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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *LICANIA TOMENTOSA* (BENTH.) FRITSCH (CRHYSOBALANACEAE)

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Abstract - This work describes the chemical composition, and evaluates the antimicrobial and antioxidant activities of a hydroalcoholic extract from the leaves of the *Licania tomentosa.* Gram positive and negative bacterial strains were used in this work. Examination of the phytochemical composition of *L. tomentosa* revealed the presence of secondary metabolites such as tannins, flavonoids, saponins, alkaloids, steroids and triterpenoids. An antibacterial assay pointed out that the extract had a lower minimal inhibitory concentration (MIC - 32 μg/mL) towards *Staphylococcus aureus* (ATCC12692). The extract also presented antibacterial activity against other assayed bacteria, with the MIC varying between 64 and 512 μg/mL. Our findings reveal that the extract presented an antioxidative capacity lower than that of BHT at the same concentration, used as positive control. Our results suggest that the levels and combinations between the secondary metabolites of this plant should be investigated to explain the demonstrated antibacterial activity.

Keywords: *Licania tomentosa*, Chrysobalanaceae, antibacterial activity, antioxidant activity

INTRODUCTION

The use of medicinal plants to combat various diseases as an alternative therapy, mainly by groups with health assistance difficulties, is common in developing countries due its accessibility and low cost. According to the World Health Organization (2002), it is important to invest in traditional medicine to improve the general health status (Silveira et al., 2007).

The Chrysobalanaceae family comprises 17 genera and about 450 species with worldwide distribution (Brummitt, 1992). Some species are used in folk medicine as hypoglycemic, anti-inflammatory, and for the treatment of diarrhea, dysentery and malaria (Castilho et al., 2000; Zuque et al., 2004; Agra et al., 2008, Ruiz-Teran et al., 2008). Other species of Chrysobalanaceae have presented cytotoxic, antitumor, antifungal, antibacterial, toxic and antioxidant activities (Suffness et al., 1988; Braca et al., 2002; Lee et al., 1996; Garo et al., 1997; Fernandes et al., 2003; Zuque et al., 2004).

Licania tomentosa, typical in the northeastern region of Brazil, is popularly known as "oiti" and is used as a hypoglycemic and diuretic (Castilho et

al., 2000; Lorenzi, 2000; Machado et al., 2006; Rossato et al., 2008). Previous studies were related molluscicidal, antitumoral and antiviral activities (Bilia et al., 2000; Miranda et al., 2002; Fernandes et al., 2003).

Therefore, considering the potential pharmacological properties of Chrysobalanaceae species, the objective of this work was to evaluate the antioxidant and inhibitory activity of *L. tomentosa* against bacterial pathogens.

MATERIALS AND METHODS

Plant material and extract preparations

Leaves of *Licania tomentosa* (Benth.) Fritsch, Chrysobalanaceae, were collected in the municipality of Juazeiro do Norte, Ceará State, Brazil, in July 2009 (7º 12' 47" S; 39º 18' 55" O). The plant material was identified and a voucher specimen was deposited with the respective number #44569 at the Herbarium Prisco Bezerra, Department of Biology, Federal University Ceara (UFC). A quantity of 280 g of aerial parts was dried at 50ºC for 72 h. The material was extracted by maceration using 1 L of 95% ethanol and water (1:1) as a solvent at room temperature, and the homogenate was allowed to stand for 72 h at room temperature. The extract was then filtered and concentrated under vacuum in a rotary evaporator (model Q-344B – Quimis, Brazil) and ultrathermal bath (model Q-214M2 – Quimis, Brazil) followed by lyophilization until complete dehydration, resulting in a yield of 10.87%.

Phytochemical content

Examination of the phytochemical composition of hydroalcoholic extracts from *L. tomentosa* was undertaken in order to detect the presence of secondary metabolites was performed following the method described by Matos (1997).

Antibacterial activity evaluation

The antibacterial activities of the extracts were investigated by employing a microdilution method, recommended by NCCCLS M7-A6 (NCCLS, 2003). Brain Hear Infusion Broth (BHI 3.8%) was used for bacterial growth (24 h, 35±2ºC). The inoculum was an overnight culture of each bacterial species in BHI broth diluted in the same media to a final concentration of approximately $1x10^8$ UFC/mL (0.5 nephelometric turbidity units (McFarland scale). After this, the suspension was diluted to $1x10^6$ UFC/mL in 10% BHI. 100 µL of each dilution were distributed in 96-well plates with extracts in different concentrations, achieving $5x10^5$ UFC/mL as the final concentration of the inoculum. Nine bacterial strains were used, clinical isolates or standard strains: *Staphylococcus aureus* MR6538, *S. aureus* ATCC12692, *Bacillus cereus* ATCC33018, *Pseudomonas aeruginosa* ATCC15442, *Klebsiella pneumoniae* ATCC10031, *Proteus vulgaris* ATCC13315, *Escherichia coli* MR27, *E. coli* ATCC25922, *E. coli* ATCC10536). The extracts were dissolved in distilled water and dimethyl sulfoxide (DMSO) to a concentration of 1024 µg/mL. Further serial dilutions were performed by the addition of BHI broth to reach a final concentration in the range of 512 at 8µg/mL. All experiments were performed in triplicate, and the microdilution trays were incubated at 35±2ºC for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of resazurine staining (0.01%) aqueous solution in each well at the end of the incubation period. The minimal inhibitory concentration (MIC) was defined as the lowest the extracts were able to inhibit bacteria growth.

