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Preliminary evaluation of antioxidant and antimicrobial activities of Luffa Operculata (L.) Cong. extracts

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ABSTRACT. *Luffa operculata* is a medicinal plant widely used in the treatment of rhinosinusitis in Brazil. The aim of this work was to carry out a preliminary analysis of the antioxidant and antimicrobial profile of *Luffa operculata* extracts. The antioxidant activity of the commercial solution, hydroalcoholic extract, infusion and ethanolic extract obtained from the commercialized fruit were evaluated for 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radicals. The antimicrobial activity was determined by minimum inhibitory concentration against the pathogens *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. It was shown that the hydroalcoholic extract exhibited the highest antioxidant and antimicrobial activity when compared to the aqueous and ethanolic extracts by the methods employed on this work**.** Thus, the extracts of *Luffa operculata* are a good source of active principles with pharmacological activity.

Keywords: Buchinha do Norte; polyphenols; DPPH; ABTS; antimicrobial activity.

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Introduction

The use of natural products for medicinal purposes is one of the oldest practices by humans, showing an important role in global health, and symbolizing the main source of many ethnic groups (Veiga Junior, Pinto, & Maciel, 2005). However, the pharmacological properties of natural products are not fully explained (Veiga Junior et al., 2005). Therefore, the population use plants for phytotherapy purposes, which is necessary scientific research regarding to their benefits actions. Such studies allow the discovery of new drugs and attest the biological effects of medicinal plants.

The biological and pharmacological properties of plants can be attributed to the presence of chemical compounds on their crude extracts (Kasote, Katyare, Hegde, & Bae, 2015). Some plants are rich in polyphenols compounds (Metodiewa, Kochman, & Karolczak, 1997), which can be responsible for the antioxidant property showed by plants extracts. The antioxidant activity intensifies the therapeutic effects of plants, through their ability to act against some oxidant agents, promoting health maintenance, as well as in the prevention of diseases (Smina, Mathew, Janardhanan, & Devasagayam, 2011).

Under oxidative stress, the presence of free radicals causes tissue damage, like as lipoperoxidation of cell membranes, activation of proinflammatory agents and aeroallergen sensitization (Diaz-Sanchez, Garcia, Wang, Jyrala, & Saxon, 1999; Halliwell & Gutteridge, 1990). Oxidative stress is associated with many diseases such as rhinosinusitis (Kinnula, 2005).

In the literature, it is shown that natural products also have antimicrobial activity (Nascimento, Locatelli, Freitas, & Silva, 2000). The search for active compounds with different mechanisms of inhibition of microorganism growth is increasing due to the high incidence of resistant microorganisms in clinical microbiology in Brazil (Montelli & Levy, 1991). *Luffa operculata* (L.) Cong, commonly known as sponge cucumber or wild loofa, is used in Brazil in the treatment of rhinosinusitis (Diretrizes Brasileiras de Rinossinusites, 2008). This plant is traditionally used by inhaling the fruit aqueous extract, which can promote the discharge of mucus and relief from nasal obstructions (Diretrizes Brasileiras de Rinossinusites, 2008).

The pharmacological mechanisms of action of this plant is not completely explained, however its nasal decongestant properties were attributed to the presence of cucurbitacins and glycosides, which promote the exudation and improve the ciliary motility, together with the emollient effect of saponins (Lorenzi & Matos, 2002). Different classes of chemical compounds have already been identified in the sponge cucumber, such as 2,3-dicaffeoylglycaric acid, a substance which is related to the antibacterial activity of the extracts. Besides that, some flavonoids, saponins, free steroids and phenolic acids have been isolated from this species (Heiser & Schilling, 1988; Lorenzi & Matos, 2002).

Previous studies have demonstrated that the use of *Luffa operculata* proved to be safe and effective in the treatment of rhinosinusitis, as well as revealed antibacterial potential (Passali et al., 2015; Scalia, Dolci, Ueda, & Sassagawa, 2015). In this sense, the aim of the present work was to evaluate the chemical composition, antioxidant and antimicrobial activities of *Luffa operculata* extracts.

