

Essential oils biological activity of the shrub *Lippia alba* (Verbenaceae)

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ABSTRACT. Introduction: *Lippia alba* is an aromatic species belonging to the Verbenaceae family. Its essential oils have been used in different industries, because of its biological properties. **Objective:** Identify the perspectives of the biotechnological applications of *Lippia alba* essential oils. **Methodology:** A scoping review was conducted on the biological activity of *Lippia alba* essential oils registered until October, 2018 in EBSCO, Embase, Pubmed, Scopus, SciELO, and Lilacs databases. **Results:** Chemotypes I and III have been reported for different biological activities from the evaluations performed on microorganisms, fish, arthropods, small mammals, and cell lines; fundamentally associated with antibacterial, antifungal, cytotoxic, antioxidant, and sedative effects, among others. Records focused mainly on the health, fishing industry, and agrifood sectors. **Conclusion:** Studies on the effect of essential oil are promising, but do not reflect a continuity of the research toward prototypes or finished commercial products. Research groups must unify evaluation methodologies and include in all studies the relationship between phytochemical and biological activity for the meta-analyses to be possible. Likewise, they must join efforts through the National System for Agricultural Innovation (SNIA, for the term in Spanish) to generate finished products that impact upon society and facilitate progress in the country's bio-economy.

Key words: *Lippia alba*, essential oils, chemotypes, aromatic plants, health protection, food industry, agrifood sector.

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SUPPLEMENTARY MATERIAL

Acronym definition: MIC: minimum inhibitory concentration; MBC: minimum bactericide concentration; LD50: lethal dosage 50; LD or LC: lethal dosage or lethal concentration; IC50: minimum inhibition concentration 50; CC50: cytotoxic concentration 50; EC50: effective concentration 50; RP50: repellency 50; RP: repellency percentage; CHO: Chinese hamster ovary cells; VERO: African green monkey kidney; A549: human lung carcinoma cell line; THP-1: human leukemic monocyte cells; Jurkat: T-cell lymphocytes with acute lymphoid leukemia; HepG2: hepatocellular liver cells; HeLa: human cervical epithelial carcinoma.

Organic products are broadly used by the global population for essential health-care (Plantlife International, 2004; Grand View Research, 2018). In 2017 the market of personal-care products reached \$12.19-billion, and further growth is foreseen (Grand view research, 2018). Medicinal and Aromatic Plants (MAPs) are a natural source of chemical substances that provide diverse biological properties (Bouyahya, Guaouguaou, Dakka, & Bakri, 2017). That is why countries several countries (i.e. the United States, Brazil, India, Colombia, and Cuba) support scientific research that let the discussion of the biological properties of MAPs and support better uses focused on bio-prospection (Tofiño-Rivera, Ortega-Cuadros, Melo-Ríos, & Mier-Giraldo, 2017).

Colombia has an outstanding floristic wealth, among which are included several promising MAPs of interest for science, such as *Lippia alba* (Mill.) N.E. Br, an aromatic species belonging to the Verbenaceae family, whose essential oils have been used in the cosmetic, food, and biomedical industries (Tofiño-Rivera et al., 2017; Linde, Colauto, Albertó, & Gazim, 2016) due to its biological properties. However, these properties are derived from the plant's phytochemical composition. The presence and concentration of secondary metabolites is influenced by agro-economic management applied to the production of biomass, the phenological cycle of the material harvested, edaphoclimatic conditions, extraction method of the essential oil (Olivero-Verbel, González-Cervera, Güette-Fernandez, Jaramillo-Colorado, & Stashenko, 2010; Linde et al., 2016), and the evaluation technique employed in some cases (Ramírez & Castaño, 2009). This scoping review was conducted to identify the perspectives of the biotechnological application of essential oils (EO) of *Lippia alba* (Mill.) N.E. Br.

A scoping review (Armstrong, Hall, Doyle, & Waters, 2011) was carried out on the scientific literature that describes the biological activity of *L. alba* EO. Likewise, a search equation was elaborated by using the terms “*Lippia alba* and essential oil”, which was used to access published articles deposited in

EBSCO, Embase, Pubmed, Scopus, SciELO, and Lilacs, since the beginning of time for each database, until October 8th, 2018. The registries recovered were filtered to eliminate duplicates using Zotero. Inclusion and exclusion criteria were applied. A select group of documents was obtained to which a detailed analysis was applied (Fig. 1). The variables of interest (year, country, author, title, institution, EO extraction technique, biological activity evaluated, evaluation method, object of study, and result obtained) were extracted and compiled in a database using Microsoft Excel 2016. IBM SPSS 23.0 was used to conduct the statistical analyses. Application of the research protocol was conducted independently by two researchers to guarantee reproducibility of the work, and the discrepancies observed were solved through a third-party concept.

