC-DAD to compare the chemical composition of propolis produced by the stingless bees, *T. angustula*, and those produced by the honeybee with *A. mellifera*. The sampling sites were the states of Minas Gerias and Paraná, and the results showed the difference in the chemical composition of the propolis produced by the two species of bees (Sawaya, 2007). These results were also substantiated by the work of Pereira et al. (2003) that showed by HT-HRGC/MS the similarity of the chemical constituents present in the dichloromethane extracts obtained from the propolis of those two species of bees. The extracts of greater polarity exhibited significant differences in their chemical compositions. The studies of Sawaya et al. (2006, 2007) with bees provide additional information about the chemical composition of propolis produced by *A. mellifera*, and stingless *T. angustula*. In that work, the authors analyzed the propolis produced by the two species of bees by ESI-MS, elaborating elucidative fingerprints not only with respect to the different chemical compositions in the samples analyzed, but also on the origin of the vegetal species that serve as sources of the chemical constituents for the production of the geopropolis. Recently, Santos et al. (2017b) presented the chemical profile by HPLC of the aqueous and hydroalcoholic extracts obtained from the propolis produced by *T. angustula*. The aqueous extract showed phenols, tannins, flavones, flavonols, xanthones, steroids and triterpenes, whereas the hydroalcoholic extract presented the same substances and catechins.

Antioxidant capacity

The antioxidant capacity of a compound can assist in the prevention of diseases related to oxidative stress, which is caused by an imbalance between the formation and neutralization of free radicals in the body through enzymatic and non-enzymatic antioxidants (Fang et al., 2002; Campos et al., 2014). An excess of free radicals in the body can result in cell membrane phospholipid oxidation, DNA and protein damage and tissue injury (Zhu et al., 2011; Campos et al., 2014). Therefore it is important to identify natural compounds and/or new substances that can neutralize these free radicals to prevent oxidative stress (Bonamigo et al., 2017b). Studies have been conducted on the propolis of different species of Brazilian stingless bees to evaluated their ability to scavenge free radicals and protect against the damage caused by oxidizing agents (Sawaya et al., 2009; Campos et al., 2014, 2015; Bonamigo et al., 2017; Torres et al., 2018). The main methods to determine antioxidant activity as well as the results of recent studies of the propolis from Brazilian stingless bee are described below.

The analytical methods and biological assays are based on the research of propolis that has evolved over the years, and has been driven to ensure the identification of the components responsible for their biological activity as well as to certify the quality of the products that can be used (Sawaya et al., 2011). These methods can be applied in different areas of research such as food, cosmetics and medicine (Mishra et al., 2012).

The chemical compounds in propolis found in Brazil vary due to the different climates in the country such as equatorial, tropical and subtropical. The presence of flavonoids and phenolic compounds are related to the antioxidant activity. The choice of the method used to evaluate this biological activity may influence the results; therefore it is recommended to use more than one method in order to ensure the results (Sawaya et al., 2011). Currently the main methods used are: the capture of free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl) and $ABTS^{+\bullet}$ (2,2-azinobis-(3-ethylbenzothiazole-6-sulphonate); FRAP (ferric reducing antioxidant power) method; the oxidative hemolysis inhibition assay and evaluation of the inhibition of lipid peroxidation in human erythrocytes (Sawaya et al., 2011; Mishra et al., 2012; López-Alarcón and Denicola, 2013; Silva et al., 2013; Shahidi and Ambigaipalan, 2015).

Antioxidant capacity of SLB (geo)propolis

Box 1 summarizes the literature data searched concerning the antioxidant capacity of Brazilian stingless bee propolis.

Sawaya et al. (2009) compared samples of propolis collected monthly from three species of *Scaptotrigona* bees from two distinct regions in Brazil (States of Maranhão and São Paulo - Northeastern and South eastern, respectively). Ethanol extracts of the propolis samples were prepared and evaluated for their antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method (DPPH). Antioxidant activity varied monthly for each species, with the highest activity (lowest Effective Dose (ED50) results) observed in the spring. The propolis of the species *S. bipunctata* presented the highest antioxidant activity and that of the species *S. depilis* presented the lowest activity. The authors reported that seasonality and geographic origin affected the composition and thus the antioxidant activity of *Scaptotrigona* bee propolis.

In a study carried out by Campos et al. (2014) the ethanol extract of propolis of *M. orbignyi* showed antioxidant activity by scavenging free radicals and exhibited anti-hemolytic action and protective actions against lipid peroxidation when incubated with human erythrocytes in the presence of the oxidizing agent 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH). The free radicals scavenging ability demonstrated by the *M. orbigny* propolis was similar to the results observed for propolis from the bee species *Apis mellifera* (Mercan et al., 2006). The results are mainly related to the presence of phenolic compounds (Campos et al., 2014). These compounds have been reported to be important antioxidants that act as hemolysis inhibitors in erythrocytes under conditions of oxidative stress (Valente et al., 2011). They are capable of donating electrons, leading to the stabilization of the radical and can also

Table 1
Antibacterial and antifungal activities of Brazilian stingless bees (geo)propolis.