Antioxidant activity

The free radical scavenging activity of the extract *Licania tomentosa* plant was evaluated as described by Mensor et al. (2001). Briefly, the plant extract was mixed with a 0.3 mM DPPH ethanol solution, to give final concentrations of 5, 10, 25, 50 and 125 µg of extract per ml of DPPH solution. After 30 min at room temperature, the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity.

Table 1. Values of the minimal inhibitory concentration (MIC) of hydroalcoholic extract of *L. tomentosa* (HAELT).

Strains	MIC (μg /mL)
Staphylococcus aureus ATCC12692	32
Bacillus cereus ATCC33018	64
Escherichia coli 27	64
Klebsiella pneumoniae ATCC10031	256
E. coli ATCC25922	512
Proteus vulgaris ATCC13315	512
E. coli ATCC10536	> 512
Pseudomonas aeruginosa ATCC15442	> 512
S. aureus 358	>512

Table 2. The DPPH free radical scavenging activity of hydroalcoholic extract of *L. tomentosa* (HAELT) and EC_{50} value in the DPPH scavenging activity.

Concentration (μg/mL)	HAELT	BHT
5	1,91±1,08	3,30±0,06
10	5,36±0,24	10,52±0,11
25	15,22±1,74	31,82±0,22
50	35,08±1,47	60,12±0,11
125	68,91±3,35	88,03±0,49
EC_{50}	73,33±4,43	35,50±0,50

BHT - butylated hydroxytoluene; DPPH - 1,1-diphenyl-2-picrylhydrazyl.

RESULTS AND DISCUSSION

This is the first report of the antimicrobial and antioxidant activities of *L. tomentosa*. The phytochemical composition of *L. tomentosa* leaf extract indicated the presence of secondary metabolites such as tannins, flavonoids, saponins, alkaloids, steroids and triterpenoids.

Kaplan and Castilho (2008) described the chemical constituents isolated from the leaves and fruits of *Licania tomentosa*. This study showed the presence of terpenes, betulinic acid, ursolic acid, oleanolic acid, palmitoleic acid, hexadecanoic acid, and other compounds. Other members of Chrysobalanaceae (Tommasi et al., 2003), such as *Chrysobalanus*, *Coupeia* and *Parinarium* also presented similar chemical composition, with tannins, flavonoids, diterpenes, terpenes and steroids as secondary metabolites (Bilia et al., 1996; Oberlies et al., 2001; Fernandes et al., 2003; Zuque et al., 2004; Castilho et al., 2005; Carvalho et al., 2008; Carvalho and Costa, 2009).

The antimicrobial activity is shown in Table 1 with MIC varying between 32 and ≥ 512 μg/mL against the pathogenic microorganisms tested. A lower MIC (32 μg/mL) was observed against *S. aureus* ATCC12692, but the extract presented an interesting MIC of 64 μg/mL against *B. cereus* ATCC33018 and *E. coli* 27. Alves et al. (2008) compared several methods for antibacterial activity screening and concluded that the microdilution broth method was the most adequate method, mainly due to its accuracy, ease and low cost (Silveira et al., 2005; Santos et al., 2007; Rotava et al., 2009). There are few studies reporting the antibacterial activity of natural products from Chrysobalanaceae. According to Zuque et al. (2004), *Coupeia grandiflora* and *Atuna racemosa* showed activity against pathogens such as *S. aureus*, *E. coli* and *P. aeruginosa*.

In this study we considered EC_{50} = 35.5 µg/mL from BHT to evaluate the antioxidant potential of natural products using different concentrations. The antioxidant activity data obtained are shown in Table 2. The hydroalcoholic extract does not present significant activity in the decomposition of DPPH radicals compared to BHT, a a synthetic antioxidant (EC_{50} = 73.33 µg/mL).

Oxidative compounds are natural byproducts of metabolism. However, when there are discrepancies between the production of oxidizing agents and their degradation, oxidative stress occurs. This problem can cause cell damage and is related to a number of diseases. Antioxidants are compounds or substances responsible for quenching free radicals (Sies, 1991; Dröge, 2002; Santos et al., 2010). The extract from *L. tomentosa* showed a good antioxidant effect that can be related to the presence of polyphenols, such as ursolic acid, and flavones such as lupeol. The activity of polyphenols against several forms of cancer, proliferative diseases, inflammation, and neurodegeneration is well-reported (Ciriolo et al., 2008) and is mainly exerted through the inhibitory and modulatory activities against a wide range of receptors, enzymes and transcriptional factors (Rice et al., 2004). Flavonoids and flavones showed an antioxidative activity by different mechanisms, including the scavenging of free radicals, chelation of metals, as well as the mediation and inhibition of enzymes. Kadam et al. (2010) have also associated these natural products with other important effects to health, where these products demonstrated anticarcinogenic and antimutagenic potentials related to their antioxidative property, which is an important effect resulting from the protection against cellular oxidative damage. The antimicrobial activities of tannins are also well documented. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins.

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are applied to fat and fatty foods to prevent oxidative deterioration. However, carcinogenic and anticarcinogenic properties have been reported for both synthetic antioxidants (Botterwerck et al., 2000). Therefore, there is an increasing interest in finding antioxidants derived from natural origins to prevent oxidative stress.

CONCLUSION

The results reported here are relevant and can be considered as the first information about the *in vitro* antimicrobial and antioxidant properties of *L. tomentosa*. Our study confirms that the extract of *L. tomentosa* presented antimicrobial and antioxidant activities. We suggest that the data obtained here may depend on the chemical composition of this species. Other studies are necessary to evaluate the actions of the isolated phytocompounds and to determine the real effect of these natural products alone or together to the demonstrated activities, creating options to use this plant as a source of natural products against infectious agents and diseases resulting from oxidative damage.

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