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BIOTECHNOLOGY

Phytochemistry and antibacterial activity of aqueous and hydroalcoholic extracts of three medicinal plants against food pathogens

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ABSTRACT. This study aimed to characterize the phytochemical compositions of three medicinal Brazilian plants' leaves and bast extracts, and to determine their antibacterial activity on three foodborne and waterborne bacterial pathogens. *Parkia platycephala*, *Pouteria ramiflora* and *Lophanthera lactescens* leaves and basts were collected and aqueous and hydroalcoholic extracts were prepared. Qualitative screening of the phytochemical extracts was performed with three replicates and in triplicate in order to identify the bioactive compounds. The Minimal Inhibitory Concentration and Minimal Bactericide Concentration were determined by microdilution in broth and *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* growth was observed on agar plates. Phytochemical composition analysis allowed for the identification of anthraquinones, catechins, saponins, tannins, sesquiterpenlactones and other lactones in the three plants' leaves and bast aqueous and hydroalcoholic extracts. Eighty-three percent of the plant extracts showed antibacterial activity against *S. aureus*, and *P. platycephala* extracts were the only ones that inhibited *E. coli* and *S. typhimurium* growth. The present study contributes significantly to the phytochemical composition characterization of three plant species commonly used in Brazilian traditional medicine. The plant extracts' *in vitro* antibacterial activity was demonstrated and catechins present in the extracts are, most likely, the bioactive compounds responsible for this action.

Keywords: catechins; foodborne and waterborne pathogen; minimal bactericide concentration; minimal inhibitory concentration.

Fitoquímica e atividade antibacteriana de extratos aquosos e hidroalcoólicos de três plantas medicinais contra patógenos de alimentos

RESUMO. Este estudo objetivou caracterizar a composição fitoquímica dos extratos de folhas e das entrecascas de três plantas medicinais brasileiras e determinar a sua atividade antimicrobiana contra três patógenos bacterianos de alimentos. Foram elaborados extratos aquosos e hidroalcoólicos, por meio de folhas e de entrecascas de *Parkiaplatycephala*, *Pouteriaramiflora* e *Lophantheralactescens*. O estudo qualitativo dos extratos foi realizado com três réplicas, em triplicata, para permitir a identificação dos compostos bioativos. A Concentração Inibitória Mínima e a Concentração Bactericida Mínima foram determinadas por microdiluição contra *Escherichia coli*, *Salmonella typhimurium* e *Staphylococcus aureus*. A análise da composição fitoquímica permitiu identificar antraquinonas, catequinas, saponinas, taninas, sesquiterpenlactonas e outras lactonas nos extratos aquosos e hidroalcoólicos das folhas e das entrecascas das três plantas. Oitenta e três porcento dos extratos das plantas apresentaram atividade antibacteriana contra *S. aureus.* Os extratos de *P. platycephala* foram os únicos que inibiram o crescimento de *E. coli* e *S. typhimurium*. Este estudo contribui significativamente para a caracterização da composição fitoquímica de três espécies de plantas, frequentemente, utilizadas na medicina tradicional brasileira. A atividade antibacteriana, *in vitro,* dos extratos das plantas foi demonstrada, e as catequinas são, provavelmente, o composto bioativo responsável por essa atividade.

Palavras-chave: catequinas; patógenos dos alimentos; concentração bactericida mínima; concentração inibitória mínima.