Reference	Stingless bees species	Propolis/Geopropolis	Extract/fraction/isolated compound	Microorganisms tested (antimicrobial activity)	Inhibitory concentrations - μg/ml	
					MIC	MBC/MFC
Souza et al. (2018b)	*Frieseomelitta longipes*	Propolis	Ethanol extracts	*Bacillus cereus*	7.8–15.6	nd
				Staphylococcus aureus	125	
				Pseudomonas aeruginosa	31.3–62.5	
				Escherichia coli	125	
				Candida albicans	2.5–250	
				Candida tropicalis	250	
Torres et al. (2018)	*Melipona quadrifasciata quadrifasciata*	Propolis	Ethanol extract	*Staphylococcus aureus*	2000–7000	nd
				MRSA		
				Enterococcus faecalis		
				Escherichia coli		
				K. pneumoniae		
Santos et al. (2017c)	*Melipona mondury*	Geopropolis	Ethanol extract	*Pseudomonas aeruginosa*	250	250
			Hexane fraction		1000	>1000
			Ethyl acetate fraction		250	>1000
			Butanol fraction		500	1000
			Ethanol extract	*Staphylococcus aureus*	250	1000
			Hexane fraction		500	>1000
			Ethyl acetate fraction		25	1000
			Butanol fraction		5–10	25
			Ethanol extract	MRSA	>1000	>1000
			Hexane fraction		>1000	>1000
			Ethyl acetate fraction		500	>1000
			Butanolicethanol fraction		250	>1000
Araújo et al. (2016b)	*Melipona fasciculata*	Geopropolis	Ethanol extract	*Pythium insidiosum*	3400	nd
Campos et al. (2015)	*Tetragonisca fiebrigi*	Propolis	Ethanol Extract	*Staphylococcus aureus*	550–650	1500–2000
				Staphylococcus epidermidis	770–880	1750–1920
				Enterococcus faecalis	880–1020	3800–5000
				Klebsiella pneumoniae	3330–3750	11580–13080
				Pseudomonas aeruginosa	5830–7910	14420–15500
				Proteus mirabilis	2250–3080	10750–12020
				Candida glabrata	7000–7910	9000–11930
				Candida albicans	7900–9250	8000–12910
Campos et al. (2014)	*Melipona orbignyi*	Propolis	Ethanol Extract	*Staphylococcus aureus*	3100	3100
				Escherichia coli	na	na
				Candida albicans	3100	50000
Cunha et al. (2013)	*Melipona scutellaris*	Geopropolis	Ethanol extract	*Streptococcus mutans*	25–50	>1600
				Staphylococcus aureus	6.25–12.5	25–50
				MSRA	6.25–12.5	25–50
				Enterococcus faecalis	800–1600	>1600
				Actinomyces naeslundii	800–1600	>1600
				Pseudomonas aeruginosa	>1600	>1600
Fianco et al. (2013)	*Scaptotrigona bipunctata*	Propolis	Ethanol extract	*Staphylococcus aureus*	nd	nd
				Escherichia coli		
Campos et al. (2011)				*Aeromonas hydrophila*	>1000	nd
				Bacillus subtillis	62.5	62.5
				Pseudomonas aeruginosa	500	500
				Staphylococcus aureus	250	250
Miorin et al. (2003)	*Tetragonisca angustula*	Propolis	Ethanol extract	*Staphylococcus aureus*	440–2010	nd
Fernandes Jr. et al. (2001)	*Nannotrigona testaceicornis*	Propolis	Ethanol extract	*Staphylococcus aureus*	nd	nd
	Tetragonisca angustula				9900 (MIC_{90})	
	Trigona spinipes				9470 (MIC_{90})	
	Scaptotrigona sp.				8230 (MIC_{90})	
	Melipona sculletaris				230 (MIC_{90})	
	Melipona mandaçaia				160 (MIC_{90})	
	Melipona sp.				170 (MIC_{90})	
	Partamona sp.				140 (MIC_{90})	

na, not active; nd, not determined; SLB, Stingless bee; MRSA, Methicillin-resistant *Staphylococcus aureus*.

stimulate antioxidant enzyme activities in erythrocytes (Campos et al., 2014). The authors concluded that taken together, the results indicate that propolis from *M. orbignyi* has therapeutic potential for the treatment and/or prevention of diseases related to oxidative stress.

Propolis from the stingless bees *Tetragonisca fiebrigi* is used in folk medicine for its nutritional and therapeutic properties. This species is present in a large part of Brazil. However, until recently there were no scientific records evidencing such properties. In the only study so far reported on the propolis generated by this species, Campos et al. (2015) evaluated the effects of the ethanol extract of propolis of this stingless bee. The ethanol extract showed antioxidant activity by evaluating its free radical scavenging effect on the 2,2′-azinobis-(3-ethylbenzothiazoline6-sulfonicacid) (ABTS) radical. The authors also observed the protective effect of the extract by inhibiting hemolysis and lipid peroxidation in human erythrocytes incubated with the AAPH oxidizing agent.

Bonamigo et al. (2017b) demonstrated the antioxidant activity *in vitro* of the propolis from the species *Plebeia droryana* found in the Cerrado biome, in the Midwest region of Brazil. The ethanol extract of the propolis of this specie was able to inhibit the DPPH free radical. These authors also tested the antioxidant activity of the extracts of this propolis by analyzing its protection against oxidative hemolysis and the ability to reduce the levels of malondialdehyde (MDA). MDA is a product of lipid peroxidation due to oxidative stress. However, this extract was not able to inhibit the MDA content generated by the oxidizing agent AAPH. These results may be related to the chemical composition of this propolis.

Torres et al. (2018) investigated the antioxidant properties of an ethanol extract of propolis from *M. quadrifasciata quadrifasciata* and *T. angustula*. The DPPH free radical scavenging activity was measured and the inhibitory concentration (IC_{50}) was determined. The authors observed that both extracts of propolis had dose-dependent antioxidant activity. However the extract of propolis from *M. quadrifasciata quadrifasciata* was ten-fold more potent in promoting antioxidant activity than the *T. angustula* extract. Analyzing the chemical composition of propolis from both species, these authors demonstrated out that the propolis extract of *M. quadrifasciata quadrifasciata* presented a higher concentration of total phenols and flavonoids, reinforcing the direct correlation between phenol concentration and antioxidant activity established in the literature (Duthie et al., 2003).

The above studies showed that the antioxidant activity present in propolis seems to depend on the genus and species of bees. That is, the genetic variability of the bee species influences the chemical composition of propolis, resulting in different biological activities (Torres et al., 2018). The differences in the chemical composition of propolis extracts in the same region may be related to species of bees and the preference for a particular plant species to elaborate the propolis (Bankova et al., 2014; Bonamigo et al., 2017b).

Together, the results presented in this review show that the propolis produced by the Brazilian stingless bee possesses antioxidant activity, indicating that this natural product exhibits promise for the treatment and/or prevention of various diseases related to oxidative stress. Consequently, this bee product is of great interest to the pharmaceutical and food industries.

Antimicrobial activity of (geo)propolis

(Geo)Propolis against bacteria

The study conducted by Santos et al. (2017c) demonstrated that the butanol fraction of geopropolis (BFGP) from *M. mondury* had bacteriostatic and bactericidal activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA) with minimal inhibitory concentrations ranging from 5 to 500 μg/ml. The *S. aureus* ATCC 29213 strain was the most sensitive microorganism to BFGP with a minimum bactericidal concentration (MBC) of 25 μg/ml. This fraction also presented high amounts of phenolic compounds and a high antioxidant capacity (Santos et al., 2017c). Previously, Fianco et al. (2013) attributed the polyphenol content of *S. bipunctata* propolis to the antibacterial activity detected against *E. coli* and *S. aureus*.