Bibliometric analysis: The search recovered 706 registries, from which 115 documents were analyzed (Fig. 1). It was identified that *L. alba* EO and their respective biological activity have been topics of interest in the scientific community since 1990. The publication of scientific articles has not been continuous. Countries with higher rate of published articles related with the subject are Brazil with 70 (60.5%), Colombia with 29 (25.2%), and India with 7 (6.2%). The institutions that have led these investigations are Universidad Federal de Santa María with 18 (15.6% publications), Universidad Industrial de Santander with 8 (6.9% publications), Universidad de Cartagena with 6 (5.2% publications), Universidade Regional do Cariri with 6 (5.2% publications), and Universidade Federal da Bahia with 3 (2.6% publications).

Biological activity of *L. alba* essential oils: This exploratory review evidences the interest by the scientific community in progressing on the knowledge of the safe use of the derivatives of these aromatic species. *L. alba* essential oils are antitumorigenic (Vera, Olivero, Jaramillo, & Stashenko, 2010; López, Stashenko, & Fuentes, 2011; Neira, Mantilla,

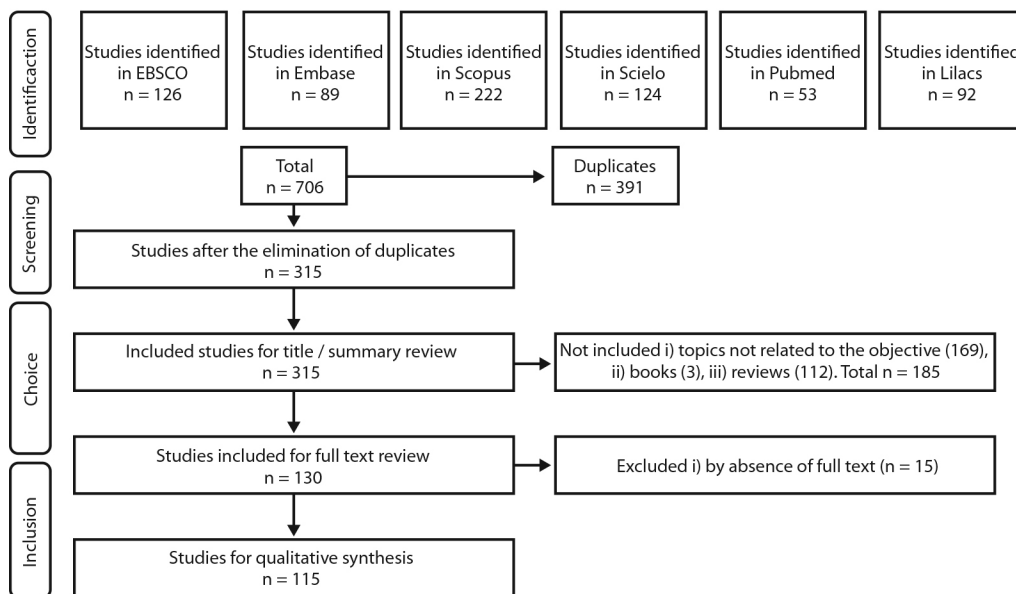


Fig. 1. Summary of the protocol of the exploratory systematic review applied and the respective results. Source: elaborated by the authors.

Stashenko, & Escobar, 2018), but are toxic for *Artemia franciscana*, on which DL 6.17-24.87 $\mu\text{g/ml}$ were presented (Olivero-Verbel, Güette-Fernandez, & Stashenko, 2009) and DL 50.4-21.05 $\mu\text{g/ml}$ (Olivero-Verbel, González-Cervera et al., 2010). In mice, oral administration with dosages of 2 000 mg/kg is lethal; 900-1 500 mg/kg is life compatible but causes signs and symptoms of neurological damage and moderate to severe motor impairment as ataxia, lethargy, salivation, and transitory convulsions (Aular, Villamizar, Pérez, & Pérez, 2016). Intraperitoneal administration of dosages $\geq 1\ 500$ mg/kg causes neurological damage, and dosages of 1 000 mg/kg cause slight hepatic damage (Olivero-Verbel, Guerrero-Castilla, & Stashenko 2010). On CHO cells, the oils did not inhibit cell proliferation at concentrations of 0.01-1 $\mu\text{g/ml}$ (Tofiño-Rivera et al., 2016), with reports for VERO CC50 cells of 25.3-200 $\mu\text{g/ml}$ (Meneses, Ocazonez, Martínez, & Stashenko, 2009; Escobar, Leal, Herrera, Martinez, & Stashenko, 2010; Moreno, Leal, Stashenko, & García, 2018; Zapata, Betancur-Galvis, Duran, & Stashenko, 2013),