Introduction

Foodborne and waterborne bacterial pathogens are a major cause of mortality in developing countries and cause significant morbidity in developed nations. Some countries carry a disproportionately heavy burden of these infectious diseases due to inadequate resources for providing sanitation and hygienic facilities, and

safe water (Faruque, 2012). Enteric infections caused by bacteria, viruses and parasites are an important public health problem. The infection is transmitted through contaminated food or water or by person-to-person transmission, as a result of the lack of adequate hygiene measures. Fever and inflammatory diarrhoea are the main symptoms in enteric infections caused by *Escherichia coli* and *Salmonella* serotypes, among other pathogens, and are responsible for the death of 2 million children per year, making them the third most common cause of death by infectious disease in the world (Sarrionandia, León, & Baamonde, 2011). Significant worldwide efforts are being made to improve food safety, including the use of antibiotics. However, selective pressure among bacteria has allowed for the development of antibiotic resistance which is acquired through horizontal gene transfer and mobile genetic elements (Singh, 2017). Considering this, it is necessary to identify new antimicrobial agents to efficiently cure and control infections caused by bacterial strains resistant to multiple drug, something that may be achieved through the medicinal properties of herbs and higher plants (Abioye et al., 2013).

This study intended to define the phytochemical composition of aqueous and hydroalcoholic extracts from *Parkia platycephala*, *Pouteria ramiflora* and *Lophanthera lactescens* leaves and basts. Moreover, it was also aimed the *in vitro* determination of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC), using the extracts of the three plants on *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* cultures.

Material and methods

Plants collection

Parkia platycephala and *P. ramiflora* leaves and basts were collected in July 2015, while *L. lactescens* leaves and bast were collected in September 2015, all in the Municipality of Palmas, Tocantins, Brazil. The three species were identified by Professor Dr. Rodney Haulien Oliveira Viana and one voucher specimen of each species was deposited in the Herbarium Tocantins (HTO) located in the NEAMB, Porto Nacional Campus, *Universidade Federal do Tocantins* (UFT), with the following registry numbers: HTO 10.951 – *P. platycephala*, HTO 10.949 – *P. ramiflora* and HTO 10.950 – *L. lactescens*.

Extracts preparation

The three plants leaves were washed, immersed in 100 ppm chloride solution for 10 minutes, rinsed with distilled water, and dried in an air-circulating oven at 48ºC until becoming brittle. The three plants' basts were grated and dried in an air-circulating oven at 48ºC, as well. After drying, each plant tissue was ground in a blender and stored in sterile hermetically sealed glass vials under the shelter of the light, inside cardboard boxes.

Aqueous extracts were prepared from the dried leaves and bast powder after dilution to 10% (m/v) with sterilized water and left to macerate at room temperature with occasional agitation for 48 hours. After maceration, the extracts were vacuum filtered in a Büchner funnel, frozen, lyophilized and stored at 2-8ºC.

Hydroalcoholic extracts were prepared from the dried leaves and bast powder after dilution to 10% (m/v) with 70% ethanol and left to macerate at room temperature with occasional agitation for 7 days. After maceration, the extracts were vacuum filtered in a Büchner funnel, the solvent was removed under low pressure in a rotary evaporator at 50ºC, concentrated in a water bath at 40ºC and stored at 2-8ºC.

Phytochemical analysis

The phytochemical extracts' qualitative screening was performed with three replicates and in triplicate in order to identify secondary metabolites such as organic acids, alkaloids, anthraquinones, azulenes, carotenoids, catechins, coumarins, steroids, triterpenes, flavonoids, cardioactive glycosides, saponins, tannins, sesquiterpenlactones and other lactones (Matos, 1988).

Antibacterial assay

In order to evaluate the plants extracts' antimicrobial activity, standard strains [American Type Collection Culture (ATCC)] were obtained from the National Institute for Quality Control in Health (Fiocruz, Rio de Janeiro, Brazil). Accordingly, Gram-negative *E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028, and Gram-positive *S. aureus* ATCC 25923 were used. The plants extracts were diluted in 1% DMSO for a final concentration of 25 mg mL^{-1}.

The MIC was determined by the microdilution technique in broth according to the National Committee for Clinical Laboratory Standards norm M7-A6 (National Committee for Clinical Laboratory Standards [NCCLS], 2003), performed with three replicates and in triplicate.