Souza et al. (2018b) reported that *Bacillus cereus* INCQS 00003 (ATCC 11778), *S. aureus* INCQS 0057 (ATCC 43300), *P. aeruginosa* INCQS 00025 (ATCC 15442) and *E. coli* INCQS 00051 (ATCC 13863) were sensitive to *F. longipes* propolis with MIC values ranging from 7.8 to 250 μg/ml. *B. cereus* inhibition by propolis (sample FL3) was comparable to that observed for the reference drug ampicillin (MIC 7.8 μg/ml). Another recent study showed the antibacterial activity of the propolis ethanol extracts of *M. quadrifasciata quadrifasciata* and *T. angustula* against Gram-positive and Gram-negative bacteria. The Gram-positive bacteria *S. aureus* ATCC 25923, MRSA (clinical isolate) and *E. faecalis* (ATCC 29212) were shown to be sensitive to the extracts; however, the best results were obtained with the propolis ethanol extract from *M. quadrifasciata quadrifasciata*. The authors demonstrated that the mode of action may involve damage to the bacterial cell membrane (Torres et al., 2018). Similar results were previously described by Campos et al. (2015) when Gram-positive and Gram-negative bacteria were treated with propolis ethanol extracts from *T. fiebrigi*. The extract was more active against the Gram-positive bacteria (MIC and MBC ranging from 0.55 ± 0.05 to 1.02 ± 0.12 mg/ml and 1.50 ± 0.14 to 5.00 ± 0.14 mg/ml, respectively). The inhibition observed followed the sequence: *S. aureus* > *S. epidermidis* > *E. faecalis* > *Proteus mirabilis* > *Klebsiella pneumonia* > *P. aeruginosa.*

Campos et al. (2011) evaluated the anti-*S. aureus* and anti-*B. subtillis* activity of a chloroform solution of propolis produced by *F. varia*. The main substance was identified as 3,5-diprenyl-4-hydroxycinnamic acid with MIC values against *B. subtillis* and *S. aureus* of 62.5 and 250 μg/ml, respectively. However Lee et al. (2018) related a severe allergic contact dermatitis associated to this propolis for a 14-year-old girl.

(Geo)Propolis against fungi

Propolis from the stingless bee *M. orbignyi* displays a broad biological activity, suggesting that this natural product could be a promising agent for the treatment and/or prevention of various infectious diseases. *M. orbignyi* propolis (ethanol extract) demonstrated antibacterial and antifungal potential, and was active against *S. aureus* and *Candida albicans*. Interestingly, the extract was able to inhibit *C. albicans* growth at 3.1 mg/ml (MIC), but the minimum fungicidal concentration (MFC) was detected at 50 mg/ml (Campos et al., 2014). Araújo et al. (2016b) compared the anti-*Pythium insidiosum* (the causative agent of pythiosis) activity of honeybee propolis and *M. compressipes fasciculata* geopropolis collected in southeast and northeast Brazil, respectively. The authors demonstrated that hydroalcoholic extract of propolis from honeybees exerted fungicidal activity against three isolates at 1 mg/ml after 24 h treatment and all other isolates at 3.4 mg/ml. The geopropolis hydroalcoholic extract of propolis from *M. compressipes* was able to inhibit two isolates at 3.4 mg/ml under the same conditions, but in this case a fungistatic effect was observed. The chemical composition of both samples (propolis and geopropolis) was not given. The phytochemical analysis is an elucidative tool to identify biologically active substances and will be necessary in further investigations of these samples.

Box 1
Antioxidant activity of the Brazilian propolis produced by different species of stingless bee.

Reference	SLB species (location)	Methods of determining antioxidant capacity			
		DPPH	ABTS + (radical scavenging assay)	Oxidative hemolysis inhibition assay (protective effect)	Evaluation of the inhibition of lipid peroxidation in human erythrocytes
Torres et al. (2018)	*Melipona quadrifasciata quadrifasciata* *Tetragonisca angustula* (Rio Grande do Sul)	*M. quadrifasciata quadrifasciata* IC_{50} = 241.8 μg/ml *T. angustula* IC_{50} = 2433.0 μg/ml	–	–	–
Bonamigo et al. (2017a,b)	*Plebeia droryana* (Mato Grosso do Sul)	At a concentration of 500 μg/ml the extract exhibited an IC_{50} of 182.4 ± 58.9 μg/ml and a maximum inhibition of 94.6 ± 0.9%. The standard (ascorbic acid), exhibited a IC_{50} of 3 ± 0.4 μg/ml and a maximum inhibition of 98 ± 0.4% at 10 μg/ml	–	–	Was not able to inhibit the MDA content generated by the action of the oxidizing agent AAPH.
Campos et al. (2015)	*Tetragonisca fiebrigi* (Mato Grosso do Sul)	–	The IC_{50} of extract (119.6 ± 20.5 μg/ml) was approximately 5 times higher than that of synthetic antioxidant BHT (standard).	After a 240 min incubation with agent AAPH (50 mM), a reduction of 46 ± 3.6% of hemolysis was observed in the highest concentration of extract (125 μg/ml)	Reduced MDA levels at all concentrations tested (50–125 μg/ml) when erythrocytes that were incubated with the oxidizing agent AAPH (50 mM).
Campos et al. (2014)	*Melipona orbignyi* (Mato Grosso do Sul)	At a concentration of 100 μg/ml the extract exhibited an IC_{50} of 40 ± 4.8 μg/ml and a maximum inhibition of 96 ± 0.6%. The standard (ascorbic acid), exhibited a IC_{50} of 3 ± 0.4 μg/ml and a maximum inhibition of 98 ± 0.4%.	–	Protected erythrocytes from the action of the hemolysis inducing agent AAPH (50 mM) during the first 120 min of incubation.	Reduced MDA levels at all concentrations tested (50–125 μg/ml) when erythrocytes that were incubated with the oxidizing agent AAPH (50 mM).
Sawaya et al. (2009)	*S. bipunctata* and *S. depilis* (São Paulo) *Scaptotrigona* ssp. (Maranhão)	The average ED_{50} value for *S. bipunctata* samples was 183 μg/ml, followed by *Scaptotrigona* ssp. with an average of 310 μg/ml and by *S. depilis* with 593 μg/ml.	–	–	–

DPPH, 2,2-diphenyl-1-picrylhydrazyl (free radical); ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride (oxidizing agent); MDA, Malondialdehyde (marker of oxidative damage of the membrane lipids); IC_{50}, Inhibitory Concentration; ED_{50}, Effective Dose (As the ED50 value represents the concentration of propolis that reduces the absorbance of DPPH by 50%; the lower the concentration, the higher the antioxidant activity of the sample); – Not performed.

(Geo)Propolis against virus

The antiviral potential of SLB (geo)propolis has received a certain amount of attention from some research groups in Brazil, due the clinical relevance of pathogenic species that afflicts both human and veterinary health. Peter et al. (2017) reported the antiviral and virucidal effects of three hydroalcoholic extracts of propolis: two of them from *A. mellifera* (brown and green propolis) and one from *T. angustula* against bovine herpesvirus type-1 (BoHV-1) and bovine viral diarrhea Virus (BVDV). The treatment of the pre-infected MDBK cell line resulted in low viability of these cells, indicating that all the propolis samples were not able to eliminate the virus. However, the pre-treatment of MDBK cell line with all of these propolis samples assured their survival. The best results were observed for the pre-treatment with 0.39 μg/ml of the *T. angustula* propolis extract. The authors hypothesized that the samples might be able to cause changes in the MDBK membrane receptors preventing the virus entry. In another study, the hydromethanol extract of geopropolis (HMG) from *S. postica* was evaluated as an antiviral agent against McIntyre *Antiherpes simplex* virus (HSV-1). The authors evaluated three systems of treatment, which included 1 h pre-treated cells, post-treated cells and pre-treated virus with different HMG concentrations (1, 10 and 100 μg/ml). In all treatment systems and concentrations tested the number of virus DNA copies dropped drastically (about 98%). This effect was attributed to the known antiviral activity of *C*-glycosylflavones, catechin-3-*O*-gallate, and 3,4-dicaffeoylquinic acid also identified in the HMG assay (Coelho et al., 2015).