A549 CI50 47.80 $\mu\text{g/ml}$ (Gomide et al., 2013), THP-1 CC50 16.8-43.17 $\mu\text{g/ml}$ (Escobar et al., 2010; Neira et al., 2018), Jurkat CI50 16.6-31.6 $\mu\text{g/ml}$, HepG2 CI50 21.5-200 $\mu\text{g/ml}$ (Zapata et al., 2013), and HeLa CI50 5-80.5 $\mu\text{g/ml}$ (Agudelo-Gómez, Gómez, Durán, Stashenko, & Betancur-Galvis, 2010; Zapata et al., 2013).

L. alba is a species cultivated in different tropical, subtropical, and temperate zones throughout the world (Linde et al., 2016); its essential oils are promising due to their chemical diversity (Linde et al., 2016; Tofiño-Rivera et al., 2017). However, this chemical composition is sensibly dependent on the geographic location and the phenology of the plant material harvested (Durán, Monsalve, Martínez, & Stashenko, 2007), which has categorized *L. alba* in seven chemotypes (Table 1; Linde et al., 2016). Essential oils from chemotypes I and III plants present antispasmodic (Viana, Vale, Silva, & Matos, 2000; Blanco et al., 2013; Sousa et al., 2015; Silva et al., 2018; Carvalho et al., 2018), anticonvulsive (Fauth, Campos, Silveira, & Rao, 2002; Maynard et al., 2011), anxiolytic (Hatano, Torricelli, Giassi, Coslope,

TABLE 1
L. alba chemotypes

Chemotype	Description of the essential oil composition (principal constituent components)
I	Citral, linalool, β -Caryophyllene (four subtypes within this chemotype)
II	Tagetenone
III	Limonene in large amounts and a variable amount of carvone or monoterpene ketones instead of carvone
IV	Myrcene
V	γ -terpinen
VI	Canfora-1,8-cineole
VII	Estragole

Source: elaborated by the authors.

& Viana, 2012; Junior et al., 2018), and anti-inflammatory effects (Sepúlveda-Arias, Veloza, Escobar, Orozco, & Lopera, 2013). On contact with the skin, they are not irritating nor alter the histopathology (Neira et al., 2018), and are sleep enhancers (Fauth et al., 2002), anesthetic, and sedative (Salbego et al., 2017a). They also have antioxidant activity, although in this property the collection site is quite determining, given that chemotype I was a good antioxidant, while other chemotype III specimens had moderate and absent effect (Stashenko, Jaramillo, & Martínez, 2004; Olivero-Verbel, González-Cervera et al., 2010).

Insecticide activity has been reported for chemotypes I and III; it was reported on *Rhipicephalus microplus*, RP50 0.47-5.07 mg/cm² (Lima et al., 2016) and CL50 8.8-27 mg/ml (Peixoto et al., 2015b; Chagas et al., 2016), on *Tribolium castaneum*, RP 34-96 % in concentrations of 0.00002-0.2 μ L/cm² (Caballero-Gallardo, Olivero-Verbel, & Stashenko, 2011) and CL50 19.7-107.8 μ L/ml, and on *Sitophilus zeamais*, CL50 15.2-70.8 μ L/ml (Peixoto et al., 2015a). Chemotype III presents dissuasion > 80 % in oviposition of *Aedes aegypti* at concentrations of 0.005-0.2 μ g/ml (Castillo, Stashenko, & Duque, 2017), and it also has larvicidal activity at variable concentrations: Vera et al. (2014) reported CI50 0.042-0.044 μ g/ml and IC95 0.089-0.099 μ g/ml; Muñoz, Stashenko and Ocampo (2014), CL50 0.11 μ g/ml and CL99 0.211 μ g/ml; Ríos, Stashenko and Duque (2017), CL50 63.61 - 72.34 μ g/

ml CL95 98.91-110.84 μ g/ml; and Aldana and Cruz (2017), CL50 of 84-367 μ g/ml and CL95 of 118-506 μ g/ml.

Antiviral activity was not evidenced for chemotype I (Agudelo-Gómez et al., 2010), but chemotype III had preventive effect on cells infected with dengue virus (serotypes DENV-1, DENV-2, DENV-3, DENV-4) (Ocazionez, Meneses, Torres, & Stashenko, 2010). The concentration of 11.1 μ g/ml reduced the viral titers of the agent causing yellow fever (VFA) (Meneses et al., 2009), and concentrations of 125 and 250 μ g/ml, at 24 and 48 h, respectively, reduced 10^{1.5} log units the viral load of an HSV-1 isolate sensitive to Acyclovir (Agudelo-Gómez et al., 2010).

