Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev Ciênc Farm Básica Apl., 2011;32(3):349-356
ISSN 1808-4532

Antioxidant and antimicrobial activities of propolis and açai (*Euterpe oleracea* Mart) extracts

Gonçalves, G.M.S.[1*], Santos, N.P.[1], Srebernich, S.M.[1]

[1]Faculty of Pharmaceutical Sciences, Pontifical Catholic University of Campinas, Campinas, SP, Brazil

Recebido 09/05/2011 / Aceito 10/10/2011

ABSTRACT

Propolis is a natural resin, collected mainly by the honey bee, *Apis mellifera*, which has been shown to have many biological roles, including antioxidant and antimicrobial effects, both conferred by phenolic compounds, especially flavonoids. The fruit of the açai palm is an important source of anthocyanins, which are also phenolics of the flavonoid group. The aim of this study was to assess the antioxidant and antimicrobial effects of a mixture of propolis and açai extracts. Antimicrobial activity was assessed by BHI broth culture with diluted extract, followed by agar subculture, while antioxidant activity was assessed by the DPPH radical scavenging assay. The results showed that around 2% of the propolis extract showed antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis*, while low concentrations of both the ethanolic propolis and aqueous açai extracts, alone and combined, exhibited antioxidant activity. In conclusion, this study showed that the antioxidant effects of propolis and açai were summed in the mixed extracts. Furthermore, this combination would show antimicrobial activity if the minimum inhibitory concentration of propolis extract established in this study were used in the formulation. Hence, these extracts could be mixed into formulations used topically to prevent skin aging and, possibly, disorders caused by microbes, such as acne.

Keywords: Propolis. Açai. Flavonoids. Antimicrobial. Antioxidant. DPPH radical.

INTRODUCTION

The search for new active substances for food, cosmetic and dermatological products and the scientific clarification of the benefits really conferred by these substances are very important for those manufacturing sectors nowadays. Considering the global trend towards sustainable development, which involves environmentally friendly, economically viable, socially just and culturally accepted production methods, as well as the importance of self care for quality of life, the use of natural active ingredients, such as açai and propolis, in skin formulations is of great interest.

Propolis is a natural resin collected mainly by the bee *Apis mellifera*. It is lipophilic, hard and fragile at room temperature and soft, malleable and sticky when heated. It is extracted by the bees from plants in the vicinity of their beehives and used to repair honeycombs and maintain the asepsis of the beehive, preventing the decomposition of organisms that die inside the beehive (Marcucci, 1995; Salatino et al., 2005; Sawaya et al., 2002).

The composition of propolis varies according to the plant species visited by the bees, substances metabolized and secreted by the bees and other components that are incorporated during its production. Despite this variability, in general all samples have antimicrobial activity, since this is the function of propolis in beehives (Marcucci, 1995; Sawaya et al., 2002)

Many studies have shown that propolis has a number of biological roles, including antioxidant and antimicrobial activity, both conferred mainly by substances belonging to the group of phenolic compounds, especially flavonoids, which make propolis an important object of study for the most diverse pharmaceutical applications, such as anti-aging and anti-acne cosmetics (Cabral et al., 2009; Havsteen, 2002; Marcucci, 1995; Salatino et al., 2005; Sawaya et al., 2002; Teixeira et al., 2010).

Açai (*Euterpe oleracea* Mart) has traditionally been cultivated in floodplains but, more recently, it is also being cultivated on dry land in the north of Brazil. The açai palm is one of the most promising palm trees because of its economic importance to small farmers and extractivists and regional industries (Homma et al., 2006). Açai is a great source of anthocyanins, phenolic compounds belonging to the group of flavonoids, which are widespread in nature and confer the colors orange, red and blue to many plant structures. Anthocyanins are not only important natural pigments, but also contain proven antioxidant properties and are very beneficial to health. However, açai needs to be stored properly to preserve its antioxidant activity, since anthocyanins are unstable when exposed to light and

Corresponding Author: Gisele Mara Silva Gonçalves - Faculdade de Ciências Farmacêuticas - PUC de Campinas - Av. John Boyd Dunlop, s/n - Jardim Ipaurussurama - CEP.13059-900 – Campinas SP - Brazil - tel.: +55 19 33436865 - fax: +55 19 33436888 - e-mail:gmsg@puc-campinas.edu.br

freezing temperatures (Castañeda-Ovando et al., 2009; Del Pozo-Insfran et al., 2004; Kuskoski et al., 2006; Pacheco-Palencia et al., 2007; Pacheco-Palencia & Talcott, 2010; Sanabria & Sangronis, 2007; Wang et al., 1996).

The use of a combination of açai and propolis extracts in food preparations, nutraceuticals or cosmetic anti-aging formulations may be promising. Hence, the objective of this study was to assess the antioxidant and/or antimicrobial activities of propolis and açai extracts separately and combined.

