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Original article

Ethnopharmacological studies of *Lippia origanoides*

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ABSTRACT

Lippia origanoides Kunth. Verbenaceae, is of great importance in the Brazilian traditional medicine. Because of it, this work had the purpose to contribute to the ethnopharmacological knowledge of *L. origanoides* through an ethnobotanical survey conducted within *quilombola* (maroon) communities of Oriximiná, Pará, Brazil. Among 254 plants cited in the survey, *L. origanoides* stood out among the ten most versatile species. The agreed main uses were to treat menstrual cramps, stomachache, and baby and postpartum colic. This could indicate a consensus of the informants to possible antispasmodic, anti-inflammatory and analgesic activities of *L. origanoides*. Therefore, anti-inflammatory and analgesic activities of *L. origanoides* extract (aerial parts) were assessed through thermal (hot plate) and chemical (formalin and acetic acid) models of nociception. A dose-dependent reduction in acetic acid-induced writhing was observed after treating mice with *L. origanoides* extract. The same extract also inhibited significantly formalin-induced licking response and proved to have a central antinociceptive effect, in the hot plate test. This work demonstrates that *L. origanoides* is used specially by *quilombola* women from Oriximiná for disorders of the genitourinary system and that biological activities of this species could contribute to these uses. Furthermore, it was also observed antispasmodic, analgesic and antimicrobial uses of other species of the genus *Lippia* (*Goniostachyum* section), rich in thymol and carvacrol.

Introduction

Lippia origanoides Kunth, Verbenaceae, is a very aromatic shrub distributed from southern North America to northern South America, with prominent occurrence in the Amazonian region of Brazil, the Guianas, Venezuela and Colombia (Maisch, 1885; Pascual et al., 2001). In Central America and Colombia, *L. origanoides* is indicated against respiratory diseases, such as flu, bronchitis, cough, and asthma; in the treatment of gastrointestinal disturbances like stomach pain, nausea, indigestion, and as carminative; as well as an antiseptic for the mouth, throat and wounds. The species is also used for cooking, as seasoning, and to whet the appetite (Morton, 1981; Stashenko et al., 2010). In Oriximiná city (Pará State), Northern Brazil, our group carried out an ethnobotanical survey of the urban population. The results showed that the leaves or aerial parts of *L. origanoides*, popularly known as "salva-de-marajó", have great importance in this locality where it is used for the treatment of stomachache, baby colic, indigestion, diarrhea, heartburn, nausea, flatus, "ladies

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belly'' (uterine inflammation), vaginal discharges, menstrual complaints and fever, as well as a general antiseptic for mouth, throat, vagina and to cleanse wounds (Oliveira et al, 2007). In continuation to our ethnobotanical studies in Oriximiná, a survey on the medicinal plants used by traditional maroon communities known as *quilombolas* (maroons) was conducted. These communities are ethnic groups with a defined historical background, specific territorial relations and presumptive black ancestry related to historical resistance to oppression . Many of these communities are still in contact with the natural biodiversity of regions distant from the urban area of Oriximiná (Oliveira et al., 2010). As a result of this survey, *L. origanoides* was identified as one of the main medicinal species, with common use that suggest its potential as an antispasmodic, especially for gastrointestinal and genitourinary disturbances (Oliveira, 2009).

Literature on chemistry and pharmacology of this species are still scarce. There are some studies on the chemical composition of the essential oil obtained from its leaves, being carvacrol and thymol the main constituents (Oliveira et al., 2007; Vicunã et al., 2010; Stashenko et al., 2010). However, there is limited knowledge about their non-volatile chemical constituents. At the moment, the flavonoid pinocembrin (Stashenko et al., 2010) and two naphtoquinones have been described for this species (Brieskorn and Pohlmann, 1976). From an ethnopharmacological perspective, there is only one study that tests the antimicrobial properties of the species that could be related to some of its traditional uses (Oliveira et al., 2007), confirming that more studies are necessary to identify the biological potential of *L. origanoides*. In this sense, this work aims to contribute to the ethnopharmacological knowledge of *L. origanoides*, using quantitative techniques in ethnobotany to determine the versatility of its use and agreement among its uses within the maroon communities of Oriximiná. In addition, some assays were performed in order to verify the antinociceptive activity of the hydroalcoholic extract of *L.origanoides* leaves that could contribute to explain some of its popular uses.

Material and methods

Characterization of the areas of study

The municipality of Oriximiná is located in the northern region of Brazil, in the State of Pará, It has an area of 107,603 km^2 and is one of largest municipalities of the Brazilian territory. It borders Suriname, Guyana, and French Guiana to the north, the cities of Faro, Juruti, and Óbidos to the South and East, and the States of Amazonas and Roraima to the West. According to the 2010 census, Oriximiná has 62,963 inhabitants; 40,182 residing in urban areas and 22,781 in rural areas (IBGE, 2010).

Currently, there are 33 known *quilombola* communities in the municipality of Oriximiná, which are divided into eight territories (Água Fria, Boa Vista, Trombetas, Erepecuru, Alto Trombetas, Jamari/Último Quilombo, Moura, and Ariramba) that, together, encompass more than 600,000 hectares (Fig. 1). The *quilombolas* are represented by their association called Associação de Comunidades Remanescentes de Quilombos do Município de Oriximiná – ARQMO (Association of the Remaining "Quilombo" Communities from the Municipality of Oriximiná). In this work, five communities representing two *quilombola* areas were chosen: Bacabal and Arancuã-de-Cima from the Trombetas region, as well as Serrinha, Jauari, and Pancada from the Erepecuru region.

