

Antimicrobial activity and phytochemical profile from the roots of *Lippia alba* (Mill.) N.E. Brown

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RESUMO: “Atividade antimicrobiana e perfil fitoquímico das raízes de *Lippia alba* (Mill.) N.E. Brown”. *Lippia alba* (Mill.) N.E. Brown (Verbenaceae) é geralmente usada na medicina popular brasileira para o tratamento de doenças gástricas, febre, asma e como tranqüilizante. Este trabalho avaliou a atividade antimicrobiana dos extratos acetato de etila, metanol e aquoso das raízes de *L. alba* usando métodos de difusão em poços e o perfil fitoquímico. Os resultados obtidos mostraram que os extratos acetato de etila e metanol apresentaram atividade antimicrobiana contra *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus aureus* (ATCC 6538) e *Klebsiella pneumonia* (ATCC 10031). Terpenóides, fenilpropanóides e açúcares foram detectados na análise fitoquímica.

Unitermos: *Lippia alba*, Verbenaceae, atividade antimicrobiana, análise fitoquímica.

ABSTRACT: *Lippia alba* (Mill.) N.E. Brown (Verbenaceae) is commonly used in the Brazilian folk medicine to the treatment of gastric illnesses, diarrhea, fever, asthma, and as a tranquilizer. This work evaluated the antimicrobial activity of ethyl acetate, methanol and aqueous extracts from the roots of the *L. alba* using plates-holes diffusion assay and the phytochemical profile. The results obtained showed that the ethyl acetate and methanol extracts presented antimicrobial activity against *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumonia* (ATCC 10031). Terpenoids, phenylpropanoids and sugars were detected in the phytochemical analysis.

Keywords: *Lippia alba*, Verbenaceae, antimicrobial activity, phytochemical analysis.

INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicine are still included as part of the habitual treatment of various maladies (Heinrich et al., 2004; Ríos et al., 2005).

Lippia alba (Mill.) N.E. Brown, also known as *Lippia geminata* HBK or *Lantana alba* (Mill), is a shrub about 3 m tall that belongs to the Verbenaceae family (Stashenko et al., 2003). In the Brazilian traditional medicine it's vulgarly known as erva-cidreira, chá-do-tabuleiro and salsa Limão (Braga, 1976; Matos, 1996). Its leaves are employed as infusion or decoction to the treatment of gastric illnesses, diarrhea, fever, asthma, cough and tranquillizing remedy (Matos, 1996; Tavares et al., 2005; Morais et al., 2005). Large variations have

been observed in the composition of *L. alba* essential oil, depending on the part of the plant employed in the distillation, on the plant's state of development and on the geographic location, the characteristics of the soil, climate, and others local conditions (Alea et al., 1997; Stashenko et al., 2003; Tavares et al., 2005). GC analyses of essential oil from three chemotypes of *L. alba* revealed the predominance of monoterpene type compounds such as citral (55.1%), β -myrcene (10.5%), and limonene (1.5%) (Matos, 1996; Julião et al., 2003). Few pharmacological studies have been done on the genus *Lippia*. Among these, the studies on the species *Lippia multiflora*, *Lippia organoides*, *Lippia sidoides*, *L. integrifolia*, *Lippia lacunosa*, *Lippia rotundifolia* and *Lippia alba* (Pascual et al., 2001; Fauth et al., 2002; Oliveira et al., 2006; Leitão et al., 2006; Barbosa-Filho et al., 2006) are included. The essential oil of *Lippia alba* showed antimicrobial activity against gram positive microorganisms, in general, with minimum inhibitory concentration (MIC) between 0.31-0.63 mg/mL (Alea et al., 1997).

Despite the popular use of *L. alba* as a medicinal plant, there are no data about the antimicrobial effect

and phytochemical profile from the roots of this vegetal specimen. Thus, the interest of this plant is justifiable because of its potential medicinal value.

MATERIAL AND METHODS

Microorganisms

Seven microbial species taken from international collections were analyzed: *Encherichia coli* (ATCC 9723), *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 6538P), *Enterococcus faecalis* (ATCC 33186), *Salmonella* sp (ATCC 8387) and *Pseudomonas aeruginosa* (ATCC14502).

Plant material

The vegetal specimens was collected in Timbaúba (7°35'S; 35°22'W), State of Pernambuco, Brazil in January 2005. The plant was identified by Prof. Dr. Haroudo Sátiro Xavier. A voucher specimen was deposited under n° 1011 at the Pharmacognosy Herbarium

of the Federal University of Pernambuco State -Brazil.

Crude extract preparation

The air dried and powdered roots (62.76 g) of *Lippia alba* (Mill) N.E. Brown were extracted separately and exhaustively with increase polarity of ethyl acetate (EEA), methanol (EME) and distilled water (EAQ) successively at room temperature (48 h for each solvent). Solvents were evaporated at 50 °C under reduced pressure affording the extracts coded as hexane extract (HE) 1.6%, ethyl acetate extract (EAc) 1.44%, methanol extract (MeE) 2.24% and water extract (WE) 3.22%.