MATERIAL AND METHODS

Material

Ripe açai fruits were harvested in the historical town of Igarapé-Miri (the "world capital" of açai production, 78 km from Belém, state of Pará, north Brazil) and processed as described by Tonon et al., (2010). Crude green propolis resin was obtained from the state of Minas Gerais, southeast Brazil.

Extraction of açai and propolis

Briefly, açai extract was prepared as follows: an aqueous solution of 0.1% hydrochloric acid and 0.1% sodium bisulfite was used as the extraction liquid for antimicrobial activity tests, whereas sterile, deionized water was used for the assay of antioxidant activity. All materials were previously disinfected with 70% ethanol.

The pulp of açai berries (*Euterpe oleracea*) was placed in a homogenizer with one of the extraction solutions mentioned above. The crude extract was filtered through a nylon net and then centrifuged to remove any solid residues that were still in suspension. The extract obtained was stored in a hermetically sealed amber glass bottle under refrigeration at 4ºC until use.

Propolis was ground and placed in a cellulose thimble sealed with filter paper and refluxed with absolute ethanol for 24h in a Soxhlet extractor. A total of 20g of propolis was used for 400mL of ethanol. After reflux, the extract was filtered and stored in an amber bottle at room temperature. All materials were previously disinfected with 70% ethanol (Araujo et al., 2002)

Assessment of antimicrobial activity – growth in liquid medium

The culture media used were brain-heart infusion (BHI) broth (HIMEDIA), tryptic soy agar (TSA) and potato dextrose agar (PDA) (Difco™). Triclosan was used as the reference antimicrobial agent. The selected microorganisms were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 13525), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231).

The inoculum was prepared as instructed by Sawaya et al. (2002), with modifications. A loopful of a colony was transferred to 5mL of BHI broth, which was then incubated at 37ºC for 18-24h. Next, sterile saline was added to the suspension, to adjust the turbidity to 0.5 on the McFarland scale (roughly 1 x 10^6 cells/mL).

The next step consisted of observing growth inhibition in BHI broth culture, followed by TSA/potato agar subculture where required, as instructed by Kalemba and Kunicka (2003), with modifications: 500µL of various concentrations of the açai and/or propolis extracts and 100µL of the suspended inoculum were added to 5mL of BHI broth in sterile test tubes. The mixtures were vortexed and incubated at 35°C for 24h for the bacteria or 25°C for 24/48 h for the yeast. The tubes were then monitored for microbial growth (turbidity). If the broth became turbid immediately after addition of the extract, a loopful was streaked on TSA agar (for bacteria) and potato agar (for yeasts) after the incubation period. These plates were then incubated as for the broth and inspected for the presence or absence of colonies. Ethanol (500mL) was used as the negative control in place of the extract. The positive control consisted of 500µL of triclosan in ethanol.

Minimum inhibitory concentrations (MICs) were calculated, based on the concentration of each extract inside the test tube.

Assessment of antioxidant activity – DPPH radical scavenging

DPPH (2,2–diphenyl–1–picrylhydrazyl) radical was purchased from Sigma-Aldrich (USA). BHT (butylated hydroxytoluene) was used as the reference antioxidant. Absorbance was read with a Varian Cary 50 Bio UV/Vis spectrophotometer.

The antioxidant activity of the extracts was assessed as instructed by Brand-Williams et al., (1995) and Sánchez-Moreno et al. (1998), with modifications.

A standard solution of 60µM DPPH in absolute ethanol was prepared in an amber bottle and used immediately to prepare dilutions at 10 µM, 20 µM, 30 µM, 40 µM, 50 µM and 60 µM, whose absorbance was read in the dark at 515 nm. From these readings, an analytical curve for DPPH was constructed.

The total antioxidant activity (TAA) of propolis or açai extract was then determined. Various concentrations of propolis or açai extract were prepared in triplicate. A solution containing açai and propolis extract combined, each with a final concentration of 0.1%, was also prepared in triplicate, to assess their combined antioxidant activity. Aliquots of 0.1mL of each concentration of each extract were transferred, in the dark, to test tubes containing 3.9mL of 60 µM DPPH solution and homogenized by vortexing. Absolute ethanol (0.1mL of absolute ethanol and 3.9mL of 60 µM DPPH solution) was used as the negative control and BHT (0.1mL BHT in ethanol and 3.9mL of 60µM DPPH solution) as the positive control. All tubes were incubated at 28°C for 30 minutes, after which the absorbance was read at 515nm, against absolute ethanol as the blank.

