



Biomedical properties and potentiality of *Lippia microphylla* Cham. and its essential oils

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ABSTRACT

Lippia microphylla Cham. (Verbenaceae) is an endemic underexploited Brazilian vegetal. This work reviewed the biological potentialities of *Lippia microphylla*, emphasizing the properties of essential oils (EOs) and analyzed scientific indicators about genus *Lippia* and *L. microphylla*. Databases from 1948 to the present were searched and a software (vantage point 7.1) associated with Derwent Innovation Index was used to identify the indicators of the genus *Lippia*, and biological activities and compounds in the *L. macrophylla* species. Ethnopharmacological records report use of *L. microphylla* leaves to treat gastrointestinal disorders, influenza, bronchitis, cough, nasal congestion, and sinusitis during vaporization, whose aromatic volatile oils are rich in monoterpenes, especially cineole, terpineol, and thymol. Other EOs have larvicidal activity on *Aedes aegypti* larvae, and antifungal, antibacterial and cytotoxic and antitumor action on human and murine cancer cells. Brazil is the country with more articles about *Lippia* species, but it deposited only 9 patents since 1993. Most of the publications about *L. microphylla* are concentrated in food and chemical sciences. This bioprospection helps to choice areas of interest for capital investment and to give support for Brazilian Institutions to establish cooperation and improve technological impact at the point of view of creation and innovation.

KEY WORDS: Antitumor action, *Lippia microphylla*, pharmacological activity, scientific indicators, terpenes

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Received: March 18, 2015

Accepted: June 01, 2015

Published: June 26, 2015

INTRODUCTION

About 80% of the worldwide population use herbal products for their basic health care (primary care), such as extracts, teas and their active principles, a market estimated at US\$ 50 billion per year [1]. Despite the interest in molecular modeling, combinatorial chemistry and other chemical synthesis techniques by institutions and pharmaceutical industries, the natural products, particularly medicinal plants, persist as an important source of new therapeutic agents against infectious (fungal or bacterial) and cardiovascular diseases, insects, cancer, and immunomodulation [2-6].

Genus *Lippia* (Verbenaceae, Lamiales/Magnoliopsida) includes about 200 species of herbs, shrubs and small trees mainly

distributed in Central and South Americas and in Africa Tropical [7-8]. Some *Lippia* species are prevalent at “Caatinga” biome, a region with approximately 1,539,000 km², distributed in nine Brazilian Northeastern states, with warm and dry climate and where grows a peculiar xerophyte vegetation. In “Caatinga flora,” there are almost 1 000 vascular plant species. Because of the extreme climate conditions most species are endemic and present particular morphological adaptations [3,4,9,10].

Among the *Lippia* species, *L. microphylla* Cham. (Syn.: *L. microphylla* Cham. and Schlecht.; *L. microphylla* Mart.; *Lantana microphylla* Mart. ex Uphof.) is recognized for its therapeutic usages [Figure 1] [11]. A few studies have explored specific therapeutic points of *L. microphylla*. Then, this



Figure 1: General aspects of *Lippia microphylla* Cham

work aimed to review its biological potentialities, emphasizing the properties of essential oils (EOs). Moreover, in order to suggest a strategic plan for genus *Lippia* and *L. microphylla*, main publication areas, its respective patents and institutions and authors were also analyzed to identify studies and orientate the development of pharmaceutical products.

For a complete and reliable review, primary and secondary resources were used, including original and review articles, books and government documents written in English, Portuguese or Spanish. Databases searched were Lilacs-Bireme (Databases on Latin American Health and Biological Sciences), MEDLINE/Index Medicus (Medical Literature Analysis and Retrieval System Online), SciELO (Scientific Electronic Library Online), Web of Science, PubMed (maintained by the National Library of Medicine) and Science Direct. A software (Vantage Point 7.1) associated with Derwent Innovation Index was used to perform bibliometric analyses, data generation, and identification of quantitative scientific indicators from 1948 to the present. Therefore, it was used the following keywords: *Lippia*, biological properties, cytotoxicity, folk use, *L. microphylla*, and EOs. Vantage Point version 7.1 is a powerful text-mining tool for discovering knowledge in search results from patent and literature databases, giving a better perspective about information and enabling to clarify relationships and find critical patterns in distinct areas of expertise.

