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# Antioxidant capacity and larvicidal activity of essential oil and extracts from *Lippia grandis*

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**Abstract:** The leaves and thin branches of *Lippia grandis* Schauer, Verbenaceae, are used for flavoring of food in the Brazilian Amazon, as substitute for oregano. In this study the constituents of the essential oil were identified and the antioxidant capacity and larvicidal activity of the oil and methanol extract and its sub-fractions were evaluated. A sensory evaluation was determined in view of absence of toxicity. The oil showed a yield of 2.1% and its main constituents were thymol (45.8%), *p*-cymene (14.3%), γ-terpinene (10.5%), carvacrol (9.9%) and thymol methyl ether (4.8%), totalizing 85%. The DPPH radical scavenging activity showed values for the EC50 between 9.0 and 130.5 μg $mL^{-1}$ and the TEAC/ABTS values varied from 131.1 to 336.0 mg TE/g, indicating significant antioxidant activity for the plant. The total phenolic content ranged from 223.0 to 761.4 mg GAE/g, contributing to the antioxidant activity observed. The crude extracts inhibited the bleaching of β-carotene and the oil showed the greatest inhibition (42.5%). The oil (LgO, 7.6±2.4 μg $mL^{-1}$) showed strong larvicidal activity against the brine shrimp bioassay. The sensory evaluation was highly satisfactory in comparison to oregano. The results are very promising for the use of *L. grandis* in seasoning and antioxidant products.

## Introduction

The genus *Lippia*, Verbenaceae, comprises nearly 200 species of herbs, shrubs and small trees spread wide in South and Central America and Tropical Africa. *Lippia grandis* Schauer is an aromatic shrub up to 1.8 m in height, known popularly as "erva-do-marajó", growing wild in areas of savannas of North Brazil. The leaves and twigs are used for flavoring of food and its tea for ailments of stomach and liver in the Amazon (Maia et al., 2001).

The genus *Lippia* is well-known for its aromatic character and more than fifty of its essential oils have been reported (Terblaché & Kornelius, 1996; Pascual et al., 2001). The main volatile constituents frequently found in the oils of *Lippia* species are thymol, carvacrol, 1,8-cineole, limonene, *p*-cymene, linalool, α-pinene and β-caryophyllene. Many *Lippia* species has shown variation in the oil composition, producing several chemical types. This is the case of *L. alba*, where the chemotypes citral, carvone, 1,8-cineole and linalool have been identified in specimens collected at different regions of Brazil (Matos et al., 1996; Zoghbi et al., 1998; Atti-Serafini et al., 2002); the case of *L. lupulina* that occurs in distant areas of the Mato Grosso state, Brazil, with the chemotypes terpinen-4-ol, 1,8-cineole/β-caryophyllene and germacrene D/β-caryophyllene/bicyclogermacrene (Zoghbi et al., 2001); the case of *L. glandulosa* that grows wild in the area of Lavrado, Roraima state, Brazil, with the chemotypes thymol and β-caryophyllene (Maia et al., 2005); the case of *L. origanoides* with three chemotypes registered at Brazilian and Colombian Amazon, where *p*-cymene, α- and γ-terpinene, thymol, carvacrol, β-caryophyllene, α-phellandrene and $\delta^3$-carene were the major constituents (Morais et al., 1972; Oliveira et al., 2007; Stashenko et al., 2008) and the case of *L. schomburgkiana* where 1,8-cineole was the main constituent found in its oil (Silva et al., 2009). Previously, we reported the oil composition of some natural varieties of *L. grandis* occurring at different localities and soil types of the Amazon. The oils showed distinct chromatographic profiles, indicating the presence of chemoypes thymol and 1,8-cineole (Silva et al., 1973; Maia et al., 2003).