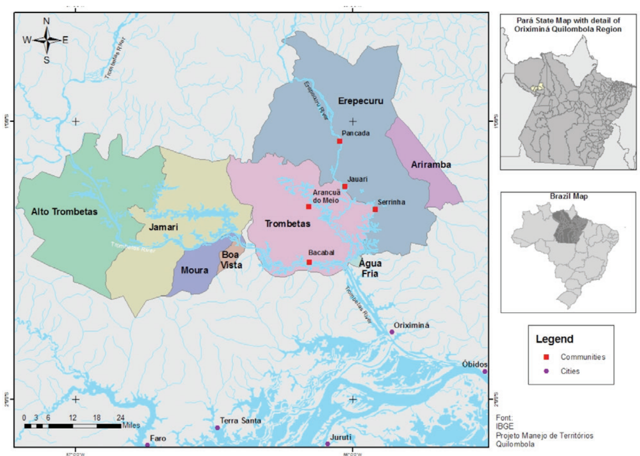

Figure 1 - Map representing the *quilombola* area and the studied communities of Oriximiná, Pará State, Brazil.

Ethnopharmacological data collection

This work was authorized by the Directing Council of Genetic Heritage (Conselho de Gestão do Patrimônio Genético) to access the traditional knowledge associated with bioprospecting purposes, by the Resolution CGEN no. 213 (6.12.2007), published in the Federal Official Gazette (DOU) of Brazil on 27 December 2007; and renewed by the Resolution CGEN no. 87/2012 (DOU 17.01.13, Section 1, page 60).

The selection of the interviewees began with the search of key informants who were respected people in the community, such as the community coordinator, matriarch or patriarch, and/or community health agent. They led to the local specialists, *quilombolas* highly experienced in the use of medicinal plants, such as extractivists, woodsmen, healers, faith healers, praying ladies, midwives, and *puxadores* or *puxadoras*, who are traditional chiropractors. And these local experts, in turn led to other ones by snowball sampling. For data acquisition, four ethnobotanical field trips were performed from June 2006 (after signing the Prior Informed Consent between UFRJ and ARQMO), until September 2008. Each field trip consisted of a residence period of 30 to 60 days in the studied communities.

The ethnobotanical data was collected through semi-structured interviews, participant observation and guided tours, also called walk-in-the-woods (Albuquerque and Lucena 2004). The formularies applied contain socio-economic data (sex, age, profession, schooling, monthly family income, number of residents) and the medicinal plants information (common name, therapeutic uses, doses, preparation methods, contraindications and sites of collection). A total of 35 *quilombolas* from the five communities studied, twenty women and fifteen men, were interviewed. They were between 19 and 87 years old, surviving mainly of subsistence fishing, hunting, and farming, and their only source of income is the extraction of the Pará nut ("Brazil nut" - *Bertholletia excelsa*), which is available for only a few months of the year.

Quantitative data analysis techniques, such as the relative importance (IR) and corrected major use agreement (MUAc), were also applied.

Major Use Agreement (MUA): this is a quantitative technique used to evaluate the agreement between the main uses cited by various informants (Oliveira et al., 2006; 2011). It is determined as the ratio between the number of informants who independently cited the species (*L. origanoides*) for a major use (MU_{LO}) and the total number of informants who mentioned that species for any use (total uses, TU_{LO}), according to equation: $MUA = MU_{LO} \times 100 / TU_{LO}$. A correction factor (CF) was applied to calculate the corrected MUA (MUAc), given by the formula: MUAc = MUA x CF. The correction factor is the ratio between the number of informants citing *L. origanoides* (TU_{LO}) and the highest number of informants citing the most-cited species. In this study, *Dipteryx odorata* was the most-cited species and was cited by 24 informants (TU_{s+}) (Oliveira et al., 2012), therefore the final equation used was: $CF = TU_{LO} / TU_{s+}$

Relative Importance (RI): This index establishes the importance of a certain plant based on its versatility; that is, a plant is as important as the number of distinct potential uses. The maximum value possible for each species is 2 (Bennet and Prance, 2000). The calculation is done according to the formula: $RI = NBS + NP$, where, NBS = number of body systems of a given plant and NP = number of properties of a given plant. The NBS is given by the number of body systems treated by a particular species, (NBS_{LO} in the case *L. origanoides*) which is divided by the total number of body systems treated by the most versatile species (NBS_{s+})[1], with the formula, $NBS = NBS_{LO} / NBS_{s+}$. NP is the number of properties assigned to a particular species (NP_{LO}) divided by the total number of properties assigned to the most versatile species (NP_{S+})[2], as the formula, $NP = NP_{LO} / NP_{s+}$. To explore this index, it is necessary to categorize the body systems and properties, for this purpose; we employed the International Classification of Diseases ICD-10 (WHO, 2010). Therefore, the employments of medicinal plants (*use properties*) are distributed into the 17 listed categories of the ICD-10 (*body systems*).

Plant material and preparation of extract

Aerial parts of *Lippia origanoides* Kunth, Verbenaceae, were collected from a cultivated specimen in October of 2002, in the city of Oriximiná, Pará State, Brazil (16°36'15,1"S and 49°16'0,70" W). Samples were identified by Dr. Fátima Regina Gonçalves Salimena at Universidade Federal de Juiz de Fora (UFJF). A voucher specimen was deposited at UFJF Herbarium, under the registry number CESJ 39532. The collected material was dried and ground, and afterwards an hydroethanolic extract was prepared with ethanol 95% (160 g, 1/5 p/v) with successive extractions. The mixture was filtered and concentrated under reduced pressure at 40°C, using a vacuum rotary evaporator, yielding 15.35 g (about 10% w/w), of a non-solid extract of greasy appearance, due to the high oil content previously described (Oliveira et al., 2007).