The amoebicidal activity of chemotype III was reported on *Acanthamoeba polyphaga* trophozoites with IC50 31.79 μ g/ml (Santos et al., 2016b); leishmanicide activity on *Leishmania braziliensis* (promastigote CE50 8.88 μ g/ml and amastigote CE50 9.19 μ g/ml) and *Leishmania chagasi* (promastigote CI50 18.9 - 100 μ g/ml) (Neira et al., 2018; Escobar et al., 2010). The trypanocidal effect is variable because for *Trypanosoma cruzi*, Moreno et al. (2018) determined that chemotype I had IC50 14.22 and 74 μ g/ml on epimastigote, trypomastigote, and amastigote, while chemotype III had IC50 88.45 and > 150 μ g/ml for the same parasite forms. For chemotype I, Escobar et al., (2010) obtained bio-activity on epimastigotes IC50 5.5-8.8 μ g/ml, and amastigote IC50 12.2 - 17.47 μ g/ml; and for chemotype III,

epimastigote IC₅₀ 8.8 µg/ml and amastigote IC₅₀ 17.47 µg/ml. Additionally, Baldissera, Souza, Mourão, Silva, and Monteiro (2017) reported that under *in-vitro* conditions, concentrations of 0.5, 1, and 2 % of chemotype I had direct correlation with the efficacy in controlling *Trypanosoma evansi*, but under *in-vivo* conditions in mice; treatment with 1.5 ml Kg⁻¹ did not show curative efficacy. Nematicidal activity of chemotypes I and III was reported on *Meloidogyne incognita* at concentrations from 0.1-2.5 ml/L, which had mean mortality percentages from 22 to 100 %, in dependent dosage; and diminished eclosion rate from 47 to 9 % (Moreira, Santos, & Innecco, 2009).

Antimicrobial and antifungal activity has been reported on periodontal-pathogenic microorganisms (Juiz et al., 2015; Bersan et al., 2014), cariogenic organisms (Bersan et al., 2014), enterobacteria (Pino, Ortega, Rosado, Rodríguez, & Baluja, 1996; Machado, Nogueira, Pereira, Sousa, & Batista, 2014), dermatophytes (Costa et al., 2014; Tangarife-Castaño et al., 2012), phytopathogens (Mena-Rodríguez, Ortega-Cuadros, Merini, Melo-Ríos, & Tofiño-Rivera, 2018; Anaruma et al., 2010), sulfate reductase (Souza et al., 2017a), important pathogens in aquaculture (Souza et al., 2017b; Majolo, Rocha, Chagas, Chaves, & Bizzo, 2017; Sutuli et al., 2015), *Candida albicans* (Mesa-Arango et al., 2010; Santos et al., 2016a; Mesa-Arango et al., 2009), aspergillosis (Mesa-Arango et al., 2010; Glamočlija, Soković, Tešević, Linde, & Colauto, 2011; Mesa-Arango, et al., 2009), Cryptococcosis (Santos et al., 2016a); gastrointestinal, cutaneous, and nosocomial infections (Pino et al., 1996; Machado et al., 2014; Porfirio et al., 2017); under the MIC interval between 4.0-9 370 µg/ml and MBC 6.5-2 500 µg/ml for bacteria (Table 2), and MIC 31.25-2 000 and MBC/MFC 500-1 250 µg/ml for fungus (Table 3).

Final considerations: *L. alba* is promising plant in Latin America (Tofiño-Rivera et al., 2017), which is included in the Colombian Vademecum of Medicinal Plants (Ministry of Health and Social Protection [MinSalud],

2008), and is approved for over-the-counter sale as antiseptic for external use and adjuvant in the treatment of anxiety of nervous origin in humans (INVIMA, 2018). According to the records found, *L. alba* essential oils are bio-inputs of interest, given their possibility of bioprospection. In biomedical sciences, research has provided effective tools to solve problems related with amoebic infections (Santos et al., 2016b), herpetic infections (Agudelo-Gómez et al., 2010), tropical diseases e.g. like dengue (Ocazonez et al., 2010), leishmaniasis (Escobar et al., 2010; Neira et al., 2018), trypanosomiasis (Escobar et al., 2010; Baldissera et al., 2017; Moreno et al., 2018), and yellow fever (Meneses et al., 2009), control of vectors, like *Aedes aegypti* (Vera et al., 2014; Castillo et al., 2017; Ríos et al., 2017; Aldana & Cruz, 2017), mycosis (Mesa-Arango et al., 2010; Santos et al., 2016a; Glamočlija et al., 2011), in-hospital diseases (Santos et al., 2016a; Pino et al., 1996; Machado et al., 2014), and diseases related with the oral cavity (Juiz et al., 2015; Bersan et al., 2014).