Recently, there has been a growing interest in the search for spices, aromatic and medicinal plants

as sources of natural antioxidants. In general, the antioxidant capacity of these plant tissues is associated with the activity of the free radical scavenging enzymes and with the contents of antioxidant substances, mainly phenol compounds. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics has become increasingly important also because of concern about potentially harmful synthetic additives. The oils and extracts, in addition to the biologically active natural compounds have been proposed for the control of human illness, and the prevention of lipid peroxidative damage implicated in several pathological disorders, such as atherosclerosis, Alzheimer`s disease, carcinogenesis and aging processes (Ruberto & Baratta, 2000; Mimica-Durik et al., 2004).

Given the use of *L. grandis* as a flavoring for food, the presence of phenol compounds in the essential oil and the future prospect of its use as a nutraceutical product, we decided to study the chemical composition of the oil, antioxidant capacity, total phenolic content and the toxicity of their crude extracts, as well as do a sensory evaluation of its leaves and twigs.

## Material and Methods

### *Plant material*

The specimen *Lippia grandis* Schauer, Verbenaceae, was collected in the National Forest of Carajás, municipality of Parauapebas, Pará state, Brazil, April 2007, which occurs in endemic form. The plant was identified by comparison with authentic voucher (MG 133.921) existing in the Herbarium of Museu Paraense Emílio Goeldi, city of Belém, Pará state, Brazil.

### *Plant processing*

The aerial parts (leaves and thin branches) were air-dried, ground and submitted to hydrodistillation (300 g, 3 h) using a Clevenger-type apparatus. The oil (LgEo) was dried over anhydrous sodium sulfate and their percentage content was calculated on basis of the plant dry weight. The moisture content of the sample was calculated after the phase separation in a Dean-Stark trap (5 g, 30 min) using toluene. The plant material (30 g) was also submitted to Soxhlet extraction with methanol and water to obtain the extracts LgMe (2.6 g) and LgAq (1.9 g), respectively. The LgMe (2.0 g) extract was suspended in a water-methanol solution (1:1) and partionated with dichloromethane and ethyl acetate, resulting in following sub-fractions: hydro-alcoholic fraction (LgMe-Hf, 0.4 g), dichloromethane fraction (LgMe-Df, 0.5 g) and ethyl acetate fraction (LgMe-Ef, 0.8 g).

### *Oil-composition analysis*

The analysis of the oil was carry out on a Thermo DSQ II GC-MS instrument, under the following conditions: DB-5ms (30 m x 0.25 mm; 0.25 µm film thickness) fused-silica capillary column; programmed temperature: 60-240 °C (3 °C/min); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 32 cm/s (measured at 100 °C); injection type: splitless (2 µL of a 1:1000 hexane solution); split flow was adjusted to yield a 20:1 ratio; septum sweep was a constant 10 mL/min; EIMS: electron energy, 70 eV; temperature of ion source and connection parts: 200 °C. The quantitative data regarding the volatile constituents were obtained by peak-area normalization using a FOCUS GC/FID operated under conditions similar to those in GC-MS, except for the carrier gas, which was nitrogen. The retention index was calculated for all the volatiles constituents using an n-alkane homologous series.

### *DPPH radical scavenging assay*

A stock solution of DPPH radical (0.5 mM) in methanol was prepared. The solution was diluted in methanol (60 µM approx.) measuring an initial absorbance of 0.62±0.02 in 517 nm and room temperature. The reaction mixture was composed by 1950 µL of DPPH solution and 50 µL of the samples diluted in different methanol portions. For each sample a methanol blank was also measured. The absorbance was measured in the reaction starting (time zero), each 5 min during the first 20 min and later at continuous intervals of 10 min up to constant absorbance value. The concentration of antioxidant required for 50% scavenging of DPPH radicals (EC50) was determined by linear regression using Windows/Excel. All experiments were triplicate. BHA (butylated hydroxyanisole) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as standard antioxidants. The radical scavenging activity of each sample was calculated by the DPPH inhibition percentage according the equation $IP_{DPPH}$ =100(A-B)/A (where A and B are the blank and sample absorbance values in the end reaction). The radical scavenging activity, expressed as milligrams of trolox equivalent per gram of each sample, was also calculated by means of the equation TE = (A-B)/(A-C) x 25/1000 x 250.29/1000 x 1000/10 x D (where A, B and C are the blank, sample and trolox absorbance values in the end reaction, and D is the dilution factor) (Silva et al., 2007a).