[1]The species with the greatest number of body systems was the Pará nut (*Bertholletia excelsa*) with a total of ten different body systems (NBSs+ = 10).
[2]The species with the greatest number of use properties was the Pará nut (*Bertholletia excelsa*) with a total of thirty different indicated properties (NPs+ = 30).

Drugs and L. origanoides *administration*

Acetylsalicylic acid (ASA) was obtained from Sigma Chemical (St. Louis, MO, USA). *L. origanoides* ethanol extract was dissolved in DMSO in order to prepare a stock solution at a concentration of 100 mg/ml. The extract was administered at concentrations of 10, 30 or 100 mg/kg in a final volume 0.1 ml (water as vehicle). The control group was administered vehicle solution. Positive control groups were administered ASA (100 mg/kg) dissolved in water just before use. The vehicle, *L. origanoides* extract, and ASA were administered through oral gavage.

Animals

All experiments were performed using male Swiss mice (18-25 g) obtained from our own animal facilities. Animals were kept in a room with controlled temperature 22 ± 2°C, with a 12/12 light/dark cycle and free access to food and water. Twelve hours before each experiment animals received only water, in order to avoid food interference with substance absorption. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), approved by the Biomedical Science Institute/UFRJ, Ethical Committee for Animal Research, and received the number DFBCICB-015.

Acetic acid-induced abdominal writhing

Mice were treated as previously described by Matheus et al. (2005). Briefly, the total number of writhings following intraperitoneal administration of 2% (v/v) acetic acid (AA) was recorded over a period of 20 minutes, starting 5 minutes after AA injection. Mice were orally pre-treated with extract or vehicle 60 minutes before administration of AA. The positive control group was administered the reference drug ASA (100 mg/kg, *p.o.*).

Formalin test

The procedure used was similar to the method described by Tsølsen et al. (1992) with some adaptations by Gomes et al. (2007). Briefly, mice received an intraplantar injection of 20 µl formalin (2.5%, v/v) into the dorsal surface of the right hind paw. The time (in seconds) that the animal spent licking the formalin-injected paw was recorded from 0 to 5 min (first phase or neurogenic phase) and 15 to 30 min (second phase or inflammatory phase) immediately after formalin injection. Animals were pre-treated with vehicle, extract (10, 30 or 100 mg/kg, *p.o.*) or ASA (100 mg/kg, *p.o.*) 60 min before intraplantar injection of formalin. The result was expressed as the time the animal spent licking the formalin-injected paw.

Hot plate test

Mice were tested according to the method described by Sahley and Berntson (1979) and adapted by Matheus et al. (2005). Animals were placed on a hot plate (Insight Equipment, Brazil) set at 55 ± 1°C. At successive intervals of 30 min after oral administration of *L. origanoides* extract, vehicle or morphine, the reaction time was recorded when the animals licked their fore- and hind-paws and jumped. Baseline was considered as the mean reaction time obtained at 60 and 30 min before administration of the *L. origanoides* extract, vehicle or morphine and was defined as the normal reaction of the animal to the temperature. When animals were kept on the hot plate for a period of time greater than three times the baseline (cut-off), they were removed to avoid possible damage to the paws. Antinociception was quantified as either the increase in baseline (%) calculated by the formula (reaction time x 100/ baseline) - 100.

Statistical analysis

All experimental groups were composed by 6-10 mice. The results are presented as mean ± S.D. Statistical significance between groups was performed by analyses of variance (ANOVA) followed by Bonferroni's test; *p* values less than 0.05 ($p < 0.05$) were considered as significant.

Results and discussion

Ethnopharmacological data

In the ethnobotanical survey with the *quilombola* communities from Oriximiná, 254 ethnospecies were reported and 233 plant species were identified, belonging to 211 genera and 72 botanical families (Oliveira et al., 2012). Among these, *L. origanoides*, locally known as *salva-de-marajó*, was cited by ten respondents, all were women. The species was cited by 28.6% of the total respondents; however, considering that out of the 35 interviewees in this survey twenty were women, 50% of them cited this species. This fact demonstrates that the knowledge and use of this medicinal plant is especially directed for the women's health, notably related to the genitourinary system disorders or to childbirth and puerperium.

Most of the interviewed women have guided or assisted in deliveries in their own communities and are experienced in the use of *L. origanoides*, locally considered as one of the most important plants for local midwives who know how to use it for both the pregnant (including for the postpartum) and non-pregnant women. It is also noteworthy that *L. origanoides* is also employed by them for babies and children, demonstrating a certain confidence of the midwives in the safe use of the species.

The versatility of *L. origanoides* can be seen in the Chart 1, which displays the plant´s uses distributed in eight body systems ($NBS_{LO} = 8$) and fourteen properties ($NP_{LO} = 14$), according the ICD-10. The NBS and NP of *L. origanoides* compared with the most versatile species (*Bertholletia excelsa*, IR = 2.0) in these communities showed a RI = 1.3.