In more detail, 100 µL of Muller-Hinton broth (MHB) were added to each microdilution plate well, followed by the addition of plants extracts at 25 mg mL^{-1} for a final concentration of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0.09 mg mL^{-1} in wells A through H, respectively. Additionally, a negative control (MHB, solvent without plants extracts and bacteria), a positive control (MHB, chloramphenicol at 1000 to 7.81 µg mL^{-1} and bacteria), a growth control (MHB and bacteria) and a broth sterility control (only MHB) were prepared and utilized. Moreover, 5 µL of standard suspension inoculum adjusted to 1.0 x 10^7 UFC mL^{-1} were added to each well and the microplates were incubated for 24 hours at 35ºC. Bacterial growth was determined visually after adding 30 µL of 0.03% (m/v) sterile resazurin followed by incubation for 1 hour. A pink colour indicated bacterial growth while a blue colour showed its absence. MIC was considered to be the minimal extract concentration capable to inhibit bacterial growth.

In order to determine the MBC, 10 µL aliquots from each microplate well without visible bacterial growth at the MIC were inoculated in the surface of a Mueller Hinton agar plate, in triplicate, and incubated for 24 hours at 35ºC. The MBC was defined as the minimal extract concentration without colony growth on the plates' surface. Additionally, the extracts showed bacteriostatic action when bacterial growth and bactericidal action when there was no growth.

Results

Phytochemical analysis

The phytochemical composition analysis of the aqueous and hydroalcoholic extracts from *P. platycephala*, *P. ramiflora and L. lactescens* leaves and basts allowed for the identification of anthraquinones, catechins, saponins, tannins, sesquiterpenlactones and other lactones, while other compounds such as alkaloids, azulenes, carotenoids, coumarins, steroids, triterpenes, flavonoids and cardioactive glycosides were not found (Table 1). Moreover, it can be observed that all three plant species presented saponins and tannins in the composition of both extracts (Table 1). Anthraquinones were only identified in bast extracts of *P. ramiflora* and *L. lactescens* while catechins were only absent from *L. lactescens* leaves (Table 1). Sesquiterpenlactones and other lactones were absent from *L. lactescens* leaves and *P. ramiflora* leaves hydroalcoholic extracts (Table 1). Mostly, the three plants presented the same type of phytochemical compounds, even considering the different plant tissues (Table 1).

Antibacterial activity

All the plant extracts showed antibacterial activity against *S. aureus* with the exception of *L. lactescens* leaves (Table 2). Additionally, *P. platycephala* bast extracts were the only ones that inhibited *E. coli* and *S. typhimurium* growth in microplate assays and *S. typhimurium* growth in Mueller Hinton agar plate assays (Table 2). Both the negative and growth controls had a positive result for bacterial growth indicating no antibacterial activity, while the positive and broth sterility controls showed no bacterial growth, demonstrating that antibiotic chloramphenicol inhibited bacterial growth and broth sterility (data not shown).

Table 1. Phytochemical composition analysis of the aqueous and hydroalcoholic extracts from *Parkia platycephala*, *Pouteria ramiflora* and *Lophanthera lactescens* leaves and basts.

Phytochemical compounds	*P. platycephala*				*P. ramiflora*				*L. lactescens*			
	Leaves		Bast		Leaves		Bast		Leaves		Bast	
	A	H	A	H	A	H	A	H	A	H	A	H
Organic acids	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	+	+	-	-	+	+
Azulenes	-	-	-	-	-	-	-	-	-	-	-	-
Carotenoids	-	-	-	-	-	-	-	-	-	-	-	-
Catechins	+	+	+	+	+	+	+	+	-	-	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-
Steroids and Triterpenes	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-
Cardioactive glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Sesquiterpenlactones and other lactones	+	+	+	+	+	-	+	+	-	-	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+

Note: A - aqueous extract; H - hydroalcoholic extract; + presence; - absence.

Table 2. *Parkia platycephala*, *Pouteria ramiflora* and *Lophanthera lactescens* extracts' MIC (mg mL^{-1}) and MBC (mg mL^{-1}).