### *ABTS radical cation decolorization assay*

The antioxidant activity was measured using an improved ABTS method (Re et al., 1999; Cai et al.,

2004). The absorbance of the reaction samples was compared to Trolox and the results were expressed in terms of Trolox Equivalent Antioxidant Capacity (TEAC). The TEAC value was calculated by the measurement of the area under the curve, plotting the percentage inhibition of the absorbance as a function of time. The calculation of the area under the curve was performed for one sample dilution which had a final percentage inhibition between 20% and 80% (Van Den Berg et al., 1999; Silva et al., 2007b). All determinations were performed in triplicate.

*β-Carotene bleaching assay*

A stock solution of β-carotene-linoleic acid mixture was prepared as following: 0.2 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade) and 20 µL of linoleic acid and 200 mg of tween 20 were added. Chloroform was completely evaporated in a vacuum rotary evaporator. Then, 50 mL of hydrogen peroxide was added with vigorous shaking. An aliquote of 2500 µL of this reaction mixture was dispensed into test tubes and 200 µL portions of the samples prepared at 4 mg $L^{-1}$ were added and the emulsion system was incubated for 120 min, at 50 °C. The same procedure was repeated with BHA, Trolox and a blank (methanol). The absorbance of these solutions at 470nm (UV-vis spectrophotometer, GBC 600) was recorded and monitored at intervals of 15 min during 120 min (Koleva et al., 2003). The antioxidant activity (AA%) was calculated in terms of percent inhibition relative to the control.

*Total phenolic content*

The amount of phenolic compounds (TP) was determined according to the Folin-Ciocalteu procedure (Singleton & Rossi, 1965; Kähkönen et al., 1999). The experimental calibration curve was prepared using 500 µL of aqueous solution of gallic acid mixed with 250 µL of Folin-Ciocalteu reagent (1.0 N) and 1250 µL of sodium carbonate (75g $L^{-1}$), resulting in final gallic acid concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg $L^{-1}$. The absorbance was measured after 30 min at 760 nm and 25 ºC (UV-vis spectrophotometer, GBC 600). The extracts were dissolved in methanol (5 mg $mL^{-1}$), diluted in water and submitted to the same procedure. The total phenolics content was expressed as gallic acid equivalents (GAE) in milligrams per gram of extract.

*Brine shrimp lethality bioassay*

It was carried out to investigate the larvicidal activity of the oil and extracts (Meyer et al., 1982; Lewan et al., 1992). Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a glass rectangular vessel (5 L) filled with sterile artificial seawater, pH 9.0 adjusted with $Na_2CO_3$, under constant aeration for 24 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary pipette and placed in vials containing 5 mL of brine solution. The oil and MeOH extract (5%) were prepared using the brine solution and DMSO. Four other solutions at different concentrations were prepared and then added to the 5 mL brine solution. The vials were maintained at room temperature for 24 h under light and the surviving larvae were counted. Experiments were carried out along with control and different concentrations (1 at 1000 µg $mL^{-1}$) using three replicates. The percentage lethality was determined by comparing the mean value of surviving larvae of the test and the control tubes. Lethal concentration (LC50) values were obtained from the best-fit line plotting concentration versus percentage lethality (Finney, 1971).

**Median lethal dose (LD50)**

Mice free from specific pathogens were subjected to single doses of aqueous extract (by oral gavages), at concentrations of 1000, 3000, and 5000 mg $kg^{-1}$. The animals were observed for a 48 h period for behavioral alterations or toxic effects. The project was approved by the Committee of Ethics in Animal Research of the University of Pará. The Median Lethal Dose (LD50) was determined by probit test (Finney, 1971).

*Sensory evaluation*

For a preliminary sensory evaluation were used the dried and ground leaves of *L. grandis*, powdered oregano (*Origanum vulgare*, Lamiaceae) and mini cheese pizzas. Both materials of *L. grandis* and oregano were spread on the mini pizza and submitted to selected tasters, separately. Then, the tasters rated the degree of acceptance based on 10-point hedonic scale, ranging from "very pleasant" to "very unpleasant" (Bender, 2005).