An IR > 1.0 reveals the good versatility of this species with uses distributed in eight categories of ICD-10. However, the main uses of *L. origanoides* are focused in the genitourinary and digestive systems, which can be confirmed by the major use agreement (MUA). In this survey 38 uses of *L. origanoides* were described by the informants, which allowed to calculate the MUA for its main therapeutical uses. The most important uses cited were as treatment against menstrual cramps (MUA = 70%; MUAc = 29.4%), to "bring down" the postpartum blood ("to clear the woman's insides"), and against flatus (MUA = 50%; MUAc = 21%); against stomach ache (MUA = 40%; MUAc = 16.8%); to treat baby colic and vaginal discharge (MUA = 30%; MUAc = 12.6%); as vaginal douche, to clear retained menstrual blood and against uterine inflammation ("ladies belly") (MUA = 20%; MUAc < 10%); to alleviate postpartum colic; against sore throat, tuberculosis ("lung disease") and "wind fallen" in umbilical cord (MUA = 10%; MUAc < 10%). Concerning the reduction of the consensus of the informants in the corrected MUA (MUAc), this fact could be irrelevant if we consider that the therapeutic uses were restricted to an all-women group, in a sample where more than 40% of respondents were composed of men that did not cite this species.

The cited uses against menstrual cramps, stomachache, baby and postpartum colics, (specially the first two uses had a calculated MUA ≥ 40%) could indicate a consensus of the informants of an antispasmodic effect, or to possible anti-inflammatory and analgesic properties of *L. origanoides*. Also, the therapeutical use against uterine inflammation/"woman inflammation" ("ladies belly") and menstrual cramps could be related to endometriosis, which causes pelvic pain and dysmenorrhea. Therefore, analgesic and anti-inflammatory effects of this plant could be related with these therapeutical uses. Another possibility is that these applications could be related with uterine cervical dysplasia or uterine cervical neoplasms. The flavonoid pinocembrin identified in *L. origanoides* has shown to have antimutagenic activity (Trakoontivakorn et al., 2001), antiproliferative and cytotoxic effects on the cervical tumor cell line (HeLa) (Barbarić et al., 2011), and induction of mitochondrial apoptosis on colon cancer cells (Kumar et al., 2007) *in vitro*. Moreover pinocembrin, thymol and carvacrol, which are present in large amounts in the leaves of *L. origanoides*, could be related with other activities. These compounds showed anticoagulant and antiplatelet activities (Enomoto et al.,, 2001) that could indicate a contribution of this plant to treat several disorders like "to bring down retained menstruation" and "to bring down the postpartum blood" ("to clear the woman's insides"). Moreover, thymol and carvacrol have been extensively investigated for their antimicrobial properties, which have already been documented in several studies (Burt, 2004). The antimicrobial activities of the essential oil of *L. origanoides* (Oliveira et al., 2007) and its ethanolic extract (Oliveira et al., 2010) have also been demonstrated *in vitro*. These results support the use of this species in vaginal douche ("banho de asseio") and to treat vaginal discharge ("corrimento").

Antinociceptive activity

In order to investigate some of the pharmacological aspects of *L. origanoides* that could be related to its uses, the hydroethanolic extract was assessed for its antinociceptive activity by the acetic acid-induced abdominal writhing, hot-plate and formalin models.

Injection of acetic acid (2%) into the peritoneal cavity of mice induced 100 ± 9.8 writhings in an interval of 20 min. A dose-dependent effect could be observed since pre-treatment

Chart 1
Uses and properties cited by the *quilombolas* from Oriximiná (Pará State, Brazil) in reference of *Lippia origanoides*, according to the International Classification of Diseases - ICD-10 (OMS, 2008).

Body systems for *L. origanoides* (NBS_{LO})	Medicinal properties of *L. origanoides* (NP_{LO})
I - Certain infectious and parasitic diseases	vaginal discharge(3), vaginal douche, pneumonia and tuberculosis (“lung disease”)(1)
VI - Diseases of the nervous system	Soothing(1)
X- Diseases of the respiratory system	Throat inflammation(1)
XI - Diseases of the digestive system	against flatus (“to pass wind”)(5), stomachache(4), baby colic(3)
XIV - Diseases of the genitourinary system	uterine inflammation (“woman inflammation” and “ladies belly”)(2), to “bring down” retained menstrual blood(2)
XV - Pregnancy, childbirth and the puerperium	to “bring down” the postpartum blood (“to clear the woman inside”)(5), postpartum colic(1)
XV - Certain conditions originating in the perinatal period	"Wind fallen" in umbilical cord(1)
XVIII - Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	menstrual cramps(7)

In the column “Medicinal properties of *L. origanoides*”, the number of times the plant was cited for each indication appears between parentheses.

of the animals with 10, 30 or 100 mg/kg of *L. origanoides* resulted in significant inhibition of total writhing of 50.5%, 61% and 90%, respectively. All doses tested displayed a similar or higher effect as ASA (Fig. 2). The algesic effects of acetic acid are due to the release of several mediators as histamine, serotonin, cytokines, and eicosanoids with an increase in peritoneal fluid levels of these (Deraedt et al., 1980). The antinociceptive activity of *L. origanoides* may be related to the reduction of the release of the inflammatory mediators or by direct receptors block resulting on a peripheral antinociceptive effect. The antinociceptive activity could also be explained by an increase on the pain threshold or by the interruption of the stimulus propagation in the pain sensing nerve fiber.