***L. microphylla* Cham: Phytochemistry and Pharmacology**

Lippia species have shown a large number of important usages in folk medicine for various diseases, particularly in the treatment of cough, bronchitis, indigestion, liver, hypertension, dysentery [12-14], worms, and skin diseases [15]. Many species have promising biological activities, including antiviral [16], antimalarial [17], anti-inflammatory, analgesic, antipyretic [18], molluscicidal against *Biomphalaria glabrata* [15], antimicrobial [19-25], insecticidal [26], and anticonvulsant [27] properties. Compounds isolated from *Lippia* also revealed *in vitro* antitumor activity on leukemia (K-562, HL-60, CEM), colon (HCT-116), breast (MCF-7),

glioblastoma (U-251), and prostate (PC -3) cell lines [28-30]. Besides its medicinal properties, the leaves of the most *Lippia* species are used for food preparation. Moreover, it is interesting to note the importance of *Leptotyphlops dulcis*, whose main component of leaves and flowers is (+)-hernandulcine, a molecule 1000-fold sweeter than sucrose [14,31,32].

Popularly called as “alecrim-da-chapada,” “alecrim-de-tabuleiro,” “alecrim-pimenta” and “alecrim-do-mato” in the Northeast Brazilian, *L. microphylla* is a deciduous shrub with a thin and brittle stem (up to 2 m in height), white flowers and with simple and aromatic leaves, which presents serrate margins and evident nerves with no more than 1 cm in length [11,33,34].

Ethnopharmacological records report the use of *L. microphylla* leaves to treat gastrointestinal disorders and influenza, bronchitis and sinusitis during vaporization resulting from boiling water. Phytochemical studies revealed the presence of quinones and flavonoids from stem and roots’ ethanol extracts [33,34]. Meanwhile, its aromatic volatile oils extracted by water vapor exhibit an EOs rich in monoterpenes, especially cineole, and terpineol, its more likely active principles.

Secondary metabolites of plants, many of them produced to protect against microorganisms and predator insects, are natural candidates for the discovery of new active products [5,6,25,35,36]. The cineole is responsible for the *Eucalyptus globulus* balsamic activity. The saturated antiseptic and balsamic vapors with cineole and other EOs found in *L. microphylla* lighten respiratory tract mucous membranes during congestion, which explains its folk use for the treatment of influenza, cough and nasal congestion. Its compounds are capable of fluidizing bronchial secretion, facilitating expectoration, and decreasing cough reflex and refreshing breath. Due to these balsamic properties, home practices of inhalation to alleviate symptoms of respiratory diseases are considered an easy way to treat them. To prepare the inhalation, leaves (50-60 g) are put in boiling water, and the person inhales the fumes through a resistant funnel, being careful to heat both parts of the face where sinus are positioned. If the EO is available, it should be used 1-2 mL per 1-2 L of boiling water [11].

Some works published previously have also highlighted the insecticidal importance of the EOs presented in *Lippia* species [37-40]. EOs of *L. microphylla*, in particular, showed significant larvicidal activity on *A. aegypti* larvae, with a LD₅₀ of 75.6 ppm [39]. Gleiser *et al.* [41] showed that *Lippia integrifolia* and *Lippia junelliana* oils are potent repellents against *A. aegypti* adult insects. Both species contained similar quantities of limonene and camphor (20.7% and 26.5%), though they differ in others such as myrcene that was detected in *L. junelliana* (14.1%) and methylheptenone in *L. integrifolia* (24.9%). Limonene is a registered active element in pesticide products used as insecticide and repellent, and cotton fabrics treated with limonene have shown repellence against *Anopheles* mosquitoes. In addition, repellent properties against *A. aegypti* have been reported for limonene, camphor, and myrcene [42,43]. Thus, it is possible that the higher repellence of *L. junelliana* compared to *L. integrifolia* is due to the additional repellent effect of myrcene

and this compound can be responsible for the insecticidal activity found in *L. microphylla*, since myrcene is also present in its leaves [44].