After the reading, the analytical curve for DPPH was used to find the concentration of DPPH in µM, and thence the amount consumed, and the result was converted to g of DPPH, by Equation 1:

*g DPPH = (µM DPPH / 1,000,000)*394.3 (molecular weight of DPPH).* (Eq.1)

It was possible to plot the absorbances of the various concentrations of the extracts on the Y axis and the concentration (mg/L) on the X axis, to determine the equation and slope of the experimental line. The TAA was calculated by replacing the absorbance equivalent to 50% of the initial DPPH concentration by "y", to find the concentration of extract needed to reduce the initial concentration of the DPPH radical by 50% (EC_{50} – mg/L).

The percent antioxidant activity, another way of expressing antioxidant activity, was calculated as follows: % antioxidant activity = [(absorbance of the control – absorbance of the sample)/ absorbance of the control]*100%.

All results were based on the final concentration of each sample in the test tube.

RESULTS

Table 1 shows the antimicrobial activity results.

Table 1. Antimicrobial activity of the extracts against the selected microorganisms.

Test sample	Microorganisms				
	Gram-positive bacteria		Gram-negative bacteria		Yeast
	S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. coli*	*C. albicans*
Ethanol	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Propolis extract 1.07%	(+) (+) (+)	(+) (-) (-)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Propolis extract 1.4%	(+) (+) (+)	(+) (-) (-)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Propolis extract 1.8%	(+) (-) (-)	(-) (-) (-)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Propolis extract 2.14%	(-) (-) (-)	(-) (-) (-)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Propolis extract 7.14%	(-) (-) (-)	(-) (-) (-)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Açai extract 8.9%	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)	(+) (+) (+)
Triclosan 0.02%	(-) (-) (-)	(-) (-) (-)	(+) (+) (+)	(-) (-) (-)	(-) (-) (-)

(+) = presence of microbial growth
(-) = absence of microbial growth.
All experiments were performed in triplicate.

The EC_{50} for antioxidant activity was obtained by plotting the absorbances of the various concentrations of the extracts and the positive control. Linear regression was then used to generate the curves. It was also necessary to construct the DPPH analytical curve. The equation of the straight line fitted to the DPPH curve was y = 0.010570x + 0.000527. The amounts of BHT, propolis extract and açai extract needed to reduce 50% of the initial DPPH (EC_{50}) are presented in Table 2.

Table 2. EC_{50} values obtained for the propolis and açai extracts and the positive control BHT (DPPH method).

Sample	EC_{50} (mg/L)	EC_{50} (g sample/ g DPPH)
Propolis extract	505.14	38.13
Açai extract	1587.75	127.61
BHT	275.35	22.13

Figure 1 shows the percent antioxidant activity of the extracts.

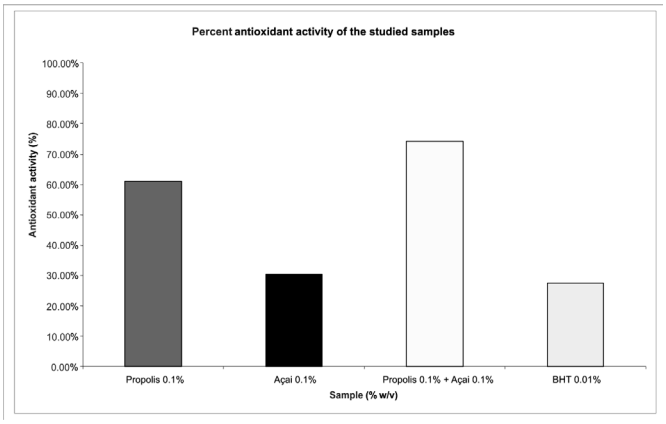

Fig. 1. Percentage of antioxidant activity of propolis and açai extracts alone and combined and the BHT control. Propolis extract at 0.1% showed a high antioxidant effect (60.93%) and açai extract had roughly half this effect (30.53%). The combination of the two extracts produced the best effect (74.30%). BHT had the lowest antioxidant effect (27.60%), at its usual concentration of 0.01%.

DISCUSSION

Antimicrobial activity is generally desired in both therapeutic and cosmetic products, in order to fight microorganisms that cause infections and to combat problems that consumers are trying to minimize or even eliminate. Currently, many mutant pathogenic microorganisms are resistant to a broad range of synthetic antimicrobial agents because of indiscriminate, prolonged and inappropriate use. Thus, natural antimicrobial agents are an attractive option for their greater efficacy and lower cost (Crisan et al., 1995).

The microorganisms selected for this study included skin flora and pathogenic microorganisms. Thus, the initially selected microorganisms were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida albicans*. Later, *E. coli* was included in the study because it is potentially pathogenic and has been used as a standard bacterium in more scientific studies than any other (Fernandes Junior et al., 1997; Fernandes Junior et al., 1994; Gonsales et al., 2006)

The antimicrobial activities of açai and propolis extracts and triclosan differed from each other. Although açai extract contains phenolic compounds in significant amounts, it showed no antimicrobial activity against any of the microorganisms tested in this study. Homma et al. (2006) emphasize the importance of processing açai pulp from its native Amazomian region properly in order to reduce the risk of microbiological contamination, which can occur at the beginning of pulp extraction.