In addition, the agroindustrial sector has focused on the use of *L. Alba* EO to control efficiently parasites that affect vegetable production (Moreira et al., 2009), improve grain conservation (Caballero-Gallardo et al., 2011; Peixoto et al., 2015a; Shukla, Kumar, Singh, & Dubey, 2009), improve the quality of products derived from livestock (Peixoto et al., 2015b; Lima et al., 2016; Chagas et al., 2016), as well as diminish damage during transport of ornamental aquatic species, like *Hippocampus reidi* (Cunha et al., 2011); anesthetics for model organisms, like *Hypsiboas geographicus* (Salbego et al., 2017a); sedatives, anesthetics, and preservatives in hedgehogs (Simões et al., 2017) and shrimp (Parodi et al., 2012), and fish of economic importance (Sena et al., 2016; Hohlenwerger et al., 2017; Cunha et al., 2010).

In aquaculture, for *L. alba* essential oils, chemotype I has reported promising results, given their sedative effect on *Rhamdia quelen* (20-40 µL/L) (Salbego et al., 2017b; Veeco et al., 2018); anesthetic effect on *Litopenaeus vannamei* (> 30 µL/L) (Parodi et al., 2012),

TABLE 2
Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)
of chemotypes I and III essential oils on bacterial species

Microorganism	Strain/isolate	MIC (µg/ml)	MBC (µg/ml)	Chemotype	Reference
<i>Aeromonas</i> spp.	Isolates, ATCC 7966	2862 - 5.000	781.3 - 6.000	I	(Suttili et al., 2015; Majolo et al., 2017; Souza, Costa et al., 2017)
<i>Aggregatibacter actinomycetemcomitans</i>	ATCC 43717	>3 200	>3 200	III	(Juiz et al., 2015)
<i>Bacillus subtilis</i>	ATCC 7001	630	1.250	III	(Pino et al., 1996)
<i>Bacteroides fragilis</i>	ATCC 25285	400 - 1 600	400 - 1 600	III	(Juiz et al., 2015)
<i>Desulfovibrio alaskensis</i>	NCIMB 13491	78 - 2 500		I	(Souza, Goulart et al., 2017)
<i>Enterobacter aerogenes</i>	ATCC 13088	2 500	2 500	III	(Pino et al., 1996)
<i>Enterococcus faecalis</i>	ATCC 19433	630 - 4 000	1 250	I and III	(Pino et al., 1996; Santos et al., 2016a)
<i>Escherichia coli</i>	ATCC 25922, 201389, 10536, Isolates	400 - 4 000	2 500 - 1 170	I and III	(Pino et al., 1996; Duarte et al., 2007; Machado et al., 2014; Santos et al., 2016a)
<i>Fusobacterium nucleatum</i>	ATCC 25586	125	125	I	(Bersan et al., 2014)
<i>Klebsiella pneumoniae</i>	ATCC 13883	2 500	2 500	III	(Pino et al., 1996)
<i>Listeria innocua</i>	ATCC 19115	580 - 1 330	1 330 - 1 170	I	(Machado et al., 2014)
<i>Listeria monocytogenes</i>	ATCC 33090	580 - 1 330	580 - 1 330	I	(Machado et al., 2014)
<i>Porphyromonas gingivalis</i>	ATCC 33277	6.5 - 250	6.5 - 250	I and III	(Bersan et al., 2014; Juiz et al., 2015)
<i>Pseudomonas aeruginosa</i>	ATCC 9027	5 340 - 9 370	5 340 - 9 370	I	(Machado et al., 2014)
<i>Salmonella choleraesuis</i>	ATCC 10708	5 340 - 9 370	5 340 - 9 370	I	(Machado et al., 2014)
<i>Serratia marcescens</i>	ATCC 8100, MBCAI 469	630 - 4 000	1 250	I and III	(Pino et al., 1996; Santos et al., 2016a)
<i>Staphylococcus aureus</i>	ATCC 6538, ATCC 25923	290 - 1 000	290 - 2 000	I and III	(Pino et al., 1996; Machado et al., 2014; Porfírio et al., 2017)
<i>Streptococcus mitis</i>	ATCC 903	250	-	I	(Bersan et al., 2014)
<i>Streptococcus sanguis</i>	ATCC 10556	250	100 000	I	(Bersan et al., 2014)

Source: elaborated by the authors.