Oils from *L. microphylla* also revealed antifungal (strains of *Aspergillus niger*, *Fusarium* spp., *Rizhopus* spp., and *Rhizoctonia solani*) and antibacterial activities (*Staphylococcus aureus*, *Shigella flexneri*, *Escherichia coli*, and *Streptococcus pyogenes*) using gel diffusion methods [20,45,46]. A tolerance of Gram-negative bacteria to EOs, such as *E. coli*, has been attributed to the existence of a hydrophilic outer layer, which may block the infiltration of hydrophobic components throughout the cell membrane. On the other hand, the inhibitory action of natural products on mold cells involves cytoplasm granulation, plasmatic membrane disrupting and inactivation and/or synthesis inhibition of enzymes. These actions can appear isolate or simultaneously, leading to the mycelium propagation, and growth inhibition [47]. In fact, oils rich in monoterpene compounds are reported to exhibit high levels of antimicrobial activity [48]. Moreover, they are probably responsible, at least in part by the antioxidant activity in *L. microphylla* extracts [49].

Volatile Components of *L. microphylla*

In the Verbenaceae family, genus *Lippia* products great quantities of volatile mixtures. Chemical studies by gas chromatographic techniques of volatile constituents from *L. microphylla* leaves resulted in the identification of α -pinene (1), sabinene (2), β -pinene (3), β -myrcene (4), p-cymene (5), 1,8-cineole (6), γ -terpinene (7), 4-terpineol (8), α -terpineol (9), anisole (10), thymol (11), and carvacrol (12) [Figure 2]. Among them, the main components are 1, 8-cineol (36%), β -pinene (11%), and thymol (11%) [44]. Silva *et al.* [50] identified the germacrene D (13) and bicyclogermacrene (14) [Figure 2] from EOs and hexanic fractions as the most common compounds in specimens collected in dry and rainy periods, while the major monoterpene was α -pinene.

Fixed Components of *L. microphylla*

Analyses of the fixed constituents of the ethanolic extracts of *L. microphylla* roots and stems result in isolation of a flavonol glycoside (15) and four quinones, two prenylated naphthoquinone dimers (16 and 17) and two furan naphthoquinones (18 and 19) [Figure 3]. Among these, microphyllaquinone (16) and a mixture of 6-methoxy- and 7-methoxy-naphtho[2,3-b]-furan-4,9-quinones (18 + 19, respectively) isolated from *L. microphylla* were evaluated for their cytotoxicity, using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method [51], which analyzes the ability of living cells to reduce the yellow dye MTT to a purple formazan product. According to Santos *et al.* [34], the molecules microphyllaquinone and the mixture demonstrated cytotoxic potential against a panel of different murine and human cancer cell lines (B-16 [murine melanoma], CEM [lymphocyte leukemia], HL-60 [promyelocyte leukemia], HCT-8 [colon adenocarcinoma], and MCF-7 [breast adenocarcinoma]), with IC_{50} values ranging from 0.69 to 3.13 $\mu\text{g/mL}$ [Table 1].