Green propolis extract in ethanol showed antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, with minimum inhibitory concentrations (MIC) of 21.43mg/mL (2.14%) and 17.85mg/mL (1.8%), respectively. However, the same extract showed no antimicrobial activity against the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, or against the yeast *Candida albicans* (Table 1).

The sensitivity of Gram-positive and resistance of Gram-negative bacteria to propolis extract are somewhat expected, since many studies mention this (Brumfitt et al., 1990; Fuentes & Hernandez, 1990; Kujumgiev et al., 1999; Parcker & Luz, 2007; Popova et al., 2005; Rezende et al., 2006; Silici & Kutluca, 2005). However, many other authors observed that propolis extract had some antimicrobial activity against Gram-negative bacteria, at times requiring higher doses than those used for Gram-positive bacteria (Bonhevi et al., 1994; Mirzoeva et al., 1997).

The antimicrobial activity of propolis is higher against Gram-positive bacteria because of the flavonoids and aromatic acids and esters present in the resin. These chemical compounds supposedly act on the structure of Gram-positive bacterial cell walls, but the mechanism of this action is still unknown (Bankova et al., 1999 e Marcucci et al., 2001)

The reason for propolis showing lower antimicrobial activity against Gram-negative bacteria is uncertain. Scientists believe that this is because of the multi-layered structure and higher fat content of the cell wall, which may be more resistant to propolis extract. Although the Gram-negative cell wall is less rigid than that of Gram-positive bacteria, the former is chemically more complex and contains an outer lipopolysaccharide layer. The antimicrobial activity and chemical composition of propolis extract vary according to its origin, which may explain the differing antimicrobial activities found in various studies (Bankova et al., 1999; Christov et al., 1999; Weston et al., 1999).

Propolis extract should have shown antimicrobial activity against the yeast *Candida albicans* but did not. It has been demonstrated that low concentrations of propolis can exhibit antimicrobial activity against *Candida albicans* (Fernandes Junior et al., 1994; Kujumgiev et al., 1999; Salomão et al., 2004; Sforcin et al., 2001). Sawaya et al. (2002) made a comparative analysis of *in vitro* microbiological methods used to test the antimicrobial effect of propolis against *Candida* species and found that the agar dilution method provided clearer results. Thus, in addition to the fact that the propolis resins used in the above-mentioned studies very probably had different origins from that used here and, consequently, different compositions, the method used to assess antimicrobial activity can also affect the results.

Other factors that may cause apparent differences in the antimicrobial activity of propolis observed in different studies are: variation in storage conditions of the resin and extract or in the extraction method, different strains of test bacteria of the same species and the concentration used and other conditions of the microbial growth assessment, such as temperature, culture medium and incubation temperature and time. These factors can lead to significant diversity in the results of similar studies.

Triclosan was used as a positive control because it is considered an antibacterial, antifungal and antiviral agent; however, it did not act against all the studied microorganisms, as it failed to inhibit the growth of the Gram-negative bacterium *P. aeruginosa*. Some strains of this bacterium have been shown to be resistant to triclosan (for example, see Braid & Wale, 2002). The growth of all the other selected microorganisms was inhibited by triclosan at a concentration of 0.02%.

Although propolis extract was not as strong as triclosan (at equal concentration) in its inhibition of microbial growth, it did have an important antimicrobial effect on both Gram-positive bacteria at concentrations around 2%. The addition of propolis extract at the active concentration to a cosmetic formulation does not affect its organoleptic characteristics and therefore favors consumer acceptance of the final product.

Considering that açai extract did not show any antimicrobial activity and that it may lead to bacterial contamination, combining the two extracts to investigate antimicrobial synergy did not seem reasonable.

The next part of the study consisted of assessing the antioxidant activity of açai and propolis extracts, alone and combined, by DPPH radical scavenging assay. Free radicals, called reactive oxygen species (ROS), are formed in the cells as a consequence of biochemical oxidation reactions. They participate in many body processes, such as the production of energy, phagocytosis and molecular synthesis. However, ROS can damage cells and the DNA if they are produced in excess under abnormal conditions, such as inflammation and external factors. ROS such as the hydroxyl radical (•OH), superoxide anion radical (O_2 •–) and hydroperoxyl radical (ROO•), can oxidize lipids and proteins and damage the DNA (Atoui et al., 2005; Barreiros et al., 2006; El-Agamey et al. 2004; Haslam, 1996; Omoni et al., 2005; Pietta, 2000; Russo et al., 2002). Such damage can be associated with many diseases, such as early aging, heart disease, inflammation, neurodegenerative diseases and cancer.