Overall there are few studies about the uncommon geopropolis produced by SLB in Brazil. Based on the data searched in this review, in relation to the antimicrobial activity, the available information concerning these natural products appears to be concentrated in the antibacterial potential. One hypothesis is related to the easier management of bacteria species and relative simple antibacterial screening assays. Moreover, no study concerning the antiprotozoal activity of Brazilian (geo)propolis produced by SLB was found in the literature search. Table 1 summarizes the antimicrobial activity of Brazilian SLB (geo)propolis.

In vitro *and* in vivo *toxicity of stingless bee (geo)propolis*

The hypothesis that natural products are safe for use must be examined carefully. Despite been a promising approach, natural product-based drug discovery requires much attention due the

Box 2
In silico toxicity analysis of the Brazilian stingless bees propolis compounds using ADMET Predictor.

#	Compounds	*In silico* toxicity
3	Ellagic acid	Mutagenicity, Skin sensibilization
9	2,2-Dimethyl-8-prenyl-2 H-1-benzopyran-6-propenoic acid	Hepatotoxicity
10	2-Heptanol	Reproductive problems, Hepatotoxicity
11	2-Heptanone	Skin sensibilization, Reproductive problems, Hepatotoxicity
12	3,5-Diprenyl-4-hydroxycinnamic acid	Skin sensibilization, Hepatotoxicity
13	3-Phenyl-*p*-coumaric acid	Hepatotoxicity
14	3-Prenyl-4-hydroxycinnamic acid	Skin sensibilization, Hepatotoxicity
15	4-Methoxybenzoic acid	-
16	Benzaldehyde	Skin sensibilization, Reproductive problems, Hepatotoxicity
17	Benzoic acid	Hepatotoxicity
18	Benzyl caffeate	Skin sensibilization, Hepatotoxicity
19	Cinnamic acid	Hepatotoxicity
20	Cinnamyl caffeate	Skin sensibilization, Hepatotoxicity
21	*cis*-Linalool oxide	Reproductive problems, Hepatotoxicity
22	Di-hexahydroxydiphenic-galloylglucose	Skin sensibilization, Hepatotoxicity
23	Di-hexahydroxydiphenic-glucose (pedunculagin)	Mutagenicity, Skin sensibilization, Hepatotoxicity
24	Ethyloctanoate	Skin sensibilization
25	Gallic acid	Skin sensibilization, Hepatotoxicity
26	Galloyl-hexahydroxydiphenic-glucose (corilagin)	Skin sensibilization
27	Hexahydroxydiphenic acid	Skin sensibilization
28	Hexahydroxydiphenic-digalloylglucose (tellimagrandin I)	Skin sensibilization, Hepatotoxicity
29	Hexahydroxydiphenic-glucose acid	Mutagenicity
30	Hotrienol	Skin sensibilization, Reproductive problems, Hepatotoxicity
31	Kaurenoic acid	Reproductive problems, Acute toxicity
32	*p*-Coumaric acid	Skin sensibilization, Hepatotoxicity
33	*p*-Cymene	Skin sensibilization
34	Thuja-2,4(10)-diene	Skin sensibilization, Reproductive problems, Hepatotoxicity
35	*trans*-Linalool oxide (furanoid)	Reproductive problems, Hepatotoxicity
36	Trigalloylglucose	Skin sensibilization
37	Trigalloyl-hexahydroxydiphenic-glucose (tellimagrandin II)	Skin sensibilization, Hepatotoxicity
38	Valoneic acid dilactone	Skin sensibilization, Hepatotoxicity, Mutagenicity

possibility of toxic effects. In fact, natural medicines, as well as allopathic medicines, can have adverse effects on health, which may occur immediately after their ingestion or in the long term, including hepatotoxic, carcinogenic and nephrotoxic effects (Zeng and Jiang, 2010). Frequently, *in vitro* assays using appropriate cellular models are chosen as the first step to assure safety of further drug tests. Various works seeking biological activities of natural products try to demonstrate that the effective concentrations exert low or zero toxicity for mammalian cells. Studies evaluating Brazilian SLB (geo)propolis cytotoxicity are few. Kujumgiev et al. (1999) screened propolis samples from different parts of the world for antimicrobial and cytotoxic activities. The cytotoxicity of propolis samples produced by meliponines collected in Brazil, *M. compressipes* and *M. quadrifasciata anthidioides*, was evaluated against primary chick embryo fibroblast (CEF). *M. compressipes* propolis, rich in flavonoids, was the less toxic with a selective index of 35. More recently, Peter et al. (2017) showed that *T. angustula* propolis was less toxic (minimal toxic concentration of 1.75 μg/ml) than honeybee brown and green propolis (0.39 and 0.78 mg/ml) for the Madin–Darby bovine kidney (MDBK) cell line. Moreover, all propolis samples in that study displayed cytotoxic effects but in very low concentrations. Dos Santos et al. (2017) carried out a preliminary assessment of the toxicity of the aqueous and hydro-alcoholic extracts from the Brazilian stingless bee *Melipona quadrifasciata* using the methodology proposed by Acharyya et al. (2009) with adaptations. The level of hemolysis of human erythrocytes is considered a determinant of cytotoxicity. All samples presented low percentage of hemolysis at the lowest concentration tested (125 μg/ml), and the hydro-alcoholic extract was the extract with the lowest hemolytic activity (0.79% ± 0.10).

In vivo assays may be important to accesses the toxicity of natural products since pluricellular organisms with several cellular differentiation levels could provide concise information of the toxic potential of drug candidates. The brine shrimp (*Artemia salina*) lethality assays is an advantageous method as there is no need to take into account any aseptic techniques, the results are acquired rapidly and it is low cost (Rajabi et al., 2015). This model was applied by Velikova et al. (2000a) to evaluate the propolis toxicity of 21 Brazilian SLB. The samples presented different levels of toxicity with 50% lethal dose (LD_{50}) ranging from 0.3 ±0.2 to >1000 μg/ml. Interestingly the less toxic and the most toxic samples belonged to the same species: *M. quadrifasciata*. Those samples differ in only the collection sites, Araripina (Pernanbuco State) and Prudentopolis (Paraná State), respectively (Velikova et al., 2000a). In fact, this is a typical example which reinforces that propolis composition, and consequently its bioactivity, is mostly dictated by the local flora. The geopropolis of *M. fasciculata*, a common SLB found in the North and Northeastern regions of Brazil, were formulated into a gel base and tested in a murine model in order to investigate eventual toxic effects after its efficacy against oral pathogens was confirmed. The geopropolis-based gel applied to the oral cavity of mice for 4 days did not show any significant alterations to the weight of their internal organs or to their histopathological analyses. In addition, the geopropolis-based gel significantly reduced cholesterol and triglyceride levels probably due the antioxidant content of *M. fasciculata* geopropolis (Liberio et al., 2011). In 2011, Araújo and co-workers performed an acute toxicological evaluation of a propolis hydroalcoholic extract (PHE) produced by *Scaptotrigona* aff. *postica* since propolis from this specie is used for the treatment of many diseases, and the breeding of stingless native bees is an activity heavily related to the economic development of Maranhão State in Brazil (Araújo et al., 2011; Maia Filho et al., 2008). The acute toxicity test of PHE ingested orally showed that it did not induce death in the animals (male and female Swiss mice, 120 days old and weighing 25–35 g), even when receiving high doses (1000, 2000 and 4000 mg kg^{-1}). Even though no death was caused, the study of acute toxicity with PHE showed that it did induce lower mobility in all the animals. However, females treated with the highest dose also showed other signs of toxicity, such as bristly hair, convulsions, tremors,