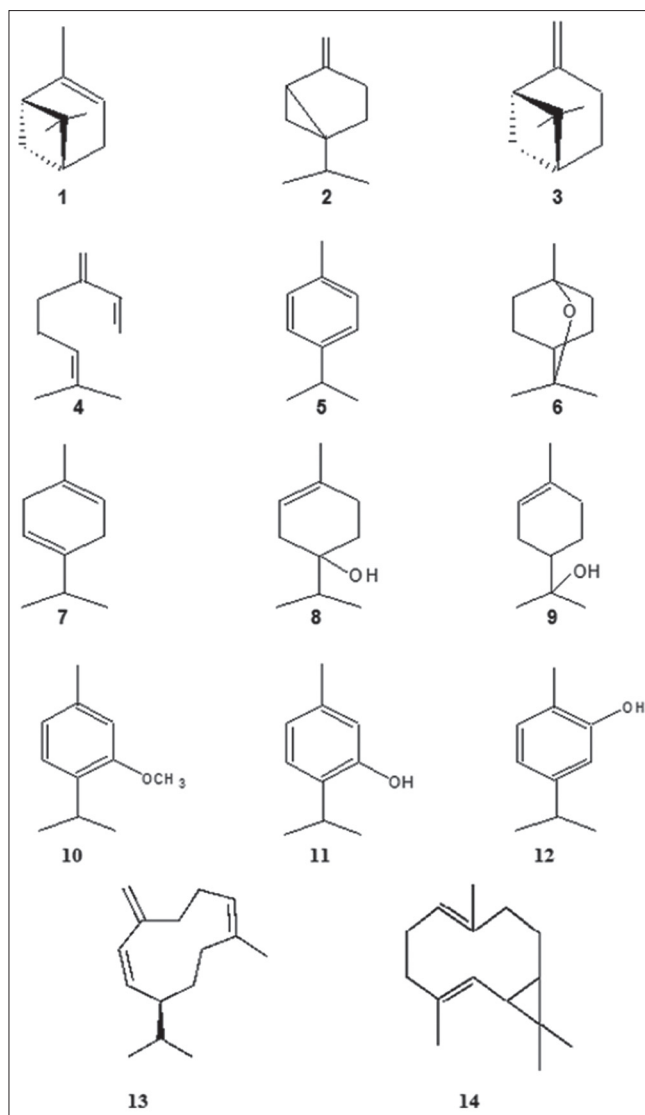


Figure 2: Volatile essential oils from *Lippia microphylla* Cham

The EO of *L. microphylla* leaves also showed *in vitro* cytotoxic action on Sarcoma 180 (IC_{50} of 100.1 [94.9-105.5] $\mu\text{g/mL}$) and human chronic myelocyte leukemia K-562 cells (IC_{50} of 51.9 [47.9-56.3] $\mu\text{g/mL}$) [Table 2]. Lytic activity was not detected in normal erythrocytes from Swiss mice at a concentration of 250 $\mu\text{g/mL}$. After 7 days of treatment, this same oil presented *in vivo* antitumor action in a dose-dependent way, and tumor growth inhibition of 38.2% and 59.8% (50 and 100 mg/kg, respectively) [52].

Interestingly, in the presence of cyclosporine A, an inhibitor of the pore formation in the mitochondrial permeability transition, it was detected reduction in the cytotoxicity on Sarcoma 180 cells with this EO (IC_{50} of 118.3 [113.7-123.1] $\mu\text{g/mL}$). Hence, declining in cytotoxicity in the presence of cyclosporine A is indicative of the intrinsic pathway involvement in the mechanism of death [Table 2], whereas after adding pore inhibitors, probably occurred the mitochondrial pores' opening blocking, which inhibited the release of pro-apoptotic

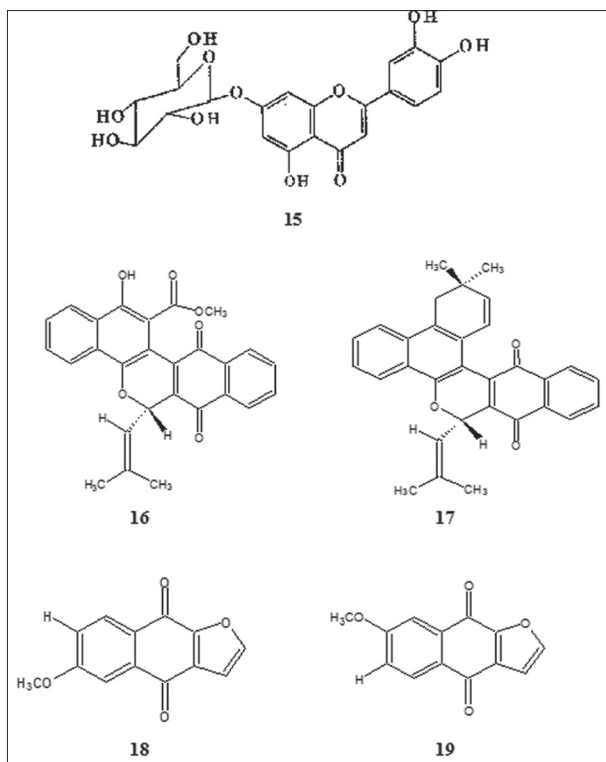


Figure 3: Fixed components of *Lippia microphylla* Cham

Table 1: Cytotoxicity of naphthoquinones isolated from *L. microphylla* and analyzed by MTT assay after 72 h exposure