Colossoma macropomum (62,5-375 µL/L) (Batista et al., 2018), *Echinometra lucunter* (150 µL/L) (Simões et al., 2017), *Rhamdia quelen* (20 and 150-450 µL/L) (Cunha et al., 2010; Toni et al., 2014; Veit et al., 2017; Salbego et al., 2017b), *Argyrosomus regius* (200 µL/L) (Cárdenas et al., 2016), *Serrasalmus rombeus* (50-200 µL/L) (Almeida, Heinzmann, Val, & Baldissarotto, 2018), *Sparus aurata* (100-300 µL/L) (Toni et al., 2015); and reduce stress in *Rhamdia quelen* (10 - 40 µL/L) (Azambuja et al., 2011; Veeck et al., 2013), *Oreochromis niloticus* (20 µL/L) (Hohlenwerger et al., 2017), *Litopenaeus vannamei* (> 30 µL/L) (Parodi et al., 2012), *Echinometra*

lucunter (150 µL/L) (Simões et al., 2017), and tambacu hybrids (*Piaractus mesopotamicus* × *Colossoma macropomum*) (200 µL/L) (Sena et al., 2016).

Chemotype I EO can be used to enhance aquaculture techniques because they maintain the freshness of fish stored in ice (Veeck et al., 2018), do not modify the organoleptic characteristics (Cunha et al., 2010; Toni et al., 2014; Hohlenwerger et al., 2017), or affect fish quality (Sena et al., 2016). However, further studies are needed, given that Veeck et al. (2018) reported that concentrations from 30-40 µL/L do not represent antimicrobial activity against mesophiles and psychrophiles, and Veit

TABLE 3
Minimum inhibitory concentration (MIC) and minimum fungicide concentration (MFC),
of chemotypes I and III essential oils on fungal species

Microorganism	Strain/isolate	MIC (µg/ml)	MFC (µg/ml)	Chemotype	Reference
<i>Aspergillus flavus</i>	ATCC 204304	180 - 1 600	-	I and III	(Mesa-Arango et al., 2009; Mesa-Arango et al., 2010)
<i>Aspergillus fumigatus</i>	ATCC 204305, ATCC9142	35 - 570	1250	I and III	(Mesa-Arango et al., 2009; Mesa-Arango et al., 2010; Glamočlija et al., 2011)
<i>Aspergillus niger</i>	ATCC6275	600	600	I	(Glamočlija et al., 2011)
<i>Aspergillus ochraceus</i>	ATCC12066	300	600	I	(Glamočlija et al., 2011)
<i>Aspergillus versicolor</i>	ATCC11730	300	600	I	(Glamočlija et al., 2011)
<i>Candida albicans</i>	MBCAI 560, ATCC 10231, CBS 562	250 - 2 000	500	I	(Duarte, Figueira, Sartoratto, Rehder, & Delarmelina, 2005; Bersan et al., 2014; Santos et al., 2016a)
<i>Candida dubliniensis</i>	ATCC 7978	500	-	I	(Santos et al., 2016a)
<i>Candida glabrata</i>	ATCC 90030	2 000	-	I	(Santos, 2016a)
<i>Candida krusei</i>	ATCC 6258, clinical isolate	140 - 2 000	-	I and III	(Mesa-Arango et al., 2009; Mesa-Arango et al., 2010; Santos et al., 2016a)
<i>Candida parapsilosis</i>	ATCC 22019, clinical isolate	280 - 2 000	-	I and III	(Mesa-Arango et al., 2009; Mesa-Arango et al., 2010; Santos, 2016a)
<i>Candida tropicalis</i>	ATCC 13803	2 000	-	I	(Santos et al., 2016a)
<i>Colletotrichum gloeosporioides</i>	Isolates	300 - 900	-	I	(Anaruma et al., 2010; Mena-Rodríguez et al., 2018)
<i>Cryptococcus gattii</i>	ATCC MYA-4560, MYA-4563, ATCC 208821	1 000 - 2 000	-	I	(Santos et al., 2016a)
<i>Cryptococcus grubii</i>	ATCC 208821	1 000	-	I	(Santos et al., 2016a)
<i>Cryptococcus neoformans</i>	ATCC MYA-4560	1 000	-	I	(Santos et al., 2016a)
<i>Fusarium oxysporum</i>	ATCC 48112	>500	-	I and III	(Tangarife-Castaño et al., 2012)
<i>Macrophomina phaseolina</i>	Isolates	800 - 1 200	-	I	(Mena-Rodríguez et al., 2018)
<i>Microsporium gypseum</i>	Human isolates	156	-	I	(Costa et al., 2014)
<i>Penicillium funiculosum</i>	ATCC10509	1 250	1 250	I	(Glamočlija et al., 2011)
<i>Penicillium ochrochloron</i>	ATCC9112	600	1 250	I	(Glamočlija et al., 2011)
<i>Phytophthora capsici</i>	Isolates	50 - 150	-	I	(Mena-Rodríguez et al., 2018)
<i>Saccharomyces cerevisiae</i>	ATCC MYA-4567	2 000	-	I	(Santos et al., 2016a)
<i>Trichoderma viride</i>	ATCC5061	600	1 250	I	(Glamočlija et al., 2011)
<i>Trichophyton mentagrophytes</i>	ATCC 24198	125 - 500	-	I and III	(Tangarife-Castaño et al., 2012)
<i>Trichophyton rubrum</i>	ATCC 28188, human isolates	31.25 - 354.98	-	I and III	(Tangarife-Castaño et al., 2012; Costa et al., 2014)