*Statistical analysis*

Samples were assayed in triplicate and the results are shown as means±standard deviation. Analysis of variance was conducted and the differences between variables were tested for significance by one-way ANOVA with Tukey's post test using Minitab version 14. Differences at $p<0.05$ were considered statistically significant. The type of relationship between variables was determined by

simple regression analysis.

## Results and Discussion

### *Oil-composition*

The leaves and thin branches of *L. grandis* provided an oil yield of 2.1% and its volatile constituents were analyzed by GC and GC-MS. Individual components were identified by comparison of both mass spectra and GC-retention data with authentic compounds which were previously analyzed and stored in the data system. Other identifications were carried out by comparison of the mass spectra with those existing in the data system libraries and cited in literature (Adams, 2007; Nist, 2005). Twenty-eight volatile constituents were identified in the oil and they are listed in Table 1. The main components were thymol (45.8%), *p*-cymene (14.3%), γ-terpinene (10.5%), carvacrol (9.9%) and thymol methyl ether (4.8%), totalizing 85%.

Two chemotypes were previously described for *L. grandis*: thymol and 1,8-cineol types (Silva et al., 1973; Maia et al., 2003). We have observed that the main components of the oils of *Lippia* species from the Amazon have varied with the part of plant, season, and collection site and oil percentage. Thus, some oils of *Lippia* species were analyzed by us confirming the presence of significant amounts of thymol, carvacrol, p-cymene, 1,8-cineole, γ-terpinene, (*E*)-caryophyllene, citral, carvone and terpinen-4-ol (Morais et al., 1972; Zoghbi et al., 1998; 2001; Maia et al., 2005; Silva et al., 2009).

### *DPPH-radical scavenging assay and total phenolic content*

The oil and methanol extract and sub-fractions obtained from *L. grandis* were assayed at various concentrations (set of four each sample) to determine the DPPH-scavenging activity, resulting in inhibition percentage varying from 7.9 to 87.7%. The EC50 value (defined as the concentration of antioxidant required for 50% of scavenging of DPPH radical) ranged from 9.0 to 130.5 μg $mL^{-1}$. EC50 values lower than 30 μg $mL^{-1}$ indicates a good potential for the radical scavenging (Ramos et al., 2003). The ethyl acetate sub-fraction (LgMe-Ef) (EC50, 9.0±0.1 μg $mL^{-1}$) was the most active, only two times smaller than Trolox (EC50, 4.0±0.1 μg $mL^{-1}$) and BHA (EC50, 4.1±0.1 μg $mL^{-1}$), therefore with the highest antioxidant activity. The hydro-alcoholic sub-fraction (LgMe-Hf) (EC50, 16.4±0.5 μg $mL^{-1}$), methanol extract (LgMe) (EC50, 19.7±1.2 μg $mL^{-1}$) and the oil (LgO) (EC50, 18.9±0.6 μg $mL^{-1}$) showed also significant antioxidant activities.

The DPPH radical scavenging was also calculated as trolox equivalent antioxidant capacity, whose results were similar those obtained for EC50, where the ethyl acetate sub-fraction (LgMe-Ef) (TEAC, 578.7±13.0 mg TE/g) was the most active, followed by the hydro-alcoholic sub-fraction (LgMe-Hf) (TEAC, 279.2±39.3 mg TE/g), methanol extract (LgMe) (TEAC, 260.2±8.0 mg TE/g) and the oil (LgO) (TEAC, 240.4±3.7 mg TE/g).

The amounts of total phenolics for the methanol extract (LgMe) (TP, 761.4±0.2 mg GAE/g), ethyl acetate sub-fraction (LgMe-Ef) (TP, 505.0±5.7 mg GAE/g) and hydro-alcoholic sub-fraction (LgMe-Hf) (TP, 336.5±3.5 mg GAE/g) were very expressive, confirming the antioxidant effects observed for the DPPH radical scavenging assay. This noteworthy antioxidant activity could be attributed to the presence of thymol, carvacrol and by more polar phenolic compounds existing in the plant. Taking into account that the dried methanol extract yielded 8.5%, the total phenolic content represents about 89.6% in the dried plant.