Plant	Plant tissue	Extract	Bacteria					
			E. coli		*S. typhimurium*		*S. aureus*	
			MIC	MBC	MIC	MBC	MIC	MBC
P. platycephala	Leaves	Aqueous	-	-	-	-	1.6	3.1
		Hydroalcoholic	-	-	-	-	1.6	1.6
	Bast	Aqueous	12.5	-	12.5	12.5	0.8	1.6
		Hydroalcoholic	12.5	-	12.5	12.5	0.8	1.6
P. ramiflora	Leaves	Aqueous	-	-	-	-	0.8	1.6
		Hydroalcoholic	-	-	-	-	1.6	3.1
	Bast	Aqueous	-	-	-	-	1.6	3.1
		Hydroalcoholic	-	-	-	-	0.8	1.6
L. lactescens	Leaves	Aqueous	-	-	-	-	-	-
		Hydroalcoholic	-	-	-	-	-	-
	Bast	Aqueous	-	-	-	-	1.6	3.1
		Hydroalcoholic	-	-	-	-	1.6	1.6

Note: MIC - Minimal Inhibitory Concentration; MBC - Minimal Bactericide Concentration; - not determined in the studied concentration range.

Discussion

The phytochemical composition analysis allowed for the identification of saponins and tannins, phytochemical compounds with multiple biological properties (Diaz Carrasco et al., 2016; Stylos, Chatziathanasiadou, Syriopoulou, & Tzakos, 2017), in all the plants extracts studied (Table 1) A similar result was reported for the aqueous fractions of *P. biglobosa* leaf, stem bark and root methanolic extracts. However, when apolar solvents were used, only alkaloids were obtained from the chloroform fraction (Udobi & Onaolapo, 2009). Since both solvents used in this study are polar, this may explain the extraction of saponins and tannins, and the absence of alkaloids in all the extracts studied.

Catechins, a major component of green tea with attributable antibacterial activity (Noormandi & Dabaghzadeh, 2015), were identified in all the extracts studied besides *L. lactescens* leaves (Table 1). Since catechins have hydroxyl groups available for hydrogen bond formation, their extraction with polar solvents is facilitated (Marques et al., 2016). In opposition to the identification of catechins in *Byrsonima* species' (Malpighiaceae) leaves (Michelin et al., 2008), this study was not able to find them in *L. lactescens* leaves (Table 1). A plausible explanation is that different genera may have different phytochemical compositions. Additionally, catechins were previously identified in fruits of species from the *Pouteria* genus (Ma, Yang, Basile, & Kennelly, 2004), supporting the presence of catechins in this study since plant tissues were collected during their fruiting season.

Other phytochemical compounds with almost ubiquitous presence in the studied extracts were sesquiterpenlactones and other lactones. They only failed to be found in *L. lactescens* leaves extracts and in the hydroalcoholic extract of *P. ramiflora* leaves (Table 1). Sesquiterpenlactones have been reported to exhibit potent bioactivities including antibiotic, antitumor, antiulcer, insect-feeding deterrent, phytotoxic, and schistosomicidal effects (Ren, Yu, & Kinghorn, 2016). Sesquiterpenes are colourless lipophilic compounds (Chadwick, Trewin, Gawthrop, & Wagstaff, 2013) and can yield three fractions of different polarities in column chromatography (Dias, Foglio, Possenti, Nogueira, & Carvalho, 2001). It is likely that the sesquiterpenlactones and other lactones identified in this study presented a polar or weakly polar charge due to the solvents used.

Anthraquinones constitute an important class of compounds with a wide range of applications. Anthraquinone derivatives show a wide array of pharmacological activities including laxative, anticancer, anti-inflammatory, anti-arthritic, antifungal, antibacterial, antiviral, antiplatelet and neuroprotective effects (Malik & Müller, 2016). In the present study, anthraquinones were found in *P. ramiflora* and *L. lactescens* bast extracts (Table 1). Similar results were previously reported for *Schinus terebenthifolius* Raddi stem bark (Lima et al., 2006), *P. bicolor* leaves, *P. biglobosa* stem bark (Adaramola, Ariwaodo, & Adeniji, 2012) and *P. ramiflora* wood and bark (Oliveira, Pereira, Muller, & Matias, 2014). Surprisingly, *P. platycephala* bast extracts did not exhibit any presence of anthraquinones. This result may be due to differences in the regions, environmental conditions, harvest time, developmental stage of the plants or extraction methods.