Many antioxidant compounds are responsible for stabilizing or deactivating free radicals and controlling their production in living beings in order to prevent damage to cell structures. The human body has some endogenous antioxidant substances but, when there is overproduction of ROS, oxidative damage may occur and accumulate. Thus, exogenous (dietary) sources of antioxidants become necessary (Atoui et al., 2005; Barreiros et al., 2006).

The products of reactions between antioxidants and free radicals are not reactive, that is, they are stable and not involved in chain reactions (Atoui et al., 2005; Barreiros et al., 2006; El-Agamey et al., 2004; Omoni et al., 2005).

The phenolic compounds present in the extracts in this study are a class of antioxidants that occur in nature and act both at the beginning and during the propagation of oxidative processes. The antioxidant activity of these compounds is associated with their reducing power and chemical structure, which can scavenge free radicals and chelate transition metals. Phenolic compounds form relatively stable intermediates in these reactions (Haslam, 1996).

The antioxidant substance used for comparison in the present study, BHT or butylated hydroxytoluene, is a very popular antioxidant in the food industry. However, since it is a synthetic compound, it can have toxic and carcinogenic effects, as reported in some scientific studies (Botterweck et al., 2000). For this reason, it is crucial to carry out experiments on the use of natural products with antioxidant activity, to enable synthetic antioxidants to be replaced by natural ones with equal or greater antioxidant efficiency and efficacy and without the deleterious health effects.

The DPPH assay, a chemical assay used to determine the capacity of a substance to scavenge free radicals, is one of the most popular methods used to assess the antioxidant capacity of bee products and fruit extracts, since it is practical, fast and the reagents are stable (Espin et al., 2000; Kuskoski et al., 2006; Teixeira et al., 2010).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical is an extremely stable, purple compound that absorbs light at 515nm when dissolved in ethanol. This radical, when reduced by antioxidant substances present in a test sample, forms a yellow compound called diphenyl picryl hydrazine, which absorbs much less strongly at that wavelength. Therefore, the reduction of DPPH radical is monitored by observing the decrease in absorbance at 515nm. The results are expressed as the percent antioxidant activity (%AA), which corresponds to the percentage of the initial DPPH consumed by the antioxidant. The amount of antioxidant necessary to reduce 50% of the initial concentration of DPPH is called the effective concentration (EC_{50}) or inhibitory concentration (IC_{50}). As the amount of DPPH scavenged by a given quantity of sample increases, the EC_{50} of the sample decreases and its antioxidant capacity increases (Brand-Williams et al, 1995; Sánchez-Moreno et al., 1998).

The maximum concentration of propolis and açai extracts in the formulations was limited to 5% because of their organoleptic characteristics. Hence, the samples were analyzed at low concentrations, alone or combined, in order to reveal a possible antioxidant synergy between them.

EC_{50} was obtained by plotting the absorbances after the reaction of DPPH with the various concentrations of the extracts and the positive control. Based on the rule that, as EC_{50} decreases, antioxidant capacity increases, the EC_{50} results show that all the samples had appreciable antioxidant activity, but neither the propolis nor the açai extract matched the capacity of the synthetic compound. Furthermore, it can be seen that the phenolic compounds present in the propolis extract had a greater antioxidant effect than the anthocyanins present in the açai extract, since the latter was less active. Summarizing, the substance with the strongest antioxidant effect was BHT, followed by the propolis extract and lastly, the açai extract.

The percent antioxidant activity (%AA) allowed comparison between the samples, including propolis and açai extracts alone and combined. BHT at 0.01%, the concentration generally used in foods and formulations, was used as a reference. The absorbances were then converted to %AA, which varied from 27.60% to 74.30%. The combination of propolis (0.1% w/v) and açai (0.1% w/v) extracts resulted in the highest antioxidant activity (74.30%). It is important to emphasize that this activity was considerably higher than the %AA of propolis extract at 0.1% (60.93%) or açai extract at 0.1% (30.53%) and also higher than the %AA of BHT at 0.01% (27.60%). Therefore, the substances arranged in decreasing order of %AA are: propolis and açai extracts combined, propolis extract, açai extract and BHT.

This study evidenced a summing of the antioxidant effects of the propolis and açai extracts. Their combined antioxidant effect at very low concentrations was very high, which is an important property when choosing antioxidant compounds for a formulation. These extracts at such small concentrations do not affect the organoleptic characteristics of the formulation; therefore, they maintain the consumer acceptance of the final product, clearly an important question when selecting antioxidants (Ramalho & Jorge, 2006).