The data regarding the DPPH inhibition, EC50 and TEAC values and total phenolic content (TP) of the oil and methanol extract and sub-fractions of *L. grandis* are shown in Table 2.

### *ABTS-radical cation scavenging assay*

The oil and methanol extract and sub-fractions showed ABTS scavenging activities at different levels, which were expressed in terms of trolox equivalent antioxidant capacity (TEAC). The highest activity was obtained by the hydro-alcoholic sub-fraction (LgMe-Hf) (TEAC, 336.0 mg TE/g) and the oil (LgO) (TEAC, 131.1 mg TE/g) was the least active. The ABTS/TEAC assay confirmed the significant antioxidant activity obtained with the DPPH/TEAC assay and showed a small change in the activity of their crude extracts. In the ABTS/TEAC assay the hydro-alcoholic sub-fraction was the most active, whereas with the DPPH/TEAC assay it was the ethyl acetate sub-fraction. The techniques used are of good precision, but their measurements are performed in different types of solubility, justifying the small changes observed. For instance, the ABTS/TEAC assay was used to analyze the methanol extracts from thirty-two selected herbs, whose medium value was 33.2 mg TE/g (Wojdylo et al., 2007). In this case, the average TEAC value is seven times lower than that obtained for the methanol extract (LgMe) (TEAC, 241.3 mg TE/g) of *L. grandis*. The ABTS/TEAC values obtained for the oil and methanol extract and sub-fractions are shown in Figure 1.

**Table 1.** Constituents identified in the oil of *Lippia grandis* Schauer, Verbenaceae.

| Constituents | RI[a] | Oil %[b] | Constituents | RI[a] | Oil %[b] |
|---|---|---|---|---|---|
| α-Pinene | 939 | 2.5 | Carvacrol, methyl ether | 1245 | 0.3 |
| Sabinene | 975 | 0.3 | Thymol | 1290 | 45.8 |
| β-Pinene | 979 | 2.4 | Carvacrol | 1299 | 9.9 |
| δ-Carene | 1002 | 0.6 | α-Copaene | 1377 | 0.9 |
| p-Cymene | 1025 | 14.3 | Neryl acetate | 1362 | 0.1 |
| (E)-β-Ocymene | 1050 | 0.6 | (E)-Caryophyllene | 1417 | 1.7 |
| γ-Terpinene | 1060 | 10.5 | *trans*-α-Bergamotene | 1435 | 0.6 |
| Terpinolene | 1089 | 0.2 | α-Humulene | 1455 | 0.5 |
| Linalool | 1097 | 0.3 | *allo*-Aromadendrene | 1460 | 0.1 |
| Umbelulone | 1171 | 0.3 | Germacrene D | 1485 | 0.1 |
| Yerpinen-4-ol | 1177 | 0.7 | α-Muurolene | 1489 | 0.1 |
| α-Terpineol | 1189 | 0.3 | δ-Cadinene | 1523 | 0.6 |
| Verbenone | 1205 | 0.1 | Caryophyllene oxide | 1583 | 0.4 |
| Thymol, methyl ether | 1235 | 4.8 | Humulene epoxide II | 1608 | 0.1 |

[a]GC-MS retention Index on DB-5ms column, relative to *n*-alkanes; [b]Relative area percentage using GC-FID.