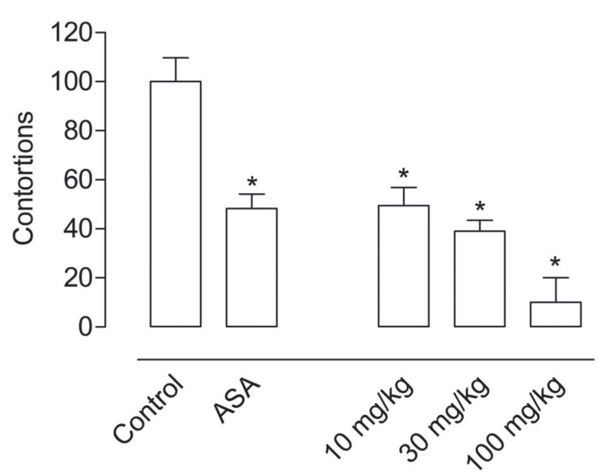

Figure 2 - Effects of *Lippia origanoides* on acetic acid-induced abdominal writhing in mice. Animals were pre-treated by oral administration of different doses of *L. origanoides*, acetylsalicylic acid (ASA, 100 mg/kg) or vehicle. The results are presented as mean ± S.D. (n = 8-10) of writhings. Statistical significance was calculated by ANOVA followed by Bonferroni’s test. * $p < 0.05$ compared to vehicle-treated mice.

In order to test if *L. origanoides* extract induces an antinociceptive effect in another model of peripheral analgesia, it was tested on the formalin model. Injection of formalin (2.5%) develops a biphasic licking response on mice-injected paw. The first phase occurs 5 min after injection and corresponds to acute neurogenic pain sensitive to drugs that interact with opioid system; and the second phase occurs between 15 and 30 min after formalin injection and corresponds to inflammatory pain inhibited by opioids and anti-inflammatory drugs. Fig. 3 shows that none of the tested doses reduced the time that the animal spent licking the formalin-injected paw at the first phase. Pre-treatment of animals inhibited in a similar and in a dose-dependent manner the second phase of formalin-induced nociception. Similarly to the previous model, all doses tested significantly reduced the licking response. Vehicle-treated group was 172.9 ± 13.2 s vs 95.6 ± 13.1 s at 10 mg/kg, 76.2 ± 12 s at 30 mg/kg and 39.2 ± 8.5 s at 100 mg/kg (Fig. 3). Substances that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibit only the phase 2 (Rosland et al., 1990). These results indicate that *L. origanoides* constituents may be acting via inhibition of any inflammatory mediator liberated in the mice paw. It

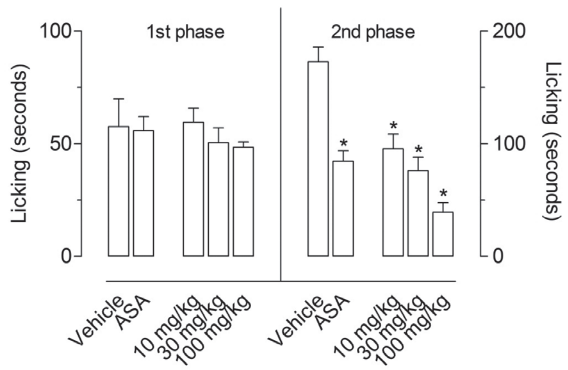

Figure 3 - Effects of *Lippia origanoides* in the formalin-induced licking response. Animals were pre-treated by oral administration of vehicle, acetylsalicylic acid (ASA, 100 mg/kg) or *L. origanoides*. The results are presented as mean ± S.D. (n = 7-10) of time, in seconds, that the animal spent licking the injected paw. Statistical significance was calculated by ANOVA followed by Bonferroni's test.* $p < 0.05$ when compared with vehicle-treated mice.

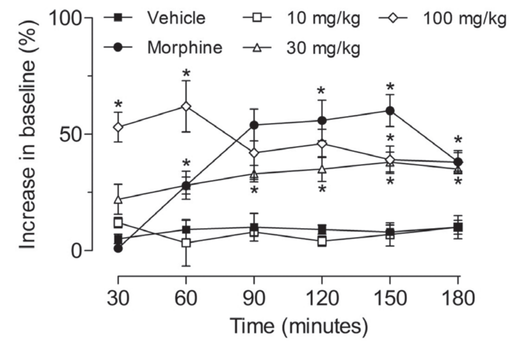

Figure 4 - Effects of *Lippia origanoides* in the hot plate model. Animals were pre-treated with oral administration of different doses of *L. origanoides*, morphine (5 mg/kg) or vehicle. The results are presented as mean ± S.D. (n = 6-10) of the increase in baseline levels. Statistical significance was calculated by ANOVA followed by Bonferroni's test. * indicates $p < 0.05$ when comparing treated mice to the vehicle-treated group.

could inhibit the formation and/or release of the mediator or directly blocking some receptor. These results also corroborate the inhibitory effect of *L. origanoides* on the acetic acid-induced writhing response.