Cancer cell line	IC ₅₀ (µg/mL)	
	16	18+19
B-16 (murine melanoma)	3.13±0.12	0.77±0.05
CEM (human lymphocyte leukemia)	2.57±0.33	1.61±0.25
HL-60 (human promyelocyte leukemia)	2.92±0.13	1.62±0.09
HCT-8 (human colon adenocarcinoma)	2.33±0.29	0.69±0.06
MCF-7 (human breast adenocarcinoma)	2.44±0.13	1.30±0.05

Data presented as IC₅₀ values ± SEM from three independent experiments with cancer lines. 16, microphyllaquinone; 18+19, mixture of 6-methoxy- and 7-methoxy-naphtho [2,3-b]-furan-4,9-quinones. Adapted from Santos *et al.* (2003), *L. microphylla*: *Lippia microphylla*, SEM: standard error of measurement

proteins and reduced the cytotoxicity of the oil. Similarly, when K-562 cells were treated with the EO in the presence of N-acetylcysteine, an antioxidant molecule and scavenger of free radicals that stimulates the biosynthesis of reduced glutathione, cytotoxicity diminution of the oil was also evidenced [52,53]. Then, it was proposed that reactive oxygen species (ROS) production is involved, at least partially, in the mechanism of cytotoxicity on K-562 cells, and activation of apoptotic intrinsic pathways in Sarcoma 180 cells. In fact, mitochondrial membrane permeabilization culminates in cytochrome *c* release. Cytochrome *c* binds to Apaf-1 (apoptotic protease-activating factor 1) and generate the catalytically active form of caspase-9, which activates caspase-3, the most important effector caspase that acts as an effective DNase to slice the genomic DNA into nucleosomes, producing fragments of 180-200 base pairs, degradation of laminin and mitotic apparatus proteins and nuclear and cellular reduction and pyknosis [54,55].

Table 2: *In vitro* antitumor activity of the essential oil from *L. microphylla* leaves determined by MTT assay after 72 h exposure

Substance	IC ₅₀ (µg/mL)	
	Sarcoma 180 (experimental murine tumor)	K-562 (human chronic myelocyte leukemia)
EOs	100.1 (94.9-105.5)	51.9 (47.9-56.3)
EOs+GSH	107.3 (102.8-111.9)	55.4 (55.4-55.6)
EOs+NA	109.2 (103.8-114.9)	94.2 (85.8-103.4)
EOs+CA	118.3 (113.7-123.1)*	51.9 (47.9-56.3)

Data presented are IC₅₀ values and 95% confidence intervals for K-562 and Sarcoma 180 cells. GSH: Reduced glutathione; NAC: N-acetylcysteine; CA: Cyclosporine A. **P*<0.05 compared to control by ANOVA followed by Turkey test. Adapted from Xavier (2011), *L. microphylla*: *Lippia microphylla*, EOs: Essential oils

Releasing of the molecules due to alterations in mitochondrial permeability transition leads to the loss of cellular homeostasis, preventing ATP synthesis, and increasing production of ROS. Studies of certain antineoplastic agents in distinct cell lines have demonstrated that some substances execute their activity by oxidative stress caused by ROS, generally occurring when the homeostasis of oxidation and reduction is altered within cells. Since ROS possess strong chemical reactivity with biomolecules such as proteins and DNA, this may result in DNA denaturation, leading to changes in protein synthesis and cell duplication. Furthermore, it is known that ROS induce activation of caspases-3 and -9 [55-57].

SCIENTIFIC DATA FOR PHYTOTHERAPIC DEVELOPMENT

Before formulating the action plan for implementation of the herbal medicine, we suggest the prospection of main institutions, researchers and areas of knowledge in a defined timeline to direct the strategic planning [58,59]. From this perspective, the prospecting is a manner to anticipate advances and can influence the orientation of technological trajectories [60]. Within the context of herbal medicines, this tool allows to target search according to which has already been produced, and to establish partnerships or cooperation that leverage innovation as determined by the requirements of public and private institutions and government agencies.

Using *Lippia* as keyword to search in the Web of Science and Derwent Innovation Index, a total of 691 articles and 94 patents were found, respectively. When the investigation was refined for *L. microphylla*, the number of manuscripts decreased for 10 and patents were not found. The first article citing *Lippia* species was published in 1948. After that, there was a vertiginous growth of manuscripts, especially in the 2000s, as seen in the Figure 4. The countries that more published about *Lippia* were Brazil, Argentina, México and United States of America [Figure 5].