**Table 2.** DPPH radical scavenging activity and total phenolics (TP) content of the oil and methanol extract and sub-fractions of *Lippia grandis* Schauer, Verbenaceae.

| Samples | Concentration (μg mL$^{-1}$) | DPPH Inhibition[a] (%) | DPPH EC50[a] (μg mL$^{-1}$) | TEAC[a] (mg TE/g) | TP[a] (mg GAE/g) |
|---|---|---|---|---|---|
| LgO | 56.3 | 86.2±3.3 | 18.9±0.6 | 240.4±3.7 | |
| | 33.8 | 70.8±1.4 | | | |
| | 22.5 | 54.1±0.8 | | | |
| | 11.3 | 39.2±0.6 | | | |
| LgMe | 40.0 | 87.7±1.1 | 19.7±1.2 | 260.2±8.0 | 761.4±0.2 |
| | 30.0 | 72.5±4.1 | | | |
| | 20.0 | 52.0±1.6 | | | |
| | 10.0 | 28.9±2.7 | | | |
| LgMe-Df | 200.0 | 64.1±4.5 | 130.5±5.7 | 48.9±0.4 | 223.0±4.6 |
| | 150.0 | 56.5±2.4 | | | |
| | 100.0 | 48.9±0.4 | | | |
| | 50.0 | 24.6±0.6 | | | |
| LgMe-Ef | 15.0 | 84.9±1.5 | 9.0±0.1 | 578.7±13.0 | 505.0±5.7 |
| | 7.5 | 43.3±1.0 | | | |
| | 5.0 | 26.9±2.7 | | | |
| | 2.5 | 7.9±1.8 | | | |
| LgMe-Hf | 25.0 | 80.5±1.4 | 16.4±0.5 | 279.2±39.5 | 336.5±3.5 |
| | 18.8 | 57.7±1.8 | | | |
| | 12.5 | 34.9±4.9 | | | |
| | 6.3 | 15.1±2.9 | | | |
| Trolox | 10.0 | 94.7±2.3 | 4.0±0.1 | | |
| | 5.0 | 80.3±5.2 | | | |
| | 2.5 | 30.8±1.1 | | | |
| | 1.3 | 17.6±1.5 | | | |
| BHA | 8.0 | 73.8±2.8 | 4.1±0.1 | | |
| | 4.0 | 63.0±2.5 | | | |
| | 2.0 | 35.2±1.3 | | | |
| | 1.0 | 17.6±0.6 | | | |

amean±standard deviation

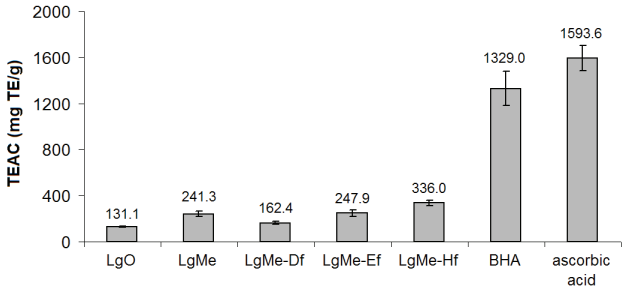


**Figure 1.** TEAC/ABTS values for the oil and methanol extract and sub-fractions of *Lippia grandis* Schauer, Verbenaceae.

*Bleaching of β-Carotene*

Oxidation of linoleic acid produces hydroperoxide-derived free radicals which attack the chromophore of β-carotene resulting in a bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene is described as a free radical scavenger and primary antioxidant (Frankel, 1980; Simic & Javanovic, 1994). The oil and methanol extract and the sub-fractions of *L. grandis* were able to inhibit the β-carotene bleaching. The oil showed the highest antioxidant activity, unlike the assays with DPPH and ABTS/TEAC where it was less active. Based on the bleaching of β-carotene, the antioxidant activities decreased in the following order: oil (LgO, 42.5%), aqueous sub-fraction (LgMe-Hf, 31.8%), ethyl acetate sub-fraction (LgMe-Ef, 27.4%), methanol extract (LgMe, 22.9%) and dichloromethane sub-fraction (LgMe-Df, 21.5%). The oil showed an antioxidant activity equal to 50% of the values observed for Trolox (87.5%) and BHA (86.9%), and about 100% of the activity of ascorbic acid (18.0%). This is in line with that proposed by Nenadis and co-workers (2003), in which the partition coefficient of the less polar combinations results in greater interaction with the lipid layer, thus showing a higher antioxidant activity. The antioxidant activities of the oil and methanol extract and sub-fractions, measured according to their abilities to prevent the oxidation of the emulsion β-carotene–linoleic acid, compared with standard antioxidants, are shown in Figure 2.