hyperaemia, a runaway reaction and aggression, in the first 4 h of observation.

In silico insights of SLB (geo)propolis toxicity

Animal models have been used for a long time for toxicity testing. However, *in vivo* assays tests are constrained by time, financial costs and ethical issues (Parasuraman, 2011; Raunio, 2011; Boekelheide et al., 2015; Parthasarathi and Dhawan, 2018). Due to technological process, *in silico* (computational) methods been developed for testing of drugs and chemicals (Mushtaq et al., 2018). *In silico* tests follow the strategy of 4 Rs (Reduction, Refinement, Replacement and Responsibility), for the laboratory use of animals (Arora et al., 2011; Ranganatha and Kuppast, 2012).

In silico toxicology employs computational resources to organize, analyze, model, simulate, visualize and predict toxicity of chemicals (Valerio, 2009; Deeb and Goodarzi, 2012; Raies and Bajic, 2016). *In silico* methods aim to complement *in vitro* and *in vivo* toxicity assays to minimize the need for animal testing, reduce the cost and time of tests, and improve toxicity prediction and safety assessment (Parthasarathi and Dhawan, 2018).

In this study, the ADMET Predictor™ software was used in order to evaluate the toxicity potential of some compounds of Brazilian stingless bee propolis (Box 2) *in silico*. For the hepatotoxicity studies, all compounds presented liver problems, since they showed elevated levels of ALP, SGOT, SGPT, GGT or LDH. On the other hand, none of them showed carcinogenic potential and cardiotoxicity and only kaurenoic acid presented acute toxicity in rats. In mutagenicity, 65% did not present mutagenic risk (compounds numbered as 3, 9–18, 20, 24, 25, 30–35); 32% did not present skin sensitization (compounds numbered as 3, 9, 11, 14, 16, 18, 20, 29, 31, 35); and 71% did not show reproductive toxicity (compounds numbered as 3,11–14, 16–19, 21–23, 25–29, 3236–38 in Box 2).

Therefore, the *in silico* analyses were able to predict compound toxicity in a rational drug development process, minimizing animal use and cost/time according to the 4 Rs strategy. Most of the compounds exhibited some toxic potential, highlighting kaurenoic acid, which presented acute toxicity in rats. Eleven compounds of third-one may be potential natural drugs, since they presented none or one toxicity parameter. Three toxicity parameters are expected for about 10% of the focused World Drug Index (WDI).

Most of the Brazilian stingless bee propolis compounds revealed a low profile for toxicity analysis *in silico*, indicating that compounds are potentially safe natural drugs. Pre-clinical studies are required to achieve a better understanding of Brazilian stingless bee propolis compounds, and this study is at an early stage of drug development.

Both propolis and geopropolis are widely consumed by different populations that use them in the treatment of several illnesses. However, as evidenced above, the (cyto)toxicity of these SLB products is controversial. The lack of studies focusing on the toxicological profile of Brazilian SLB (geo)propolis revels that this is a promising field of research within the natural products field.

Research into (geo)propolis in Brazil appears to be a promising field since many questions still wait for answers. The studies presented here tested crude extracts or performed initial steps of extract partitions. Literature lacks studies describing the mode of action of these extracts and the substances responsible for their biological activities. Our attention was called to the low number of toxicological and antimicrobial studies considering the traditional use of (geo)propolis. Thus, we hope that this review will stimulate further investigations into Brazilian SLB (geo)propolis.

Conclusion

The biological potential of Brazilian native SLB (geo)propolis was demonstrated. As described in detail in the present review species of the genus *Melipona*, *Frieseomelitta*, *Scaptotrigona*, *Trigona* and *Tetragonisca* produce propolis with similar chemical profiles, constituted mainly by terpenoids and phenolics, notably flavonoids. In contrast, species with atypical constituents in the chemical constitution of propolis produced by stingless bees are *Frieseomelitta longipes* and *Scaptotrigona bipunctata*. In this context, the propolis of *Frieseomelitta longipes* presented polyphenylated benzophenones, whereas the propolis produced by *S. bipunctata* contains piperidinic alkaloids. The findings compiled here provide strong evidences that the propolis and their chemical constituents display interesting antioxidant capacity and antimicrobial effects. Despite the controversial data concerning the toxic potential of (geo)propolis, the *in silico* analysis performed in this review suggests that most of the substances found in these products are safe for consumption.

Authors' contributions

IAR and CSC designed the work. IAR, FCL and GBLS collected and discussed data concerning the native stingless bee geographic distribution and behavior. ACFA and JRAS collected and discussed data concerning the phytochemical composition of (geo)propolis. CSC and EHBC collected and discussed data concerning the antioxidant capacity of (geo)propolis. IAR, FCL and MMBA collected and discussed data concerning the antimicrobial activity of (geo)propolis. IAR, BAV and TFSD collected and discussed data concerning the toxicity of (geo)propolis. BAV and TFSD performed and discussed the *in silico* analysis of (geo)propolis. ABV critically revised the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2018.11.007.

References

Acharyya, S., Patra, A., Bag, P.K., 2009. Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant Vibrio cholerae. Trop. J. Pharm. Res. 8, 231–237.

Araújo, K.S.S., Santos Júnior, J.F., Sato, M.O., Finco, F.D.B.A., Soares, I.M., Barbosa, R.S., Alvim, T.C., Ascêncio, S.D., Mariano, S.M.B., 2016a. Physicochemical properties and antioxidant capacity of propolis of stingless bees (Meliponinae) and Apis from two regions of Tocantins Brazil. Acta Amaz. 46, 61–68.

Araújo, M.J., Bosco, S.M., Sforcin, J.M., 2016b. Pythium insidiosum: inhibitory effects of propolis and geopropolis on hyphal growth. Braz. J. Microbiol. 47, 863–869.