Antioxidant activity may vary with the origin of the sample, concentration, assessment method (reaction time and solvent) and affinity of the antioxidants. The origin of the sample has a direct impact on the concentrations of the antioxidant compounds it contains, especially in the case of propolis. The great advantage of the DPPH assay is that the results are not affected by the polarity of the compounds to be analyzed (Cabral et al., 2009; Koleva et al., 2002; Moure et al., 2001). Rufino et al. (2010) compared the antioxidant activity of tropical fruits ascertained by many different methods and concluded that it is not easy to compare the methods. These same authors concluded that açai has a low antioxidant activity, despite the results of the present study. This difference may be due to the treatment given to the açai samples, including less efficient extraction of anthocyanins.

The rather low percentage of antioxidant activity shown by BHT in this study may have been due to its quality. Inappropriate storage, for example, may impair the quality of the compound, slowly reducing its antioxidant effect over time. Galotta et al. (2008) and Jian-Hua et al. (2010) reported that the %AA of BHT at its usual concentration is roughly 80%, exceeding those of the extracts reported here. Nevertheless, the propolis and açai extracts combined did show a high %AA, quite comparable with the %AA of BHT. Therefore, a combination of 0.1% propolis extract and 0.1% açai extract could represent an important and low-cost substitute for BHT in today's products, since BHT is synthetic and potentially carcinogenic.

The present study corroborates the work of Hogan et al (2010), who assessed the antioxidant activity of açai extracts, rich in anthocyanins, and found it to be high. Those authors found that açai extract also showed antiproliferative activity against C-6 rat brain glioma cells, while other extracts from other plants did not, making açai extract stand out in relation to many other tested extracts. Since açai and propolis extracts both have high antioxidant activity, their combined use might help to reduce the incidence and mortality rates of some types of cancer and investigating this hypothesis will be the next step of the present research.

In conclusion, considering all the results, the summed effects of propolis and açai extracts were seen only in the antioxidant activity. Thus, the addition of propolis and açai extracts at low concentrations to a formulation can grant it both antioxidant activity, conferred by the sum of effects of propolis and açai, and appreciable antimicrobial activity against the staphylococci tested, conferred by propolis, as long as the minimum concentration established in this study for such activity is employed.

This study also evidenced the importance of standardizing samples of natural products. Propolis resin is an example of a raw material that needs to be standardized. Since it is a natural substance whose composition is affected by its geographical origin, among other factors, it would be best to determine the compounds that confer its antimicrobial and antioxidant activities, in order to

quantify them and assess them individually. These active compounds could then be concentrated, in order to improve their antimicrobial and/or antioxidant effects and avoid possible toxic effects from other substances present in the extract.

ACKNOWLEDGEMENTS

We thank the Pontifical Catholic University of Campinas, PUC-Campinas, for sponsoring the study (Introduction to Science grant provided by Fapic/Dean's Office).

RESUMO

Atividades antioxidante e antimicrobiana dos extratos de própolis e açaí (Euterpe oleracea mart)

A própolis é uma resina natural que apresenta diversas ações biológicas devido a presença de fenólicos, especialmente flavonóides, em sua composição. O fruto do açaí (*Euterpe oleracea* Mart) é fonte de antocianinas, que também pertencem ao grupo dos flavonóides. O objetivo deste estudo foi avaliar o efeito antioxidante e antimicrobiano dos extratos de açaí e propolis em associação. A atividade antimicrobiana foi avaliada por ensaio em caldo enriquecido e a atividade antioxidante foi avaliada pelo método do sequestro de radicais DPPH. Os resultados mostraram que o extrato de própolis em pequenas concentrações apresentou atividade sobre o crescimento de *S. aureus* e *S. epidermidis*, e que ambos os extratos apresentaram atividade antioxidante. Foi possível concluir que ocorreu sinergismo entre os extratos de própolis e de açaí em relação à atividade antioxidante, bem como esta associação poderá apresentar atividade antimicrobiana caso o extrato de própolis seja utilizado em quantidade suficiente na formulação. Assim, esses extratos podem ser usados em formulações para uso tópico para prevenir o envelhecimento da pele e, possivelmente, transtornos causados pelos microrganismos empregados no estudo.
Palavras-chave: Própolis. Açaí. Flavonoides. Antimicrobiano. Antioxidante. Radical DPPH.

REFERENCES

Araujo CEP, Shimizu MT, CUNHA IB, Marcucci MC, Ramos OHP, Sawaya ACHF. Análise química, toxicológica e antiulcerogênica preliminar de uma amostra de própolis da região do Paraná. Lecta-USF, 2002; 20(1):47-52.

Atoui AK, Mansouri A, Boskou G, Kefalas P. Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chemistry,2005; 89(1):27-36.

Bankova V, Christov R, Popov S, Marcucci MC, Tsvetkova I, Kujumgiev A. Antibacterial activity of essential oils from Brazilian propolis. Fitoterapia, 1999; 70(2):190–193.

Barreiros ALBS, David JM, David JP. Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. Quimica Nova, 2006; 29(1):113-123.