Material and methods

Plant extract preparations

The dried material (fruit) and the extract (hydroalcoholic 65%) of *Luffa operculata* 10% (COM) for the nasal use with the indication of use for sinusitis and rhinitis were obtained commercially in the city of Ponta Grossa, Brazil. The aqueous extract (INF) was obtained by infusion by covering and soaking the leaves with boiling water for 30 minutes, filtered and cooled to room temperature. It was also prepared the hydroalcoholic extract 65% (HEtOH) and ethanolic (EtOH) extract by maceration, addition of the extractor liquid in light shelter and occasional agitation for 7 days. The standardized concentration was 10% when compared to the commercially obtained extract**.**

Determination of total phenolic compounds

Total phenolic content of the *Luffa operculata* extracts was determined by using Folin-Ciocalteu's reagent (Singleton, Orthofer, & Lamuela-Raventós, 1999). 5 µL of samples of each extract were mixed with 10% (v/v) Folin-Ciocalteu reagent and 7.5% (m/v) aqueous solution of Na_2CO_3. The absorbance was measured at 765 nm after 15 minutes of incubation at 45ºC. Total phenolic content of each extract was determined from the analytical curve of gallic acid standard and the results were expressed as milligram of gallic acid per gram of extract (mg GA g^{-1}).

Determination of total flavonoid contents

Total flavonoid content of the *Luffa operculata* extracts was determined by complexation with aluminum ion Al(III) (Kosalec, Bakmaz, Pepeljnjak, & Vladimir-Knezević, 2004). 5 µL samples of each extract were mixed with 10% (w/v) $AlCl_3$ and 0.1 mol L^{-1} CH_3COOK. The absorbance was measured at 420 nm. Total flavonoid content for each extract was determined from the analytical curve of quercetin standard and the results were expressed as milligram of quercetin per gram of extract (mg QER g^{-1}).

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging activity

Antioxidant activity of *Luffa operculata* extracts was evaluated by $ABTS^{\bullet+}$ method as described by (Pellegrini, Re, Yang, & Rice-Evans, 1999) by using ascorbic acid as standard. First, 20 µL of samples of extracts at different concentrations (1–100 µg mL^{-1}) were mixed with sodium phosphate buffer (pH 7.4) and $ABTS^{\bullet+}$ solution. After 30 minutes of incubation in the absence of light, the absorbance was measured at 734 nm. The results were expressed as IC_{50} values.

2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity

Antioxidant activity of the *Luffa operculata* extracts was also evaluated by $DPPH^{\bullet}$ method (Brand-Williams, Cuvelier, & Berset, 1995) by using ascorbic acid as standard compound. Samples containing 20 µL of extracts at different concentrations (1–100 µg mL^{-1}) were mixed with $DPPH^{\bullet}$ ethanolic solution, followed by the addition of ethanol. The absorbance was measured at 518 nm after 30 minutes of incubation in the absence of light. All the results were expressed as IC_{50} values.

Antimicrobial activity

An initial screening of the antibacterial potential for *Luffa operculata* extract was performed by testing the inhibition of a gram positive (*S. aureus*), gram negative (*E. coli*) and yeast fungus (*C. albicans*). Antimicrobial activity of *Luffa operculata* was analyzed using the minimum inhibitory concentration technique (MIC), determinate by microdilution technique that was adapted from the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2014). The strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* 25922 e *Candida albicans* 10231 were used. The extracts were diluted in 2.5 % DMSO and in Muller-Hinton broth for bacteria and in Muller-Hinton broth with 2% of glucose for yeast fungus, in the final concentrations of 2000-625 μg mL^{-1}. The strains were prepared in sterile physiological solution, which contained 1.0-2.0 x 10^8 UFC mL^{-1} for bacteria and 1.0-2.0 x 10^6 UFC mL^{-1} for yeast, equal to the MacFarland's scale 0.5 standard. Thereafter, it was diluted (1:10) and 50 μL of the microorganisms and added to the antimicrobial activity test. Next, the plaques were incubated at 35ºC for 24 hours. After incubation, the optic density (equal to the turbidity produced by the growth of microorganisms in broth) was measured at 630 nm.

Statistical analysis

The results were expressed as mean±standard deviation for IC_{50} values and total content of phenolics and flavonoids. Tukey and Bonferroni tests were performed for antioxidant and antimicrobial activities. The software GraphPad Prism 5.0 was used for statistical analysis.

Results

Total phenolic compounds and flavonoids

The total content of phenolics and flavonoids are observed in Table 1. It can be verified that the HEtOH and INF samples exhibited a similar content of polyphenols, being the COM sample the one with lowest polyphenols quantities.

Table 1. Total content of phenolic, flavonoid and antioxidant activity of Luffa operculate extracts.