Araújo, M.J.A.M., Búfalo, M.C., Conti Jr., B.J., Trusheva, A.F., Bankova, B., Sforcin, V.J.M., 2015. The chemical composition and pharmacological activities of geopropolis produced by *Melipona fasciculata* Smith in Northeast Brazil. J. Mol. Pathophysiol. 4, 12–20.

Araújo, M.J.A.M., Mattar, N.S., Reis, A.S., Serra, I.C.P.B., Fialho, E.M.S., Assunção, A.K.M., Dutra, R.P., Nogueira, A.M.C., Libério, S.A., Guerra, R.N.M., Lopes, A.S., Ribeiro, M.N.S., Nascimento, F.R.F., 2011. Pharmacognostic and acute toxicological evaluation of Scaptotrigona aff. postica propolis extract in pre-clinical assays. Nat. Prod. Res. 25, 1037–1046.

Arora, T., Mehta, A.K., Joshi, V., Mehta, K.D., Rathor, N., Mediratta, P.K., Sharma, K.K., 2011. Substitute of animals in drug research: an approach towards fulfillment of 4R's. Indian J. Pharm. Sci. 73, 1–6.

Bankova, V., Popova, M., Trusheva, B., 2014. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem. Cent. J. 8, http://dx.doi.org/10.1186/1752-153X-8-28.

Bankova, V.R., Christov, R., Marcucci, M.C., Popov, S., 1998. Constituents of Brazilian geopropolis Z. Naturforsch C. 53, 402–406.

Bankova, V.R., Christov, R., Popov, S., Marcucci, M.C., Tsvetkova, I., 1999. Antibacterial activity of essential oils from Brazilian propolis. Fitoterapia 70, 190–193.

Barth, O.M., Luz, C.F.P.D., 2003. Palynological analysis of Brazilian geopropolis sediments. Grana 42, 121–127.

Bartolomeu, A.R., Frión-Herrera, Y., da Silva, L.M., Romagnoli, G.G., de Oliveira, D.E., Sforcin, J.M., 2016. Combinatorial effects of geopropolis produced by *Melipona fasciculata* Smith with anticancer drugs against human laryngeal epidermoid carcinoma (HEp-2) cells. Biomed. Pharmacother. 81, 48–55.

Boekelheide, K., Brent, R., Charnley, G., Cheung Jr.S.G., V.G., 2015. HHS Public Access 13, 51–138.

Bonamigo, T., Campo, J.F., Oliveira, A.S., Torquato, H.F.V., Balestieri, J.B.P., Cardoso, C.A.L., Paredes-Gamero, E.J., Souza, K.P., Santos, E.L., 2017b. Antioxidant and cytotoxic activity of propolis of *Plebeia droryana* and *Apis mellifera* (Hymenoptera Apidae) from the Brazilian Cerrado biome. PLOS ONE 12, e0183983.

Bonamigo, T., Campos, J.F., Alfredo, T.M., Balestieri, J.B.P., Cardoso, C.A.L., Paredes-Gamero, E.J., Souza, K.P., Santos, E.L., 2017a. Antioxidant, cytotoxic, and toxic activities of propolis from two native bees in Brazil: *Scaptotrigona depilis* and *Melipona quadrifasciata* anthidioides. Oxid. Med. Cell Longev., http://dx.doi.org/10.1155/2017/20171038153.

Campos, J.F., dos Santos, U.P., da Rocha, P.S., Damião, M.J., Balestieri, J.B.P., Cardoso, C.A.L., Paredes-Gamero, E.J., Estevinho, L.M., Souza, K.P., Santos, E.L., 2015. Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless bee *Tetragonisca fiebrigi* (Jataí) Evid. -Based Compl. Alt. Med., http://dx.doi.org/10.1155/2015/2015296186.

Campos, J.F., dos Santos, U.P., Macorini, L.F.B., Mestriner, A.M., Balestieri, J.B.P., Gamero, E.J.P., Cardoso, C.A.L., Souza, K.P., dos Santos, E.L., 2014. Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (Hymenoptera, Apidae). Food Chem. Toxicol. 65, 374–380.

Campos, V.A.C., Júnior, S., Oliveira, D.F., Carvalho, H.W.P.D., Machado, A.R.T., Tirelli, A.A., 2011. Antibacterial activity of propolis produced by *Frieseomelitta varia*. Cienc. Agrotecnol. 35, 1043–1049.

Cao, X.P., Chen, Y.F., Zhang, J.L., You, M.M., Wang, K., Hu, F.L., 2017. Mechanisms underlying the wound healing potential of propolis based on its in vitro antioxidant activity. Phytomedicine 34, 76–84.

Cisilotto, J., Sandjo, L.P., Faqueti, L.G., Fernandes, H., Joppi, D., Biavatti, M.W., Creczynski-Pasa, T.B., 2017. Cytotoxicity mechanisms in melanoma cells and UPLC-QTOF/MS2 chemical characterization of two Brazilian stingless bee propolis: the uncommon presence of piperidinic alkaloids. J. Pharm. Biomed. Anal. 149, 502–511.

Coelho, G.R., Mendonça, R.Z., Vilar, K.S., Figueiredo, C.A., Badari, J.C., Taniwaki, N., Namiyama, G., Oliveira, M.I., Curti, S.P., Silva, P.E., Negri, G., 2015. Antiviral action of hydromethanol extract of geopropolis from *Scaptotrigona postica* against Antiherpes Simplex Virus (HSV-1). Evid. -Based Compl. Alt. Med., http://dx.doi.org/10.1155/2015/296086.

Cunha, M.G., Franchin, M., Galvão, L., de Ruiz, A., de Carvalho, J.E., Ikegaki, M., Alencar, S.M., Koo, H., Rosalen, P.L., 2013. Antimicrobial and antiproliferative activities of stingless bee *Melipona scutellaris* geopropolis. BMC Complement. Altern. Med. 13, 13–23.

Cunha, M.G., Rosalen, P.L., Franchin, M., de Alencar, S.M., Ikegaki, M., Ransom, T., Beutler, J.A., 2015. Antiproliferative constituents of geopropolis from the bee *Melipona scutellaris*. Planta Med. 82, 190–194.

de Souza, S.A., da Silva, T.M.G., da Silva, E.M.S., Camara, C.A., Silva, T.M.S., 2018. Characterization of phenolic compounds by UPLC-QTOF-MS/MS of geopropolis from the stingless bee *Melipona subnitida* (jandaíra). Phytochem. Anal. 29, 549–558.

Deeb, O., Goodarzi, M., 2012. In silico quantitative structure toxicity relationship of chemical compounds: some case studies. Curr. Drug Saf. 7, 289–297.

dos Santos, L., Hochheim, S., Boeder, A.M., Kroger, A., Tomazzoli, M.M., Dal Pai Neto, R., Maraschin, M., Guedes, A., de Cordova, C.M.M., 2017. Chemical characterization, antioxidant, cytotoxic and antibacterial activity of propolis extracts and isolated compounds from the Brazilian stingless bees *Melipona quadrifasciata* and *Tetragonisca angustula*. J. Apic. Res. 56, 543–558.