Bonvehi JS, Coll FV, Jordá RE. The composition, active components and bacteriostatic activity of propolis in dietetics. Journal of American Oil Chemists Society, 1994; 71(5): 529-532.

Botterweck AAM, Verhagen H, Goldbohm RA, Kleinjans J, Van Den Brandt PA. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: Results from analyses in The Netherlands cohort study. Food and Chemical Toxicology, 2000; 38(7):599-605.

Braid JJ, Wale MCJ. The antibacterial activity of triclosan-impregnated storage boxes against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Shewanella putrefaciens* in condictions simulating domestic use. Journal of Antimicrobial Chemotherapy JAC, 2002; 49(1):87-94.

Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology, 1995; 28(1):25-30.

Brumfitt W, Hamilton-Miller JMT, Franklin I. Antibiotic activity of natural products: 1. Propolis. Microbios, 1990; 62(250):19-22.

Cabral ISR, Oldoni TLC, Prado A, Bezerra RMN, Alencar SM, Ikegaki M, Rosalen PL. Composição fenólica, atividade antibacteriana e antioxidante da própolis vermelha brasileira. Quimica Nova, São Paulo, 2009; 32(6):1523-1527.

Castañeda-Ovando A, Pacheco-Hernández ML, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: A review. Food Chemistry, 2009; 113(4):859-871.

Christov R, Bankova V, Tsvetkova I, Kujumgiev A, Delgado Tejera A. Antibacterial furofuran lignans from Canary Islands propolis. Fitoterapia, 1999; 70(1):89-92.

Crisan I, Zaharia CN, Popovici F, Jucu V, Belu O, Dascalu C, Mutiu A, Petrescu A. Natural propolis extract NIVCRISOL in the treatment of acute and chronic rhinopharyngitis in children. Romanian Journal f Virology, 1995; 46(3-4):115-133.

Del Pozo-Insfran D, Brenes CH, Talcott ST. Phytochemical Composition and Pigment Stability of Açai (*Euterpe oleracea* Mart.) Journal of Food Chemistry, 2004; 52(6): 1539-1545.

El-Agamey A, Lowe GM, Mcgarvey DJ, Mortensen A, Phillip DM, Truscott TG, Young AJ. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archives of *Biochemistry* and Biophysics, 2004; 430(1):37-48.

Espin JC, Soler-Rivas C, Wichers HJ, Garcíaviguera C. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. Journal of Agricultural and Food Chemistry, 2000; 48(5): 1588-1592.

Fernandes Junior A, Lopes CAM, Sforcin JM, Funari SRC. Population analysis of susceptibility to propolis in reference strains of *Staphylococcus aureus* and *Escherichia*

coli. Journal of Venomous Animals and *Toxins* including Tropical Diseases, 1997; 3(2): 287-94.

Fernandes Junior A, Sugizaki MF, Fogo ML, Lopes CAM, Funari SRC. *In vitro* susceptibility of *Candida albicans* to propolis. Proc IV Iberolatinamerican Meeting Apic, Ministerio de Agricultura, Ganaderia y Recursos Renovables, Rio Cuarto, Argentina, 1994. 209- 211.

Fuentes AMO, Hernandez NR. Accion antimicrobiana de los extractos alcoholicos de propoleo. Revista Cubana de Farmacia,1990; 24(1):34-44.

Galotta ALQA, Boaventura MAD, Lima LARS. Antioxidant and cytotoxic activities of 'açaí' (*Euterpe precatoria* Mart.). Quimica Nova, 2008; 31(6): 1427-1430.

Gonsales GZ, Orsi RO, Fernandes Júnior A, Rodrigues P, Funari SRC. Antibacterial activity of propolis collected in different regions of Brazil. Journal of Venomous Animals and *Toxins* including Tropical Diseases, 2006; 12(2): 276-284.

Haslam E. Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action, Journal of Natural Products, 1996; 59(2): 205–215.

Havsteen BH. The biochemistry and medical significance of the flavonoids. Farmacology and Therapeutics, 2002; 96(2/3): 67-202.

Hogan S, Chung H, Zhang L, LI J, Lee Y, Dai Y, Zhou K. Antiproliferative and antioxidant properties of anthocyanin-rich extract from açai. Food Chemistry, 2010; 118, 208-214.

Homma AKO, Nogueira OL, Menezes AJEA, Carvalho JEU, Nicoli CML, Matos GB. Açaí: Novos Desafios e Tendências. Amazônia: Ci. & Desenv., 2006; 1(2): 7-23.

Jian-Hua X, Ming-Yong X, Shao-Ping N, Ming-Yue S, Yuan-Xing W, Chang L. Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. Food Chemistry, 2010; 119(4): 1626–1632.

Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 2003; 10(10): 813-829.

Koleva TA, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochem Anal,2002; 13(1): 8-17.

Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology, 1999; 64(3):235-240.