	Total phenolics (mg GA g^{-1})*	Total flavonoids (mg QER g^{-1})*	$ABTS^{\bullet+}$ IC_{50} (μg mL^{-1})*	$DPPH^{\bullet}$ IC_{50} (μg mL^{-1})*
COM	66.20 ± 2.02^{C}	58.30 ± 0.91^{C}	27.30 ± 2.89^{D}	53.47 ± 4.60^{D}
HEtOH	88.40 ± 0.89^{A}	79.24 ± 0.92^{A}	9.88 ± 1.74^{B}	14.02 ± 0.82^{B}
EtOH	70.80 ± 1.02^{B}	66.90 ± 2.10^{B}	19.56 ± 3.29^{C}	29.75 ± 4.02^{C}
INF	83.30 ± 2.62^{A}	72.15 ± 0.59^{A}	12.84 ± 1.86^{B}	14.37 ± 3.40^{B}
Ascorbic acid	-	-	4.84 ± 1.80^{A}	5.05 ± 2.12^{A}

*Data presented as average ± standard deviation. Letters (^{A,B,C,D}) indicate a significant statistical difference between the averages ($p < 0.05$).

Antioxidant activity

Table 1 shows the IC_{50} values obtained in the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ methods for the extracts. Of the analyzed extracts, HEtOH extract exhibited the highest antioxidant activity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. However, it presented a significant difference ($p < 0.05$) to the same concentration extract commercially obtained. The IC_{50} values observed for the COM extract were higher, which means a higher concentration of the extract is necessary to reach an antioxidant activity, suggesting a weaker antioxidant activity. As for the INF extract, no significant difference was found ($p < 0.05$), indicating a potential activity close to the HEtOH extract, and a good antioxidant activity. The EtOH also had a significant difference compared to other extracts.

Antimicrobial activity

Table 2 shows the percentage of inhibition for microorganism growth. The HEtOH extract showed a MIC of 1.000 μg mL^{-1} for *E. coli*, 500 μg mL^{-1} for *S. aureus* and 2.000 μg mL^{-1} for *C. albicans,* being *S. aureus* the most sensitive pathogen to the extract, and *C. albicans* the most resistant. While the other extracts did not present inhibition of 100% of the microorganisms and under a 2.000 μg mL^{-1} concentration, all extracts reached an inhibition of at least 50 % (IC_{50}).

Table 2. Percentage inhibition of microorganism growth.

		% inhibition of microorganism growth					
		2.000 μg mL^{-1}	1.000 μg mL^{-1}	500 μg mL^{-1}	250 μg mL^{-1}	125 μg mL^{-1}	62.5 μg mL^{-1}
E. coli	HEtOH	100**	100*	82**	67**	62**	58**
	EtOH	84**	71**	67**	43**	31**	21^{n}
	COM	62**	56**	46**	26**	11^{n}	9^{n}
	INF	92*	88**	76**	68**	52**	31**
S. aureus	HEtOH	100**	100**	92,8*	87**	72**	66**
	EtOH	94*	89**	77**	56**	47**	32**
	COM	82**	76**	46**	38**	26**	22**
	INF	90*	81**	69**	53**	52**	38**
C. albicans	HEtOH	100*	88**	82 **	77**	56**	46**
	EtOH	86**	75**	47**	43**	36**	29**
	COM	48**	37**	35**	32**	8^{n}	6^{n}
	INF	80**	68**	46**	34**	21**	12^{n}

Data presented in %. *MIC, ** $p < 0.05$, ^{n}not significant

Discussion

Within plants, there is a variety of different classes of phenolic compounds with distinct polarities and chemical properties. Therefore, an adequate selection of solvents plays an essential role in determining the nature and quantity of extracted compounds (Bhebhe, Füller, Chipurura, & Muchuweti, 2016). From our results (Table 1), it was observed that HEtOH presented more phenolic and flavonoid levels than the EtOH extract. This behavior is in accordance with some works reported in literature (Pérez-Jiménez & Saura-Calixto, 2006), since it is known that the addition of water creates an increase in polarity, which facilitate the extraction of the mentioned compounds.