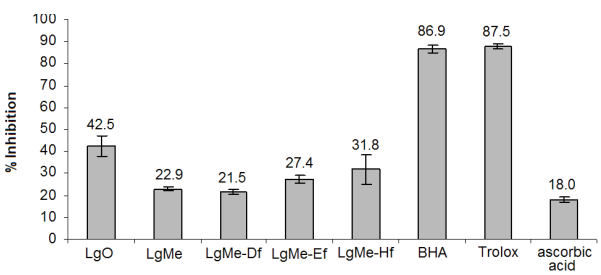


**Figure 2.** Bleaching of β-Carotene. Inhibition of the oil and methanol extract and sub-fractions of *Lippia grandis* Schauer, Verbenaceae.

*Brine shrimp bioassay*

The oil and the methanol extract of *L. grandis* showed high larvicidal activity against the brine shrimp assay, according Meyer and co-workers (1982), who classified crude extracts and pure substances into toxic (LC50<1000 μg $mL^{-1}$) and nontoxic (LC50>1000 μg $mL^{-1}$). The oil (LgO, 7.6±2.4 μg $mL^{-1}$) was the most toxic, showing evidences for significant biological activity. The methanol extract (LgMe, 121.6±5.3 μg $mL^{-1}$) showed only a moderate toxicity. The results for the brine shrimp assay are shown in Table 3.

**Table 3.** Brine shrimp bioassay (*Artemia salina*). Lethal concentration of the oil and methanol extract of *Lippia grandis* Schauer, Verbenaceae.

| Samples | Concentration (μg $mL^{-1}$) | Mortality (%) | LC50 (μg $mL^{-1}$) |
|---|---|---|---|
| LgO | 5.0 | 40.0 | 7.6±2.4 |
| | 10.0 | 90.0 | |
| | 25.0 | 100.0 | |
| LgMe | 25.0 | 10.0 | 121.6±5.3 |
| | 100.0 | 40.0 | |
| | 1000.0 | 100.0 | |

**Preliminary sensory evaluation**

The sensory evaluation was based on previous acute-toxicity study. The acute-toxicity analysis did not shows any toxic symptoms, changes in behavior or mortality of mice at doses of 1000, 3000, and 5000 mg $kg^{-1}$ of the aqueous extract. These results indicated that the median lethal dose (LD50>2500 mg $kg^{-1}$) of the aqueous extract of *L. grandis* has no toxicity. The grinded leaves of *L. grandis* were compared with oregano (*Origanum vulgare* L.) in the acceptance test, tried out by tasting judges, chosen at random. The same visual similarity was provided to the plant samples identified as "mini cheese pizzas enriched with herbs" and submitted to tasters, separately. The tasters judged the samples according to 10-points hedonic scale, ranging from "very pleasant" to "very unpleasant" (Bender, 2005). Thirty tasters, aged between 19 and 50 years, participated in the acceptance test. The percentage acceptance for the products was 77.3% for pizza oregano and 78.0% for pizza *Lippia grandis*, based on averages which were approximately at same level of acceptance.

**Conclusion**

The essential oil of *Lippia grandis* Schauer, Verbenaceae, collected in the National Forest of Carajás, state of Pará, Brazil, indicated a composition where thymol, *p*-cymene, γ-terpinene, carvacrol and thymol methyl ether were the main constituents. The values

obtained for DPPH inhibition, total phenolics content, ABTS/TEAC assay and bleaching of β-carotene showed significant antioxidant capacity for the oil and methanol extract and sub-fractions. We have in mind that the antioxidant activity can be attributed to the presence of thymol, carvacrol and by more polar phenolic compounds existing in the plant. The oil showed also high larvicidal activity against *Artemia salina*, so indicating a significant biological property. The aqueous extract was not toxic and the sensorial evaluation of *Lippia grandis* was highly positive as flavoring agent. These results can be considered as promising for the use of this plant in spice products like oregano.

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