Kuskoski EM, Asuero AG, Morales MT, Fett R. Frutos tropicais silvestres e polpas de frutas congeladas: atividade antioxidante, polifenóis e antocianinas. Ciência Rural, 2006; 36(4):1283-1287.

Marcucci MC, Ferreres F, García-Viguera C, Bankova VS., De Castro SL, Dantas AP, Valente PH, Paulino N. Phenolic compounds from Brazilian propolis with pharmacological activities. Journal of Ethnopharmacology, 2001; 74(2):105–112.

Marcucci MC. Propolis, chemical composition, biological properties and the therapeutic activity. Apidologie, 1995; 26(2):83-99.

Mirzoeva OK, Grishanin RN, Calder PC. Antimicrobial action of propolis and some of its components: the effects on growth, membrane potencial and motility of bacteria. Microbiology Research, 1997; 152(3): 239-246.

Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, Dominguez H, Nunez MJ, Parajo JC. Natural antioxidants from residual sources. *Food Chemistry*, 2001; 72(2):145-171.

Omoni AO, Aluko RE. The anti-carcinogenic and anti-atherogenic effects of lycopene: a review. *Trends* in *Food Science & Technology*, 2005; 16(8):344-350.

Pacheco-Palencia LA, Hawken P, Talcott ST. Juice matrix composition and ascorbic acid fortification affects on the phytochemical, antioxidant and pigment stability of açaí (*Euterpe oleracea* Mart.) Food Chemistry, 2007; 105(1):28-35.

Pacheco-Palencia LA, Talcott ST. Chemical stability of açaí fruit (*Euterpe oleracea* Mart) anthocyanins as influenced by naturally occurring and externally added polyphenolic cofactors in model systems, Food Chemistry, 2010; 118(1):17-25.

Pietta PG. Flavonoids as Antioxidants. Journal of Natural Products, 2000; 63(7):1035- 1042.

Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine, 2005; 12(3): 221-228.

Ramalho VC, Jorge N. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. Química Nova, 2006; 29(4): 755-760.

Rezende GPSR, Pimenta FC, Costa LRRS. Antimicrobial activity of two Brazilian commercial propolis extracts. Brazilian *Journal* Of *Oral* Sciences, 2006; 5(16): 967-970.

Rufino MSM, Alves RE, Brito ES, Pérez-Jiménez J, Saura-Calixto FG, Mancini-Filho J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chemistry, 2010; 121(4): 996–1002.

Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia, 2002; 73(1): S21-S29.

Salatino A, Teixeira EW, Negri G, Message D. Origin and Chemical Variation of Brazilian Propolis. Articles from *Evidence-based* Complementary and *Alternative Medicine*, 2005; 2(1): 33-38.

Salomão K, Dantas AP, Borba CM, Campos LC, Machado DG, Aquino Neto FR, Castro SL. Chemical composition and microbicial activity of extracts from Brazilian and Bulgarian propolis. *Letters* in *Applied Microbiology*, 2004; 38(2): 87-92.

Sanabria N, Sangronis E. Caracterización del açaí o manaca (*Euterpe oleracea*, Mart.): um fruto del Amazonas. Archivos Latinoamericanos de nutrición, 2007; 57(1): 1-6.

Sánchez-Moreno C, Larrauri JA, Saura-Calixto FM. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 1998; 76: 270-276.

Sawaya ACHF, Palma AM, Caetano FM, Marcucci MC, Silva Cunha IB, Araújo CEP, Shimizu MT. Comparative study of in vitro methods used to analyse the activity of propolis extracts with different compositions against species of Candida. Letters in Applied Microbiology,2002; 35: 203-207.

Sforcin JM, Fernandes-Júnior A, Lopes CAM, Funari SRC, Bankova V. Seasonal effect of Brazilian propolis on *Candida albicans* and *Candida tropicalis*. Journal of Venomous Animals and *Toxins* including Tropical Diseases, 2001; 7(1):139-144.

Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology, 2005; 99(1): 69-73.

Silva JFM, Souza MC, Matta SR, Andrade MR, Vidal FVN. Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities. Food Chemistry, 2006; 99(3): 431-435.

Teixeira EW, Message D, Negri G, Salatino A, Stringheta PC. Seasonal Variation, Chemical Composition and Antioxidant activity of Brazilian Propolis Samples. Evid Based Complement Alternat Med., 2010; 7(3): 307–315.

Tonon RV, Brabet C, Hubinger MD. Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpe oleracea* Mart) juice produced with different carrier agents. Food Research International, 2010; 43(3): 907-914.

Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J. Agric. Food Chemistry, 1996; 44(3):701-705.

Weston RJ, Mitchell KR, Allen KL. Antibacterial phenolic components of New Zealand manuka honey. Food Chemistry, 1999; 61(3): 295–301.