The presence of polyphenols in the extracts is in accordance with previous studies, that have identified the presence of these compounds in *Luffa operculate* (Schilling & Heiser, 1981; Silva, Costa, Souza, Lopes, & Ueda, 2018). In the COM sample, a lower level of these compounds was observed, even with the same concentration as HEtOH, which could be explained by deficient inspection and quality control of the natural products (Newman & Cragg, 2016), allowing for errors in the indicated concentration, as well as an incorrect storage, causing degradation of the compounds, a process which has been described in the literature by (Devlin & Harris, 1984).

Antioxidant activity by using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities were employed in order to verify the free radical sequestration capacity of natural products (Alves, David, David, Bahia, & Aguiar, 2010) and these methods are correlated with the total content of phenolic compounds and flavonoids (Waterman & Mole, 1994). In this work, it was demonstrated that the extracts which showed the highest content of polyphenols on their chemical composition exhibited the lowest IC_{50} values on these methods.

HEtOH extract showed a higher antioxidant activity, with the lowest IC_{50} values levels, in the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays (Table 1) indicating a superior antioxidant activity to the one found in *Luffa cylindrica* (IC_{50} of 26.46 μg mL^{-1} for $DPPH^{\bullet}$; (Sharma et al., 2012) and *Luffa acutangula* Var. *amara* (IC_{50} of 43.76 μg mL^{-1} and 84.00 μg mL^{-1} for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, respectively; (Kalaskar & Surana, 2014). However, the COM sample presented a significant difference ($p < 0.05$), with higher IC_{50} values. Higher concentrations of extract are necessary to reach the same antioxidant effect, and therefore, a lower antioxidant potential.

Generally, the extracts presented a good activity in scavenging free radicals, indicating a potential pharmacological activity in preventing oxidative stress and worsening, or development of inflammatory diseases such as rhinosinusitis (Heffner & Repine, 1989; Metodiewa et al., 1997). It has been demonstrated that antioxidants tended to improve and reduce inflammation in patients with respiratory diseases after treatment with these compounds. This can be explained probably due to the decrease in inflammatory stimuli released by free radicals mediation (Valko et al., 2007). Although the initial results are promising and indicate that the employment of such compounds may be useful in the treatment of inflammatory respiratory diseases, it is necessary further investigations to validate its use (Barnes, 2008).

An initial screening of the antibacterial potential for *Luffa operculata* extract was performed by testing the inhibition of a gram positive (*S. aureus*), gram negative (*E. coli*) and yeast fungus (*C. albicans*). Differently from what happens to antibiotics, there are few instances reported in the literature for a potential mechanism of action to natural products. The compounds isolated from plants are substances

which could act on the intermediate metabolism, activating enzymes, altering the activity of inhibitors that influence the nutrients in the medium, interfering in enzymatic processes at a nuclear or ribosomal level, and causing changes to membranes or even interfering with secondary metabolism (Cowan, 1999). The crude extracts have a promising antimicrobial activity, which can be related to synergism between phytochemical constituents of plants (Delgado-Adámez, Fernández-León, Velardo-Micharet, & González-Gómez, 2012; Lee & Lee, 2010).

The compounds present in the *Luffa operculata* extracts suggest a relative antimicrobial activity, with a good response against *S. aureus*, especially for HEtOH, with a MIC of 500 μg mL^{-1}, while for *E. coli* a MIC of 1.000 μg mL^{-1} was observed. The greater sensitivity of *S. aureus* is in accordance to literature, which indicates a greater sensitivity of this bacterium to secondary metabolites (Ferreira et al., 2010). The double membrane present in gram-negative bacteria makes up a complex casing, responsible for the lower sensitivity of these microorganisms to plant extracts (Francescato, Deuschle, Mallmann, Alves, & Heinzmann, 2007), as well as lower antifungal activity in natural compounds (Estevez-Braun, Estevez-Reyes, Moujir, Ravelo, & Gonzalez, 1994).

The results obtained for the extracts COM is in accordance with other essays that used commercial extract of *Luffa operculate* (Scalia et al., 2015). In general, the results demonstrated a biological potential of inhibition of bacteria and fungi for the extracts, which may be further explored with more tests employing additional microorganisms.

Conclusion

It was possible to observe that HEtOH was the extract that presented the best results for the applied tests, while the COM showed worse performance. It is considered that the present *in vitro* study contributed to the expansion of the knowledge regarding the antioxidant and antimicrobial activities of the *Luffa operculata* extracts. It showed promising potential for new studies and possible application as antioxidant or antimicrobial of natural origin, besides generating a screening of potential order of activity of the extracts. Thus, hey may be important sources of compounds for biological activities.

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