It is important to note that this rising of articles approaching *Lippia* species overlaps with the implantation of the National Program on Biodiversity, whose objectives include investments and management of funds to produce new medicines [58]. In fact, identifying which are the most important needs and

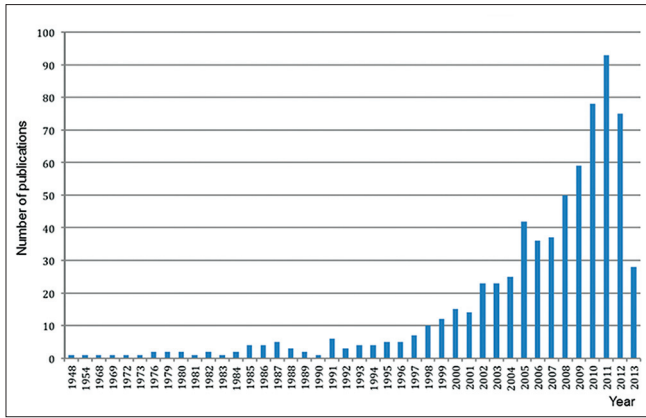


Figure 4: Temporal evolution on the number of published articles involving the genus *Lippia* (Vantage Point 7.1)

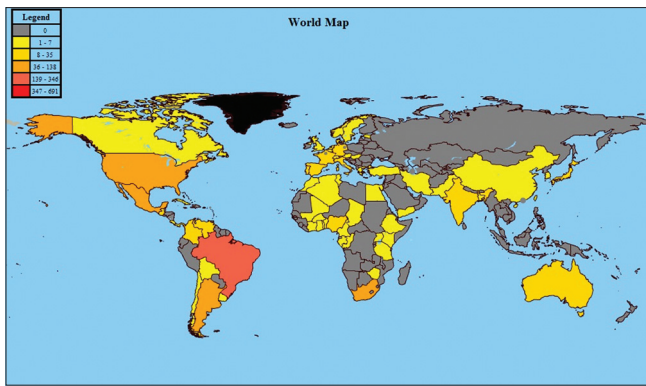


Figure 5: World participation in publications belonging to the genus *Lippia* (Vantage Point 7.1)

opportunities for Research and Development (R and D) in the future, from planned interventions in innovation systems can be an important step in established programs in Brazil as the National Program on Medicinal Plants and Phytotherapics. This program, approved in 2006, aims to ensure safe access and rational use of medicinal plants and phytotherapics by the population based on the list of regulated plants by ANVISA (National Agency of Sanitary Surveillance in Brazil) [61].

In relation to *Lippia* species, *L. alba* (142 articles) and *L. sidoides* (97 articles) are the species that have received more attention [Figure 6]. On the other hand, *L. microphylla* has a few reports about its biology and pharmacology as described above. These results are confirmed by the Figure 7 that presents the areas of publications on *L. microphylla*, most of them concentrated in food and chemical sciences.

Figure 8 shows the interaction between Brazilian and international institutions. This data is extremely important to identify universities involved in the study about *L. microphylla*, and their partnerships and cooperation in order to direct funding, define methods and technology to implement the appropriated findings. Research groups at the Federal University of Ceará and Federal University of Paraíba are the Brazilian institutions with more articles about *L. microphylla*. EMBRAPA Foundation works without partnerships or, at least, it did not

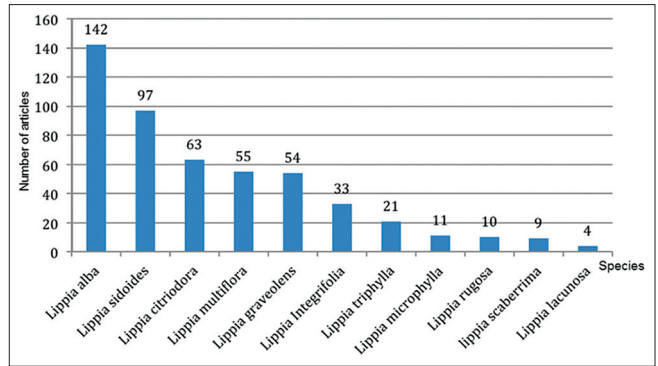


Figure 6: Number of articles about the major species belonging to the genus *Lippia* (Vantage Point 7.1)