Duthie, G.G., Gardner, P.T., Kyle, J.A., 2003. Plant polyphenols: are they the new magic bullet? Proc. Nutr. Soc. 62, 599–603.

Dutra, R.P., Abreu, B.V.B., Cunha, M.S., Batista, M.C.A., Torres, L.M.B., Nascimento, F.R.F., Ribeiro, M.N.S., Guerra, R.N.M., 2014. Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee *Melipona fasciculata* Smith. J. Agric. Food Chem. 62, 2549–2557.

Fang, Y., Yang, S., Wu, G., 2002. Free radicals, antioxidants and nutrition. Nutrition 18, 872–879.

Felipe Neto, C.A.L., 2015. Influence of landscape structure on production and quality of honey bee Jandaira (Melipona subnitida, Apidae: Meliponini) in the Caatinga. MSc. Dissertation, Universidade Federal Rural do Semi-Árido. Mossoró 62.

Fernandes Jr., A., Leomil, L., Fernandes, A.A.H., Sforcin, J.M., 2001. The antibacterial activity of propolis produced by *Apis mellifera* L. and Brazilian stingless bees. J. Venom. Anim. Toxins 7, 173–182.

Ferreira, J.M., Fernandes-Silva, C.C., Salatino, A., Message, D., Negri, G., 2018. Antioxidant activity of a geopropolis from Northeast Brazil: chemical characterization and likely botanical origin. Evid. -Based Compl. Alt. Med., 10.1155/2017/402472.

Fianco, A.L.B., Falcão, M.A., Cassel, E., Milão, D., 2013. Determinação da atividade antimicrobiana e teor de polifenóis totais de extratos etanólicos de própolis das abelhas sem ferrão Tetragonisca angustula (Jataí) e Scaptotrigona bipunctata (Tubuna). Rev. Liberato. 14, 1–112.

Freitas, M.O., Ponte, F.A.F., Lima, M.A.S., Silveira, E.R., 2008. Flavonoids and triterpenes from the nest of the stingless bee *Trigona spinipes*. J. Braz. Chem. Soc. 19, 532–535.

Gastauer, M., Campos, L.A.O., Wittmann, D., 2011. Handling sticky resin by stingless bees (Hymenoptera Apidae). Rev. Bras. Entomol. 55, 234–240.

Guzmán-Gutiérrez, S.L., Nieto-Camacho, A., Castillo-Arellano, J.I., Huerta-Salazar, E., Hernández-Pasteur, G., Silva-Miranda, M., Argüello-Nájera, O., Sepúlveda-Robles, O., Espitia, C.I., Reyes-Chilpa, R., 2018. Mexican propolis: a source of antioxidants and anti-inflammatory compounds, and isolation of a novel chalcone and ε-caprolactone derivative. Molecules 23, E334.

Hrncir, M., Jarau, S., Barth, F.G., 2016. Stingless bees (Meliponini): senses and behavior. J. Comp. Physiol. A. Neuroethol. Sens. Neural Behav. Physiol. 202, 597–601.

Jaffé, R., Pope, N., Torres Carvalho, A., Madureira Maia, U., Blochtein, B., de Carvalho, C.A., Carvalho-Zilse, G.A., Freitas, B.M., Menezes, C., Ribeiro, M.F., Venturieri, G.C., Imperatriz-Fonseca, V.L., 2015. Bees for development: Brazilian survey reveals how to optimize stingless beekeeping. PLoS ONE 10, e0121157.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., Popov, S., 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. J. Ethnopharmacol. 64, 235–240.

Kustiawan, P.M., Phuwapraisirisan, P., Puthong, S., Palaga, T., Arung, E.T., Chanchao, C., 2015. Propolis from the stingless bee *Trigona incisa* from East Kalimantan Indonesia, induces in vitro cytotoxicity and apoptosis in cancer cell lines. Asian Pac. J. Cancer. Prev. 16, 6581–6589.

Landim, C.D.C., 2009. Abelhas: morfologia e função de sistemas, first ed. Unesp, São Paulo.

Lee, S.X., Boontaveeyuwat, E., Thaiwat, S., Rustin, M., McFadden, J., 2018. Severe allergic contact dermatitis mimicking angioedema caused by propolis used as a traditional remedy. Contact Dermatitis 79, 185–186.

Liberio, S.A., Pereira, A.L., Dutra, R.P., Reis, A.S., Araújo, M.J., Mattar, N.S., Silva, Ribeiro, M.N., Nascimento, F.R., Guerra, R.N., Monteiro-Neto, V., 2011. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee *Melipona fasciculata* Smith. BMC Complement. Altern. Med. 11, 108–117.

López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal. Chim. Acta 763, 1–10.

Maia Filho, E.M., Maia, C., de, C.R., Bastos, A.C.S.C., Novais, T.M.G., 2008. Efeito antimicrobiano in vitro de diferentes medicações endodônticas e própolis sobre *Enterococcus faecalis*. RGO 56, 21–25.

Mercan, M., Kivrak, B., Duru, M.E., Katircioglu, H., Gulcan, S., Malci, S., Acar, G., Salih, B., 2006. Chemical composition effects onto antimicrobial and antioxidant activities of propolis collected from different regions of Turkey. Ann. Microbiol. 56, 373–378.

Michener, C.D., 2013. The Meliponini. In: Vit, P., Pedro, S.R.M., Roubik, D. (Eds.), Pot-honey. Springer, New York, pp. 3–17.

Miorin, P.L., Levy Junior, N.C., Bretz, A.R., Marcucci, C.W.A.M.C., 2003. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. J. Appl. Microbiol. 95, 913–920.

Mishra, K., Ojha, H., Chaudhury, N.K., 2012. Estimation of antiradical properties of antioxidants using DPPH - assay: A critical review and results. Food Chem. 130, 1036–1043.

Molnár, S., Mikuska, K., Patonay, K., Sisa, K., Daood, H.G., Némedi, E., Kiss, A., 2017. Comparative studies on polyphenolic profile and antimicrobial activity of propolis samples selected from distinctive geographical areas of Hungary. Food Sci. Technol. Int. 23, 349–357.

Mushtaq, S., Das, Y.K., Aksoy, A., 2018. Alternative methods to animal experiments Turkiye Klinikleri. J. Med. Sci. 38, 161–170.

Parasuraman, S., 2011. Toxicological screening. J. Pharmacol. Pharmacother. 2, 74–79.

Parthasarathi, R., Dhawan, A., 2018. In silico approaches for predictive toxicology. In: Dhawan, A., Kwon, S. (Eds.), In Vitro Toxicology. Academic Press, pp. 91–109.

Patricio, E.F.L.R.A., Cruz-López, L., Maile, R., Tentschert, J., Jones, G.R., Morgan, E.D., 2002. The propolis of stingless bees: terpenes from the tibia of three Frieseomelitta species. J. Insect. Physiol. 48, 249–254.