Source: elaborated by the authors.

et al. (2017) obtained that 375 ml/L do not prevent physico-chemical changes produced by the electric stunning or hypothermia in fillets of *Rhamdia quelen* during frozen storage. Some important recommendations have

been considered: 1) to transport live fish, 15 µL/L of essential oil for *Argyrosomus regius*, or 100-300 µL/L for *Sparus aurata*, or 62.5-375 µL/L for *Colossoma macropomum* are not recommended because these do not prevent the

response to stress (Toni et al., 2015; Cárdenas et al., 2016; Batista et al., 2018); 2) prior sedation is not recommended with 200 $\mu\text{L/L}$ during three minutes with *L. alba* EO to reduce initial agitation, nor is treatment recommended with 30-40 $\mu\text{L/L}$ EO for transport because it does not avoid oxidative stress in liver (Salbego et al., 2014); for *Argyrosomus regius*, 15 $\mu\text{L/L}$ is not recommended to transport live fish, given that it does not inhibit stress (Cárdenas et al., 2016); 3) concentrations of 1 600 and 3 200 $\mu\text{L/L}$ of chemotype III oils are effective against *Anacanthorus spathulatus*, *Notozothecium janauachensis*, and *Mymarothecium boegeri*, natural parasites of *Colossoma macropomum*; but these dosages are toxic to fish gills, therefore, there is a need to propose strategies to take advantage of the antihelmintic effect of these products without affecting the host organism, using cutting-edge biotechnological techniques, like nanotechnology (Soares et al., 2016).

Chemotypes I and III seem to be the most common. Chemotype I has more reports on the biological effect; however, it must be considered that the cell and molecular characteristics of each microorganism or organism also interfere with the EO spectrum, given that on *Tribolium castaneum* and *Sitophilus zeamais* parasites, chemotype III was more effective than chemotype I (Peixoton et al., 2015a), but on *Candida krusei* and *Aspergillus fumigatus*, chemotype I was more effective (Mesa-Arango, et al., 2009). Additionally, chemotype I had anesthetic effect on *Hypsiboas geographicus* (Salbego et al., 2017a), but not on *Neohelice granulata* (Souza et al., 2018), and chemotype III was anti-inflammatory in the RAW 264.7 murine cell line (Sepúlveda-Arias et al., 2013), but this effect was not registered on *Oreochromis niloticus* (Rodrigues-Soares et al., 2018).

One of the critical variables associated with energizing an industry through *L. alba* EO corresponds to the phytochemical characterization of the plant material from which it is extracted, given that the availability of raw material with standardized conditions constitutes a critical point for the bio-industry

(BIOinTropic, Universidad EAFIT, & Silo, 2018). We can highlight the lack of a comprehensive productive model for the agroindustrial use of *L. alba* because only specific studies are identified on a single management practice and for a specific area of the country (Zambrano, Buitrago, Durán, Sánchez, & Bonilla, 2013).

Regarding this work, it was found that 18.3 % of the articles did not relate the biological activity of the EO with its composition, given that no indication is provided of the chemotype or majority components. In this sense, under a general vision of the registries recovered, exploratory or preliminary works were observed without the continuity of the evaluation, according to the Colombian technical standards and norms by the WHO to advance in the formulation of bio-products (Decree 677, 1995; Decree 2510, 2003; WorWHO, 2004; ICONTEC, 2011), which is reflected in the lack of patents registered or pending, although research groups exist in Colombia that include *L. alba* as study model (Tofiño-Rivera et al., 2017). A vector and confluent effort as a country is needed here through strategies defined within the National Agricultural Innovation System (SNIA, for the term in Spanish) to encourage development based on bio-economy and, thus, take advantage of the diversity of *L. alba* chemotypes registered throughout the country -citral in Bolívar and Cesar, limonene in Arauca, and carvone in Cundinamarca, Tolima, Boyacá, Valle del Cauca, Santander, Antioquia, Quindío, and Cesar (Durán et al., 2007; Mesa-Arango et al., 2009; Olivero-Verbel, Guerrero-Castilla et al., 2010; Tofiño-Rivera et al., 2016)-, considering the specific uses referred by the scientific literature and which have been systematized in this document.