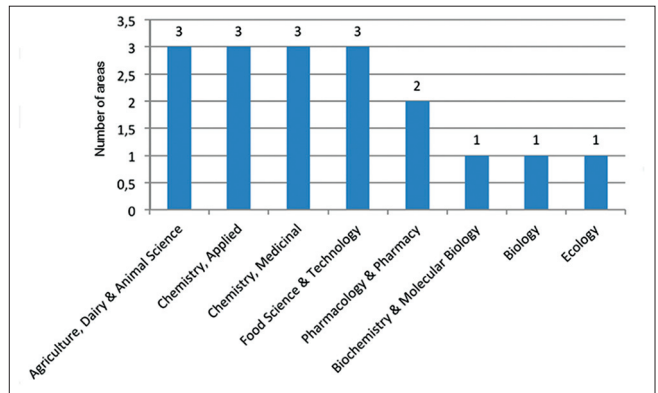


Figure 7: Areas of publications about *Lippia microphylla* (Vantage Point 7.1)

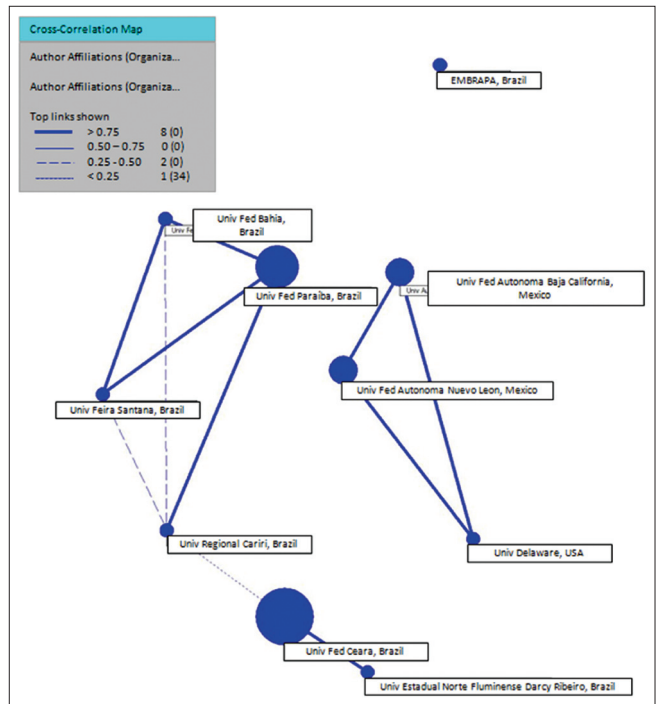


Figure 8: Institutions and their collaborations about *Lippia microphylla* publications (Vantage Point 7.1)

publish works with other institutions yet. Universities of USA (Delaware) and Mexico often work together. The academic

institutions around the world are the leading centers for generation of new patentable technologies, as observed in this study. However, Brazil has low competitiveness and shows little effort to innovate in the area of technological inventions, probably due to some failures in the innovation system (cooperation between government, business, and institutions to promote an effective system of production and development of medicines). Then, Brazil does not have a valuable protecting structure of *Lippia* species, reflecting the lack of incentives to safeguard the technologies developed using industrial property.

Finally, in 2004, it was promulgated a Brazilian law about Technological Innovation (number 10,973), which was regulated in 2005 (Decree 5,563). This law normalizes the incentives for the involvement of Scientific and Technological Institutions (Institutos de Ciência e Tecnologia - ICT's) in the innovation process for innovation in companies, for the independent inventor and conception of investment funds for innovation. It is the first Brazilian law that deals with the relationship between Universities and/or Research Institutions and companies with the creation of Technological Innovation Centers (Núcleos de Inovação Tecnológica - NIT's), helping the institutional maturity to make strategic management of intellectual property in the Brazilian ICT's [62].