In the present study, antibacterial activity was observed with all plant extracts besides *L. lactescens* leaves (Table 2), suggesting that they lack some bacterial growth inhibitory compound shared by the other extracts. Indeed, when comparing the extracts phytochemical analysis results it is possible to see that neither catechins, nor sesquiterpenlactones and other lactones, were found in *L. lactescens* leaves (Table 1). However, sesquiterpenlactones and other lactones were also absent from *P. ramiflora* hydroalcoholic leaves extract (Table 1), which

showed antibacterial activity (Table 2). Additionally, it is also noticeable that only *L. lactescens* leaves extracts had no activity on *S. aureus* (Table 2). Altogether, these results suggest that catechins are likely responsible for the antibacterial activity documented in this study (Table 2), supporting a previous report on catechins antibacterial activity (Noormandi & Dabaghzadeh, 2015). Catechins may have antibacterial activity through different mechanisms, such as blocking the connection of the conjugated R plasmid, may bind to the ATP site of the DNA gyrase β subunit of bacteria thus inhibiting the activity of the gyrase enzyme, may interfere with the expression of β-lactamases, may inhibit the extracellular release of toxins, their bactericidal action may be due to hydrogen peroxide generation, and/or catechin-copper (II) complexes may damage the cytoplasmic membrane (Noormandi & Dabaghzadeh, 2015). Overall, the present study supports the relevant role of catechins in antibacterial activity.

The fact that *S. aureus* was the most affected bacteria by the three plants extracts used (Table 2) is also significant, suggesting that Gram-positive bacteria are more sensitive to these extracts, even including extracts obtained from leaves, than the Gram-negative bacteria, perhaps due to the absence of the outer membrane in Gram-positive bacteria (Horiuchi et al., 2007, Fontanay, Grare, Mayer, Finance, & Duval, 2008). Similar results were reported by other authors (Abioye et al., 2013, Ajaiyeoba, 2002, El-Mahmood & Ameh, 2007, Udobi & Onaolapo, 2009, Dzoyem et al., 2017).

Interestingly, only *P. platycephala* bast extracts showed antibacterial activity against *E. coli* and *S. typhimurium* (Table 2). The only reasonable explanation is that *P. platycephala* bast extracts retained a phytochemical compound absent from the other plants extracts studied and that this compound was responsible for *E. coli* and *S. typhimurium* growth inhibition. Considering that the plant tissues studied were collected during their fruiting season and that *P. platycephala* ethanolic seed extracts showed the presence of flavonoids and polyphenols (Farias et al., 2013), then *P. platycephala* bast extracts may have a unique catechin responsible for the antibacterial activity identified. Certainly, the phytochemical composition of *P. platycephala* fruits, as well as the individualized identification of catechins in *P. platycephala* bast and fruit is required.

Conclusion

The present study contributes significantly to the phytochemical composition characterization of three medicinal plant species from the Brazilian flora commonly used in traditional medicine. Additionally, *in vitro* antibacterial activity was demonstrated with 83% of the plant extracts used against *S. aureus*, while only *P. platycephala* bast extract showed a significant effect on *E. coli* and *S. typhimurium* growth inhibition. The phytochemical composition and antibacterial activity analyses correlation allowed to determining that catechins presence is most likely the bioactive compound responsible for the results obtained. Altogether these results suggest a promising future use of the studied plants tissues on the three tested foodborne bacterial species frequently responsible for enteric infections in humans, possibly replacing the currently used antibiotics in situations with multi-resistant bacterial infections.

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