Fig. 4 shows the effects of *L. origanoides* on the hot plate test. A significant increase on the baseline was observed 30 min after oral administration. As expected, administration of the vehicle did not induce any antinociceptive effect. The pre-treatment with *L. origanoides* induced an increase in the antinociceptive effect that reached maximal values at 60 min. The activity persisted in high levels (with 100 mg/kg) until the end of assay. The treatment of mice at 30 mg/kg dose it an intermediate antinociceptive effect was observed. A significant effect was observed 30 min after administration. A drastic increase was not observed and the effect plateaued between 30 and 40% increase in the baseline. Morphine-induced (5 mg/kg) activity increased until reach a plateau at 90 min after oral administration (Fig. 4). Our results indicate that *L. origanoides* has central antinociceptive activity. The hot plate test has been used by many investigators and has been found to be suitable for evaluation of central acting analgesics but not of peripheral acting ones (Tjølsen et al., 1992). Characteristic differences occurred in the time course and maximal effects of the antinociceptive action of *L. origanoides*. A rapid onset with an early maximum effect is characteristic of the time course of action of opioid agonists (*e.g.*, morphine), which mediate analgesia via opioid receptors under both normal and inflammatory conditions (Aceto et al., 1997). The administration of *L. origanoides* produced a time course of drug action in the hot plate test similar to morphine. One possible explanation for the rapid onset of action might be the solubility of the substances present in the extract, which allows them to rapidly reach the brain. The inhibitory effect observed with *L. origanoides* on the models of analgesia could also be due to a decrease on the activity of the C fibers, or for decrease in the production of inflammatory mediators responsible for the C fiber stimulation (Rossi et al., 1993). As *L. origanoides* is a complex extract with several components, the observed activity can be attributed to the overall effects of the constituents or activity similarities to non-steroidal anti-inflammatory or opioids drugs, therein in a complementary manner.

Lippia origanoides belongs to the *Lippia* section *Goniostachyum*, founded originally by Schauer in 1847 (O´Leary et al., 2012) that includes several species with morphological similarities. The section can now be distinguished into four groups, one of which is headed by *L. origanoides* and in which 28 taxa have been considered as synonyms, including the well-known for their traditional uses *L. sidoides, L. affinis, L. graveolens* and *L. salviifolia* (O´Leary et al., 2012). Another group is headed by *L. grata*, considering *L. gracilis* as synonym. Interestingly, they all have in common the thymol and carvacrol content and, in the same way of *L. origanoides* (Table 1) they have also been used in Brazilian and several other countries' folk medicine for the treatment of menstrual problems, against genitourinary and digestive disorders, and as a general antiseptic, which strengthens the idea that chemical constituents of plants in this genus have potential antispasmodic, analgesic, anti-inflammatory and antimicrobial properties.

The analgesic and anti-inflammatory effects of other *Lippia* species have already been described (Table 1), for their essential oils (Mendes et al., 2010), as for the alcoholic and aqueous extracts of *L. gracilis* (Guimarães et al., 2012). These activities have been associated with the aromatic monoterpenes for the first study and with the presence of the flavonoid naringenin for the latter. The anti-inflammatory activity of thymol, obtained from *L. gracilis*, was recently demonstrated by Riella et al. (2012). Furthermore, *Origanum* and *Thymus* species, also rich in thymol and carvacrol, have been reported in the literature to have potential antispasmodic activity for the treatment

Table 1
Species of *Lippia* section *Goniostachyum* and others, rich in thymol and carvacrol listed with some of their most common uses.

Scientific name	Distribution	Popular name	Uses	Thymol (%)	Carvacrol (%)	References
L. gracilis	Brazil	alecrim-da-chapada	Respiratory diseases, headache, cutaneous diseases, wounds, as a mouth antiseptic and in baths	0-30,6	0-47,7%	Riella et al., 2012; Gomes et al., 2011
L. grandis Martius and Schau.	Brazil	erva-do-marajó, salva, malva-do-marajó	Liver ailments and menstrual disorders	65,8	4,5	Maia et al., 2001
L. graveolens H.B.K.	Texas and Central America	mexican origanum	analgesic, anti-inflammatory, antipyretic, dysentery, menstrual, antispasmodic disorders, respiratory diseases	0,22-68,4	0,47-48,0	Terblanché and Kornelius, 1996; Senatore and Rigano 2001; Salgueiro et al. 2003; Vernin et al., 2001; Tucker and Maciarello, 1994
L. micromera Shauer	South America	----	gastrointestinal disorders, respiratory disorders	-	52	Terblanché and Kornelius, 1996; Pascual et al., 2001; Lawrance, 1984
L. nodiflora L. Greene	Africa	-------	Analgesic, anti-inflammatory and antipyretic, menstrual disorders, antimicrobial, antispasmodic, respiratory diseases, syphilis and gonorrhea	2,74	3,22	Terblanché and Kornelius, 1996; Pascual et al., 2001; Forestieri et al., 1996
L. origanoides H.B.K.	Colombia, Venezuela, Brazil	alecrim-d'angola	gastrointestinal disorders, respiratory diseases	20,6-38,35	0-0,36	Morais et al., 1972; Gallino, 1987; Pascual et al., 2001

of menstrual cramps and stomachache (Chishti et al., 2010). Moreover, it also deserves attention the antispasmodic activity demonstrated for thymol and carvacrol (Begrow et al., 2010) as well as *Origanum acutidens* (Goze et al., 2010). The anti-inflammatory activity of pinocembrin identified in *L. origanoides* (Stashenko et al., 2010) was also described in the literature (Rasul et al., 2013).

Conclusion

The ethnopharmacological data obtained demonstrated the use versatility of *L. origanoides*, especially for the treatment of gastrointestinal and genitourinary disturbances, although their use has been restricted to women in the studied group. This fact can be better understood by use consensus among informants, as it was for the treatment of menstrual cramps and other women's health disorders. The analgesic activity demonstrated here may contribute to understand some uses of *L. origanoides*, as antispasmodics to treat menstrual cramps, stomachache, "inflammation of the uterus"/"inflammation of the woman", among others. Furthermore, it was found in the literature a similarity between the uses of *L. origanoides* with other species that have chemical similitudes, and yet many of them are morphologically similar also in the case of species included within the same section (*Goniostachyum*) of the genus *Lippia*.