Despite a high Brazilian scientific production, these findings did not trigger large impacts on the economic development. Numbers of patents is far from the quantity of published articles, existing an abysmal between which is published which is patented, and which would become a product or service to generate work and wealth to the country [Figure 9]. Japan made its first deposit in 1990 and, currently, it is the largest holder of patents (36). Brazil arose in 1993 and deposited only 9 patents. First was recorded in 1987 [Figure 10]. Thus, these data are not consistent with the Brazilian scientific production about *Lippia* species and the number of published articles, suggesting a lack of encouragement in researches for the development of inventions involving *Lippia* species. Moreover, the scientific production in Brazil is recent (last 100 years), and is concentrated in public universities and research centers, and in honorable exceptions, in private institutions, as a result of the Brazilian public policies in Science and Education [63]. Certainly, this is the capital challenge in the Brazilian national innovation system for the transfer of technology generated in universities and research centers to industry, in a way that new processes and products may be generated from these institutions.

The growing interest in plant products with different purposes is linked, in part, to the low cost of drug production based on active principles isolated from natural products when compared to investments for synthesis in the laboratory [64]. In the context of the herbal market evolution, estimated at US\$ 22 billion and corresponding to 3.7% of the global market, this growth accompanies the pharmaceutical industry, considered one of the most lucrative in the world. Brazil is considered the seventh largest market, and from 2006 to 2010 grew by 14%. In 2015, Brazil will be the sixth largest market in this sector [65]. Hence, Brazilian pharmaceutical and bio prospecting areas require constant and high investments in research and development

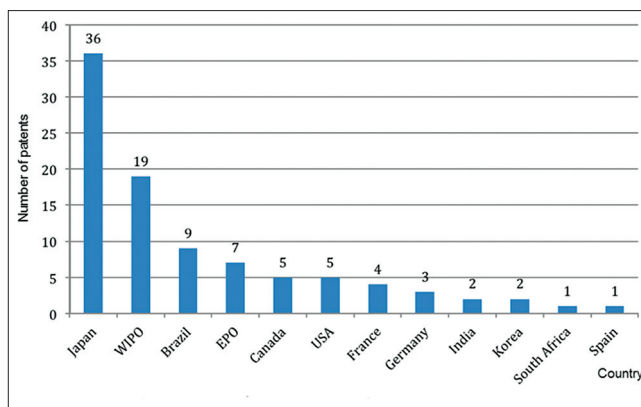


Figure 9: Countries with patents about *Lippia* species (Vantage Point 7.1). WIPO = World Intellectual Property Organization; EPO = European Organization; USA = United States of America

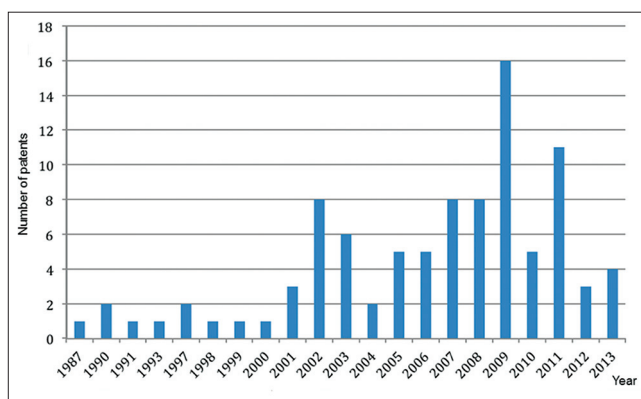


Figure 10: Number of patents about genus *Lippia* deposited from 1987 (Vantage Point 7.1)

of new products, since the natural resources have money-making value, attract investors and catch the attention to the preservation of endemic species.

CONCLUSION

Lippia species are a source of remarkable bioactive substances (such as EOs) with economic potential for local communities. Specifically, *L. microphylla* is an endemic underexploited Brazilian vegetal with great medicinal properties that has gained much attention in the general population and scientific community. Then, this bio prospection helps to build perspectives of the bioactivity areas of real interest for capital investment and to give support for Brazilian institutions to establish cooperation and partnerships in order to change the scientific reality, where basic and applied researches in pharmaceutical sectors are ineffective and without technological impact to create and innovate.

ACKNOWLEDGMENTS

We wish to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI) for financial support in the form of grants and fellowship award.

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Source of Support: Nil, Conflict of Interest: None declared.