Pedro, S.R., 2014. The stingless bee fauna in Brazil (Hymenoptera: Apidae). Sociobiology 61, 348–354.

Pereira, A.S., Bicalho, B., Neto, F.R.A., 2003. Comparison of propolis from *Apis mellifera* and *Tetragonisca angustula*. Apidologie 34, 291–298.

Peter, C.M., Picoli, T., Zani, J.L., Latosinski, G.S., Lima, M., Vargas, G.D., Hübner, S.O., Fischer, G., 2017. Atividade antiviral e virucida de extratos hidroalcoólicos de própolis marrom, verde e de abelhas Jataí (*Tetragonisca angustula*) frente ao herpesvírus bovino tipo 1 (BoHV-1) e ao vírus da diarreia viral bovina (BVDV). Pesq. Vet. Bras. 37, 667–675.

Raies, A.B., Bajic, V.B., 2016. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip. Rev. Comput. Mol. Sci. 6, 147–172.

Rajabi, S., Ramazani, A., Hamidi, M., Naji, T., 2015. Artemia salina as a model organism in toxicity assessment of nanoparticles. Daru 23, http://dx.doi.org/10.1186/s40199-015-0105-x.

Ranganatha, N., Kuppast, I.J., 2012. A review on alternatives to animal testing methods in drug development. Int. J. Pharm. Pharm. Sci. 4, 28–32.

Raunio, H., 2011. In silico toxicology - non-testing methods. Front. Pharmacol. 2, http://dx.doi.org/10.3389/fphar.2011.00033.

Santos, H.F.D., Campos, J.F., Santos, C.M.D., Balestieri, J.B.P., Silva, D.B., Carollo, C.A., de Picoli Souza, K., Estevinho, L.M., dos Santos, E.L., 2017a. Chemical profile and antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities of geopropolis from the stingless bee *Melipona orbignyi*. Int. J. Mol. Sci. 18, E953.

Santos, L., Hochheim, S., Boeder, A.M., Kroger, A., Tomazzoli, M.M., Neto, R.D.P., Maraschin, M., Guedes, A., Cordova, C.M.M., 2017b. Chemical characterization, antioxidant, cytotoxic and antibacterial activity of propolis extracts and isolated compounds from the Brazilian stingless bees *Melipona quadrifasciata* and *Tetragonisca angustula*. J. Apic. Res. 56, 543–558.

Santos, T.L.A., Queiroz, R.F., Sawaya, A.C.H.F., Lopez, B.G.C., Soares, M.B.P., Bezerra, D.P., Rodrigues, A.C.B.C., de Paula, V.F., Waldschmidt, A.M., 2017c. Melipona mondury produces a geopropolis with antioxidant, antibacterial and antiproliferative activities. An. Acad. Bras. Cienc. 89 (3 Suppl), 2247–2259.

Sawaya, A.C.H.F., Calado, J.C.P., dos Santos, L.C., Marcucci, M.C., Akatsu, I.P., Soares, A.E.E., Abdelnur, P.V., Cunha, I.B.S., Eberlin, M.N., 2009. Composition and antioxidant activity of propolis from three species of Scaptotrigona stingless bees. J. Apiproduct Apimedical Sci. 1, 37–42.

Sawaya, A.C.H.F., Cunha, I.B.S., Marcucci, M.C., Aidar, D.S., Silva, E.C.A., Carvalho, C.A.L., Eberlin, M.N., 2007. Electrospray ionization mass spectrometry fingerprinting of propolis of native Brazilian stingless bees. Apidologie 38, 93–103.

Sawaya, A.C.H.F., Cunha, I.B.S., Marcucci, M.C., Rodrigues, R.F.O., Eberlin, M.N., 2006. Brazilian propolis of *Tetragonisca angustula* and *Apis mellifera*. Apidologie 37, 398–407.

Sawaya, A.C.H.F., da Silva Cunha, I.B., Marcucci, M.C., 2011. Analytical methods applied to diverse types of Brazilian propolis. Chem. Cent. J. 5.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - A review. J. Funct Foods. 18B, 820–897.

Silva, E.C.C., Muniz, M.P., Nunomura, R.D.C.S., Nunomura, S.M., Zilse, G.A.C., 2013. Phenolic constituents and antioxidant activity of geopropolis from two species of Amazonian stingless bees. Quim. Nova 36, 628–633.

Souza, E.C.A., da Silva, E.J.G., Cordeiro, H.K.C., Filho, N.M.L., da Silva, F.M.A., dos Reis, D.L.S., Porto, C., Pilau, E.J., da Costa, L.A.M.A., de Souza, A.D.L., Menezes, C., Flachauim, A., 2018b. Chemical compositions and antioxidant and antimicrobial activities of propolis produced by *Frieseomelitta longipes* and *Apis mellifera* bees. Quim. Nova 41, 485–491.

Souza, S.A., Camara, C.A., Silva, E.M.S., Silva, T.M.S., 2013. Composition and antioxidant activity of geopropolis collected by *Melipona subnitida* (Jandaíra) bees. Evid. -Based Compl. Alt. Med., http://dx.doi.org/10.1155/2013/801383.

Torres, A.R., Sandjo, L.P., Friedemann, M.T., Tomazzoli, M.M., Maraschin, M., Mello, C.F., Santos, A.R.S., 2018. Chemical characterization, antioxidant and antimicrobial activity of propolis obtained from *Melipona quadrifasciata quadrifasciata* and *Tetragonisca angustula* stingless bees. Braz. J. Med. Biol. Res. 51, 1–10.

Valente, M.J., Baltazar, A.F., Henrique, R., Estevinho, L., Carvalho, M., 2011. Biological activities of Portuguese propolis: protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth in vitro. Food Chem. Toxicol. 49, 86–92.

Valerio, L.G., 2009. In silico toxicology for the pharmaceutical sciences. Toxicol. Appl. Pharmacol. 241, 356–370.

Velikova, M., Bankova, V., Marcucci, M.C., Tsvetkova, I., Kujumgiev, A., 2000a. Chemical composition and biological activity of propolis from Brazilian Meliponinae. Z. Naturforsch C. 55, 785–789.

Velikova, M., Bankova, V., Tsvetkova, I., Kujumgiev, A., Marcucci, M.C., 2000b. Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. Fitoterapia 71, 693–696.

Villas-Bôas, J., 2012. Manual Tecnológico: mel de abelhas sem ferrão, first ed. Instituto Sociedade, População e Natureza (ISPN). Brasília (DF), Brasil.

Zeng, Z., Jiang, J., 2010. Analysis of the adverse reactions induced by natural product-derived drugs. Br. J. Pharmacol. 159, 1374–1391.

Zhu, W., Chen, M., Shou, Q., Li, Y., Hu, F., 2011. Biological activities of Chinese propolis and Brazilian propolis on streptozotocin-induced type 1 diabetes mellitus in rats. Evid. -Based Compl. Alt. Med., 10.1093/ecam/neq025.