Phytochemical profile

The chromatographic analyses were made by TLC on Si gel (MERCK-Germany, 105553) developed by different solvent systems: EtOAc-HCOOH-AcOH-H₂O (100 : 11 : 11 : 26, v:v), EtOAc-HCOOH-AcOH-H₂O (100 : 0.5 : 0.5 : 0.5, v:v), Et₂O-toluene-AcOH 10 % (50 : 50 : 50, v:v). Toluene-AcOEt (97 : 3 v/v), n-BuOH-Me₂CO-Buffer Phosphate pH = 5.0 (40 : 50 : 10 v/v)

Table 1. Antimicrobial activities of the ethyl acetate, methanol and aqueous extracts of *Lippia alba*.

| Microorganism | Extracts | | | | | | Positive controls Tetracycline |
|--|----------|-----|----------------|-----|-----|-----|-----------------------------------|
| | EEA | | (mg/mL) EME | | EAQ | | |
| | 50 | 100 | 50 | 100 | 50 | 100 | |
| <i>Encherichia coli</i> (ATCC 9723) | - | - | - | - | - | - | 25 |
| <i>Klebsiella pneumonia</i> (ATCC 10031) | - | 12 | - | - | - | - | 25 |
| <i>Staphylococcus aureus</i> (ATCC 6538) | 11 | 13 | - | 12 | - | - | 25 |
| <i>Staphylococcus aureus</i> (ATCC 6538P) | 12 | 13 | - | 13 | - | - | 25 |
| <i>Enterococcus faecalis</i> (ATCC 33186) | - | - | - | - | - | - | 28 |
| <i>Salmonella</i> sp (ATCC 8387) | - | - | - | - | - | - | 23 |
| <i>Pseudomonas aeruginosa</i> (ATCC14502) | NT | NT | NT | NT | - | - | 19 |

Diameter of zone (mm), (-) negative; NT: not tested

Table 2. Minimum inhibitory concentration (MIC) exhibited by the EAA and EME.

| Microorganism | MIC (mg/mL) | |
|---|-------------|-----|
| | EAA | EME |
| <i>Klebsiella pneumonia</i> (ATCC 10031) | >2 | >2 |
| <i>Staphylococcus aureus</i> (ATCC 6538) | 0,5 | 2 |
| <i>Staphylococcus aureus</i> (ATCC 6538P) | 1 | >2 |

Table 3. Chromatography condition of phytochemistry screening.

| | | | |
|---|---|------------------------------------|---------------------------------|
| Alkaloids | A | Dragendorff | (Wagner, 1996) |
| Monoterpenoid, sesquiterpenoids and diterpenoids | B | Vanillin sulphuric acid | (Wagner, 1996) |
| Triterpenoids / steroids | C | Lieberman / Burchard | (Harbone, 1998) |
| Iridoids | A | Vanillin sulphuric acid | (Wagner, 1996) |
| Saponins | A | Anisaldehyde | (Wagner, 1996) |
| Sugars | D | 2,3,5 Triphenyltetrazolium chlorid | (Wallenfels, 1950) |
| Coumarins | E | 2-Aminoethyldiphenyl borinate | (Wagner, 1996) |
| Flavonoids | A | 2-Aminoethyldiphenyl borinate | (Markhan, 1982) and (Neu, 1956) |
| Phenylpropanoids | A | 2-Aminoethyldiphenyl borinate | (Wagner, 1996) |
| Condensed proanthocyanidins and leucoanthocyanidins | A | Vanillin chloridric | (Roberts, 1957) |
| Quinones | C | KOH 10% | (Harbone, 1998) |
| A-EtOAc-HCOOH-AcOH-H ₂ O (100 : 11 : 11 : 26 v/v) | | | |
| B-Benzene-AcOEt (97: 3 v/v) | | | |
| C- EtOAc-HCOOH-AcOH-H ₂ O (100 : 0,5 : 0,5 : 0,5 v/v) | | | |
| D- n-BuOH-Me ₂ CO-Buffer Phosphate pH = 5,0 (40 : 50 : 10 v/v) | | | |
| E- Et ₂ O-toluene-AcOH 10 % (50 : 50 : 50 v/v) | | | |

It was verified the presence or absence of terpenoids, steroids, saponins, sugars, flavonoids, phenylpropanoids, alkaloids, coumarins, condensed proanthocyanidins, leucoanthocyanidins and quinones (See Table 3).

Plate-hole diffusion assay

The microorganism cultures were grown in Müller Hinton agar at 37 °C. After 18 h of growth, each microorganism culture, were dissolved in NaCl 0.9% solution sterile until a concentration of 0.5 at MacFarland scale, was inoculated on the surface of Müller Hinton (MH) agar plates (100 µL). The methodology used was the plate-holes diffusion assay (Ieven et al, 1979; Caetano et al., 2002). The plates were incubated at 37 °C for 24 h; after this period, the zones of growth inhibition around the discs and the holes were measured.

Tetracycline was the positive control (1 mg/mL). All determinations were made in duplicate.

Minimum inhibitory concentration (MIC)

The concentrations of 0.031, 0.062, 0.125, 0.25, 0.5, 1 and 2 mg/mL by the EAA and EME were tested against the microorganisms: *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 6538P).

RESULTS AND DISCUSSION

A total of 7 microorganisms were tested and the results are summarized in Table 1, following by the Minimum inhibitory concentration in Table 2. The EAQ extract of *L. alba* did not show any activity.

As can be observed in Table 1, the EEA and EME possessed the antimicrobial activity against some microorganism tested. In the assays against microorganisms by plates-holes diffusion method, the zones obtained were between 11 to 13 mm.

The EEA and EME extracts of *L. alba* were activity against *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumonia* (ATCC 10031).

The MIC values obtained were ranged between 0.5 - 2 mg/mL (Table 2). The best results were observed for the EEA extract against *Staphylococcus aureus* (ATCC 6538).

The phytochemical profile from the roots of *L. alba* detected the presence of terpenoids, phenylpropanoids, and sugars. On the other hand, It wasn't verified the presence of coumarins, condensed proanthocyanidins, leucoanthocyanidins, saponins, flavonoids, alkaloids, steroids and quinones.

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