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Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening

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A B S T R A C T

The aim of this study was to investigate the effect of the dry and rainy season on the antibacterial activity and chemical composition of the Brazilian red propolis. The samples were collected in rainy (RP-PER) and dry (RP-PED) seasons and analyzed by HPLC-DAD. The extracts were tested alone and in association with antibiotics against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The HPLC analysis identified luteolin and quercetin as the main compounds. Seasonal variation was observed according to concentrations of the compounds. The MIC values against *E. coli* ranged from 128 μg/mL to 512 μg/mL (EC 06 and EC ATCC). The red propolis showed MIC values of 512 μg/mL against both strains of *P. aeruginosa* used in our study (PA03 and PA24) and against strains of Gram-positive bacteria *S. aureus* the MICs ranged from 64 μg/mL to ≥1024 μg/mL (SA10). A synergistic effect was observed when we combined the RP-PED with gentamicin against all the strains tested. When we combined the RP-PED with Imipenem, we only observed synergistic effect against *P. aeruginosa*. According to our synergistic activity results, the utilization of red propolis collected in the drier periods can be used as an adjuvant against multiresistant bacterial infections.

1. Introduction

Propolis is a resinous mixture produced by honeybees for various purposes. It is used to avoid intruders by protecting the entrance of the hive and sealing the holes; to prevent contamination inside the hive by bacteria, viruses or parasites because of its antiseptic effect; as well as to cover intruders who died inside the hive in order to avoid their decomposition (Salatino et al., 2005; Righi et al., 2010). Many plant constituents are found in propolis composition e.g. plant resins, pollen and bud excretions. Additionally, it is also found bee products as beewax and bees secretion, so it has been considered an organotherapy product (Daugsch et al., 2007; Mendonça et al., 2015; Anvisa, 2005). After collecting the plant parts, the resin is chewed, then salivary enzymes are added and the partially digested material is mixed with beeswax (Burdock, 1998).

In Brazil, we have 13 groups of propolis based on physiochemical characteristics (Park et al., 2000; Daugsch et al., 2007). The characteristic of each different type of propolis is dependent of some factors as e.g. plant source and edaphoclimatic conditions (Silva et al., 2015). The most recent type of propolis discovered in northeast Brazil has a reddish color and is located in beehives along the river shores (Daugsch et al., 2007). The Brazilian red propolis is found in the states of Alagoas, Paraíba, Pernambuco, Sergipe, Bahia and Roraima (Daugsch et al., 2007; López et al., 2014). However, red propolis can also be found in other countries as Cuba, China, Mexico and Venezuela (Trusheva et al., 2004; Daugsch et al., 2007; Izuta et al., 2009; Lotti et al., 2010; Piccinelli et al., 2011). One species has been suggested to be the botanical source for the Brazilian red propolis: *Dalbergia ecastophyllum*. However, in other countries, other botanical sources are associated to red propolis as *Clusia major*, *C. minor* and *C. scrobiculata* in Venezuela and *C. rosea* in Cuba

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(Tomas-Barberan et al., 1993; Cuesta-Rubio et al., 2002; Trusheva et al., 2004, 2006).

Previous studies have reported many biological activities for the Brazilian red propolis like antioxidant (Alencar et al., 2007; Righi et al., 2010; Frozza et al., 2013), antimicrobial (Bispo-Junior et al., 2012; Cabral et al., 2009; Righi et al., 2010), anticancer (Awale et al., 2008; Li et al., 2008), anti-inflammatory (Bueno-Silva et al., 2013; Cavendish et al., 2015), cytotoxicity (Alencar et al., 2007), repair of wounds (Batista et al., 2012) and antinociceptive (Cavendish et al., 2015). These properties are attributed to the phenolic compounds, which are present in high amount in Brazilian red propolis, but the substances involved in those biological activities are still being researched. Some studies showed that isoflavones found in red propolis have a participation in many activities as e.g. antimicrobial, antifungal, anticancer and antioxidant (Alencar et al., 2007; Freires et al., 2016). Flavanones, isoflavones, chalcones and pterocarpans have displayed important activity against cancer cell lines (Li et al., 2008). Neovestitol and vestitol isolated from Brazilian red propolis inhibited neutrophil migration in mouse thus showing an anti-inflammatory activity (Bueno-Silva et al., 2013).

In the last decade, many researchers focused their efforts on the discovery of chemicals that participates in the composition of the Brazilian Red Propolis. Among the red propolis samples from the states of Alagoas, Paraíba and Sergipe, it has been found flavones, isoflavones, flavonols, chalcones, aurones, pterocarpans and xanthones (Awale et al., 2008; Li et al., 2008; López et al., 2014; Mendonça et al., 2015). However, some chemical compounds are more frequently found in those samples and between them, there are formonetin, biochanin A, quercetin, pinocembrin, daidzein, medicarpin, homopterocarpin and liquiritigenin (Alencar et al., 2007; Cabral et al., 2009; Zulueta et al., 2009; Righi et al., 2010; Piccinelli et al., 2011; Oldoni et al., 2011; Frozza et al., 2013; Cavendish et al., 2015; Lopez et al., 2015). These major compounds are also present in the exudate of *Dalbergia ecastophyllum*, the mean source for the production of red propolis in the Northeast of Brazil. On the other hand, López et al. (2014) showed that probably a second plant species participates as one of the mean source of resins for the red propolis in Brazil.