Authors' contributions

All authors participated in the design of the study. GGL and SGL guided the PhD thesis of DRO and provided financial support for it. DRO conducted fieldwork and collected plant samples. PDF was responsible for the biological activities. DRO, GGL, PFD and SGL contributed in analysis of the data and wrote the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

Aceto, M.D., Harris, L.S., Bowman, E.R., 1997. Etorphines: µ-opioid receptor-selective antinociception and low physical dependence capacity. Eur. J. Pharmacol. 338, 215-223.

Albuquerque, U.P., Lucena, R.F.P., 2004. *Métodos e Técnicas na Pesquisa Etnobotânica*. Recife: Livro Rápido/Núcleo de Publicações em Ecologia e Etnobotânica Aplicada.

Barbarić, M., Mišković, K., Bojić, M., Lončar, M.B., Smolčić-Bubalo, A., Debeljak, Ž., Medić-Šarić, M., 2011. Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells. J. Ethnopharmacol. 135, 772-778.

Begrow, F., Engelbertz, J., Feistel, B., Lehnfeld, R., Bauer, K., Verspohl, E.J., 2010. Impact of thymol in thyme extracts on their antispasmodic action and ciliary clearance. Planta Med. 76, 311-318.

Bennet, B.C., Prance, G.T., 2000. Introduced plants in the Indigenous Pharmacopoeia of Northern South America. Econ. Bot. 54, 90-102.

Brieskorn, C.H., Pohlmann, R., 1976. The ocorrence of isomeric catalponol and tectol dimethyl ether in the root of *Lippia origanoides* H.B.K. Archiv der Pharmazie. 309, 829-36.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. Int. J. Food Microbiol. 94, 223-253.

Chishti, S., Kaloo, Z.A., Sultan, P., 2013. Medicinal importance of genus *Origanum:* A Review. J. Pharmacogn. Phytother. 5, 170-177.

Deraedt, R., Jougney, S., Delevalcee, F., Falhout, M., 1980. Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 51, 17-24.

Enomoto, S., Asano, R., Iwahori, Y., Narui, T., Okada, Y., Singab, A.N., Okuyama, T., 2001. Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. Biol. Pharm. Bull. 24, 307-310.

Forestieri, A.M., Monforte, M.T., Ragusa, S., Trovato, A., Iauk, L., 1996. Antiinflamatory, analgesic and antipyretic activity in rodents of plants extracts used in african medicine. Phytoth. Res. 10, 100-106.

Gallino, M.A., 1987. Una Verbenacea Essenziera Ricca in Timolo: *Lippia origanoides* H.B.K. Essenze Derivati Agrumari 57, 628-629.

Gomes, N.M., Rezende, C.M., Fontes, S.P., Matheus, M.E., Fernandes, P.D., 2007. Antinociceptive activity of Amazonian Copaiba oils. J. Ethnopharmacol. 109, 486-492.

Gomes, S.V.F., Nogueira, P.C.F., Moraes, V.R.S., 2011. Aspectos químicos e biológicos do gênero *Lippia* enfatizando *Lippia gracilis* Schauer. Ecletica Quimica 36, 64-77

Goze, I., Alim, A., Cetinus, S.A., Etin, A., Durmus, N., Atas, A.T., Vural, N., 2010. In vitro antimicrobial, antioxidant, and antispasmodic activities and the composition of the essential oil of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. J. Med. Food. 13, 705-709.

Guimarães, A.G., Gomes, S.V.F., Moraes, V.R.S., Nogueira, P.C.L., Ferreira, A.G., Blank, A.F., Santos, A.D.C., Viana, M.D., Silva, G.H., Quintans Junior, L.J., 2012. J. Nat. Med. 66, 428-434.

IBGE 2010. *Censo Demográfico 2010*, Instituto Brasileiro de Geografia e Estatística. http://www.ibge.gov.br/, accessed January 2011.

Kumar, M.A., Nair, M., Hema, P.S., Mohan, J., Santhoshkumar, T.R., 2007. Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells. Mol. Carcinog. 46, 231-241.

Lawrence, B.M., 1984. The botanical and chemical aspects of *Oregano*. Perfumer. Flavorist. 9, 41-51,

Maia, J.G.S., Zoghbi, M.G.B., Andrade, E.H.A., 2001. *Plantas aromáticas na amazônia e seus óleos essenciais*. Museu Goeldi. Coleção Adolpho Ducke. Belém, Goeldi Editoração. 196 p.

Maisch, J.M., 1885. On some useful plants of the natural order of Verbenaceae. Am. J. Pharm. 57, 189-99.

Matheus, M.E., Berrondo, L.F., Vieitas, E.C., Menezes, F.S., Fernandes, P.D., 2005. Evaluation of the antinociceptive properties from *Brillantaisia palisotii* Lindau stems extracts. J. Ethnopharmacol. 102, 377-381.

Mendes, S.S., Bomfim, R.R., Jesus, H.C.R., Alves, P.B., Blank, A.F., Estevam, C.S., Antoniolli, A,R,, Thomazzi, S,M,m 2010. Evaluation of the analgesic and anti-inflamammtory effects of the essential oil of *Lippia gracilis* leaves. J. Ethnopharmacol. 129, 391-397.

Morais, A.A., Mourão, J.C., Gottlieb, O.R., Silva, M.L., Marx, M.C., Maia, J.G.S., Magalhães, E.M.T., 1972. Óleos essenciais da Amazônia contendo timol. Acta Amazon. 2, 45-46.