Unlike synthetic substances (Célis, 2018) and other natural standardized substances in which the concentration used over the same or different organisms will permit a meta-analysis for a finer verification of the biological effect of the substance in context (Valero et al., 2011), this possibility would not be rigorous in the case of the set of documents recovered in this research on the biological activity of *L. alba*

EO because although many of the investigations refer to the chemotype or the majority component, the same concentration of EO, or the same chemotype, may have large variations in the concentrations of its majority or minority components. This variation is dependent on the equation that explains the phenotypic expression of an attribute $F = G + A + GXA$: chemotype -genetics G-, bioclimatic offer of the plantation -A-, and the genotype x environment interaction -GXA-, which are conditioners of the plant's physiological response over the secondary metabolism, upon the bioclimatic offer, agro-economic management, and harvest time (Palacio-López & Rodríguez-López, 2008). In addition to this heterogeneity exhibited by the raw material, the extraction method also affects the final composition of the EO, which hinders the possibility for a meta-analysis for the bioactivity of *L. alba* from a set of studies with high variability in the concentrations of the bioactive components of the EO (Durán et al., 2007; García-Perdomo, 2015; Barrientos, Reina, & Chacón, 2012; Delgado, Sánchez, & Bonilla, 2016). It would, then, be important to have a set of studies with detailed phyto-chemistry of the EO and which use the individual standardized bioactive components as controls (Ortega-Cuadros, Tofiño-Rivera, Merini, & Martínez-Pabón, 2018). Other exploratory revision works have registered the variable biological activity of the *L. alba* EO, specifically in the results of microbial control, dependent on the extraction technique used and the quality of biomass harvested. This work also highlighted high heterogeneity in assessment techniques of the biological activity of the *L. alba* EO used by the scientific community (Ortega-Cuadros & Tofiño-Rivera, 2019).

It is concluded that *L. alba* EO is a substance of broad spectrum of use, which is why industrial development is feasible based on this raw material. Nevertheless, it is necessary to establish within the SNIA research programs that prioritize the most consistent effects registered in this review, in accordance with national and global demands. Besides, progress is necessary on preliminary tests at

pilot scale established by the Colombian norms to advance in the generation of commercial formulations. One of the first steps in this respect corresponds to the methodological harmonization, given that in some studies there was no appropriate control of the variables interfering on the biological quality of the *L. alba* EO, no detailed phyto-chemical analyses were presented, nor were adequate controls involved to facilitate a subsequent meta-analysis that guides the prioritizing of investment resources in the most consistent application lines. Another significant element is the generation of strategic alliances to elaborate protocols to standardize agro-economic management conditions of the plants in production zones, as well as articulation with the industry for scaling of the protocols formulated.

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RESUMEN

Actividad biológica de los aceites esenciales del arbusto *Lippia alba* (Verbenaceae). **Introducción:** *Lippia alba* es una especie aromática perteneciente a la familia Verbenaceae, cuyos aceites esenciales han sido empleados en diferentes industrias dada sus propiedades biológicas. **Objetivo:** identificar las perspectivas de aplicación biotecnológica de los aceites esenciales de *Lippia alba*. **Métodos:** se realizó una revisión sistemática exploratoria de la literatura sobre la actividad biológica de aceites esenciales de *Lippia alba* registrada hasta octubre 2018 en las bases de datos EBSCO, Embase, Pubmed, Scopus, SciELO, y Lilacs. **Resultados:** los quimiotipos I y III han sido reportados para diferentes actividades biológicas a partir de evaluaciones realizadas en microorganismos, peces, artrópodos, pequeños mamíferos, y líneas celulares; fundamentalmente asociadas con efectos antibacterial, antifúngico, citotóxico, antioxidante y sedante, entre otros. Los registros se enfocaron principalmente a los sectores salud, industria pesquera y agroalimentaria. **Conclusión:** los estudios sobre el efecto del aceite esencial son promisorios, pero no reflejan una continuidad de la investigación hacia prototipos o productos comerciales acabados. Los grupos de investigación deben unificar metodologías de evaluación e incluir en todos los estudios la relación entre fitoquímica-actividad biológica, para que los metaanálisis sean posibles. De igual manera, deben aunar esfuerzos por medio del Sistema Nacional de Innovación Agropecuaria (SNIA), para generar productos acabados que impacten en la sociedad y faciliten el avance de la bioeconomía del país.

Palabras clave: *Lippia alba*, aceites esenciales, quimiotipos, plantas aromáticas, protección de la salud, industria de alimentos, sector agroalimentario.

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