The red propolis of some Northeastern Brazilian states (e.g. Alagoas, Paraíba and Sergipe) have been tested for antibacterial activity against Gram-negative bacteria – *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebisiella pneumoniae*, *Escherichia coli*, *Proteus mirablis*, *P. morganii*, *Neisseria meningitides*, *Haemophilus influenzae* and *Enterobacter aerogenes* – (Righi et al., 2010; Lopez et al., 2015) and Gram-positive bacteria – *Bacillus subtilis*, *Enterococcus faecalis*, *Estreptococcus pyogens*, *Staphylococcus aureus* and *S. epidermidis* (Alencar et al., 2007; Cabral et al., 2009; Righi et al., 2010; Lopez et al., 2015). Trusheva et al. (2006) attributed the antibacterial activity found in the Brazilian red propolis to the flavonoids, especially isoflavonoids and prenilated benzophenones. However, studies about the antimicrobial activity of the red propolis from other Brazilian states remains very scarce.

Resistance of microorganism to commercial antimicrobials drugs has been reported in the literature, so scientists all over the world are gathering efforts to find an alternative way to treat resistant microbial infections in humans diseases (Mandal et al., 2010; Eumkeb et al., 2012). The association between synthetic antimicrobial agents and natural substances have been studied and some combinations have had synergistic effect to inhibit bacterial growing (Matias et al., 2010; Hassan et al., 2016). Propolis has been combined with some kind of drugs, e.g. anticancer (Guo et al., 2015), antibacterial (Wojtyczka et al., 2013), antifungal (Pippi et al., 2015) and in some studies, it was observed a synergistic effect (Fernandes et al., 2005; Wojtyczka et al., 2013). The association of Brazilian red propolis with antifungals showed synergistic effect against *Candida* sp, however studies with red propolis and antimicrobials agents remains scarce (Pippi et al., 2015).

The major effect that can affect the chemical composition of propolis is the geographical location. Every place on earth has its own particularities concerning to the diversity of plants, climate, soil, and these factors together strongly influences the chemical composition of propolis (Teixeira et al., 2008; Sampaio et al., 2016). However, seasonality is also an important factor that can determine the propolis composition due to the influence of phenological factors in biosynthesis of plant metabolites (Teixeira et al., 2008). Seasonal variation of chemical compounds from propolis were previously described for Brazilian propolis, but was not observed a significant variation for antibacterial activity (Sforcin et al., 2000; Jorge et al., 2008; Teixeira et al., 2008).

The Northeast of Brazil has two seasons (drier and rainy) that are very distinct between them and one of these differences comprises the amount of rain during the year (APAC, 2016). Accordingly, these seasonal variations, as mentioned above, can influence the biosynthesis of plant metabolites and consequently affect the resin that is secreted by plants, which is used by the honeybees to produce propolis (Teixeira et al., 2008). Therefore, we aimed to investigate the effect of the dry and rainy season on the antibacterial activity and chemical composition of the Brazilian red propolis to bring the possibility whether a better antibacterial activity could be assessed for the red propolis alone or in association with standard drugs.

2. Material and methods

2.1. Propolis sampling and processing

Red propolis produced by Africanized *Apis mellifera* was collected in apiaries located in Tamandaré, state of Pernambuco. Those samples were collected in two moments: rainy season (between April and august of 2014) and dry season (between December/2015 and March/2016). Those periods are historically known by have the higher and the lowest precipitation in the state of Pernambuco (APAC, 2016), respectively. Raw red propolis from Pernambuco Rainy (RP-PER) and Dry (RP-PED) season were stored at −20 °C and then the propolis samples were dissolved in hydroethanolic solution (ethanol 54%) for 72 h. Afterward, the extract was filtered and concentrated using a rotary vacuum evaporator (model Q-344B-Quimis, Brazil). The concentrated solution was frozen and then lyophilized to obtain a fine powder of the red propolis hydroethanolic extract. For the microbiological assays, the dry extract of red propolis was solubilized in dimethyl sulfoxide (DMSO) and afterward, the DMSO-red propolis solution was diluted in sterile water to reach the concentration of 1024 μg/mL.

2.2. Chemical, apparatus and general procedures for HPLC-DAD

All chemical were of analytical grade. Acetonitrile, methanol, phosphoric acid, chlorogenic acid, caffeic acid, *p*-coumaric acid and ellagic acid were purchased from Merck (Darmstadt, Germany). Quercetin, apigenin, vitexin, rutin and luteolin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). The standards vitexin and rutin were used only for RP-PER samples and the p-coumaric acid for the RP-PED samples. High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.