Morton, J.F., 1981.Atlas of Medicinal Plants of Middle America. Vol. I. Springfield, Illinois, USA: Thomas Publisher, p. 745-750.

O'Leary, N., Denham, S.S., Salimena, F., Múlgura, M.E., 2012, Species delimitation in *Lippia* section *Goniostachyum* (Verbenaceae) using the phylogenetic species concept. Bot. J. Linn. Soc. 170, 197-219.

Oliveira, D.R., Leitão, G.G., Santos, S.S., Bizzo, H.R., Lopes, D., Alviano, C.S., Alviano, D.S., Leitão, S.G., 2006. Ethnopharmacological study of two *Lippia* species from Oriximiná, Brazil. J. Ethnopharmacol. 108, 103-108.

Oliveira, D.R., 2009. *Bioprospecting of vegetable species of the traditional knowledge associated in quilombola communities from Oriximiná-PA*. Rio de Janeiro, 303 p. Tese de Doutorado, Núcleo de Pesquisas em Produtos Naturais, Universidade Federal do Rio de Janeiro.

Oliveira, D.R., Leitão, S.G., O'Dwyer, E.C., Leitão, G.G., ARQMO, 2010. Authorization of the traditional knowledge associated access for bioprospecting purposes: The case of UFRJ and the Association of the Oriximiná Quilombola Communities - ARQMO. Rev. Fitos. 5, 59-76.

Oliveira, D.R., Leitão, G.G., Castro, N.G., Vieira, M.N., ARQMO, Leitão, S.G., 2012. Ethnomedical knowledge among the "Quilombolas" from the Amazon region of Brazil with a special focus on plants used as nervous system tonics. In: Rai, M., Rastrelli, L., Marinof, M., Martinez, J.L., Cordell, G. (eds.) *Medicinal plants: Diversity and Drugs*. Edited by, USA: Science Publishers.

Pascual, M.E., Slowing, K., Carretero, E., Mata, D.S., Villar, A., 2001. *Lippia:* traditional uses, chemistry and pharmacology. A review. J. Ethnopharmacol. 76, 201-214.

Riella, K.R., Marinho, R.R., Santos, J.S., Pereira-Filho, R.N., Cardoso, J.C., Albuquerque-Junior, R.C.L., Thomazzi, S.M., 2012. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from *Lippia gracilis*, in rodents. J. Ethnopharmacol. 143, 656-663.

Rasul, A., Millimouno, F.M., Eltayb, W.A., Ali, M., Li, J., Li, X., 2013. Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. BioMed Res. Int. DOI: 10.1155/2013/379850.

Rosland, J.H., Tjolsen, A., Maehle, B., Hole, D.K., 1990. The formalin test in mice. Effect of the formalin concentration. Pain 42, 235-242.

Rossi, G.C., Pasternak, G.W., Bodnar, R.J., 1993. Synergistic brainstem interactions for morphine analgesia. Brain Res. 624, 171-180.

Sahley, T.L., Berntson, G.G., 1979. Antinociceptive effects of central and systemic administration of nicotine in the rat. Psychopharmacol. 65, 279-283.

Salgueiro, L.R., Cavaleiro, C., Pinto, E., Pina-Vaz, C., Rodrigues, A.G., Palmeira, A., Tavares, C., Gonçalves, M.J., Martinez-de-Oliveira, J., 2003. Chemical composition and antifungal activity of the essential oil of *Origanum virens* on *Candida* species. Planta Med. 69, 871-874.

Senatore, F., Rigano, D., 2001. Essential oil of two *Lippia* spp (Verbenaceae) growing wild in Guatemala. Flavour Frag. J. 16, 169-171.

Stashenko, E.E., Martínez, J.R., Ruíz, C.A., 2010. *Lippia origanoides* chemotype differentiation based on essential oil GC/MS and principal component analysis. J. Sep. Sci. 33, 93-103.

Terblanché, F.C., Kornelius, G., 1996. Essential oil constituents of the genus *Lippia* (Vernenaceae) - a literature review. J. Essential Oil Res. 8, 471-485.

Tjølsen, A., Berge, O.C., Hunskaar, S., Rosland, J.H., Hole, K., 1992. The formalin test: an evaluation of the method. Pain 51, 5-17.

Trakoontivakorn, G., Nakahara, K., Shinmoto, H., Takenaka, M., Onishi-Kameyama, M., Ono, H., Yoshida, M., Nagata, T., Tsushida, T., 2001. Structural analysis of a novel antimutagenic compound, 4-hydroxypanduratin A, and antimutagenic activity of flavonoids in a Thai spice, fingerroot (*Boesenbergia pandurata* Schult.) against mutagenic heterocyclic amines. J. Agric. Food. Chem. 49, 3046-3050.

Tucker, A.O., Maciarello, M.J., 1994. Oregano: Botany, Chemistry, and Cultivation In: Spices, Herbs and Edible Fungi, Charalambous, G., Elsevier Science B.V., p. 439-456.

Vernin, G., Lageot, C., Gaydou, E.M., Parkanyi, C., 2001. Analysis of the essential oil of *Lippia graveolens* HBK from El Salvador. Flavour Frag. J. 12, 219-226.

Vicunã, G.C., Stashenko, E.E., Fuentes, J.L., 2010. Chemical composition of the *Lippia origanoides* essential oils and their antigenotoxicity against bleomycin-induced DNA damage. Fitoterapia 81, 343-349.

WHO, 2010. International Classification of Diseases (ICD-10). World Health Organization, Geneva, Switzerland, http://apps.who.int/classifications/apps/icd/icd10online/.