Tzasna HERNÁNDEZ, Margarita CANALES, José Guillermo AVILA, Ana Maria GARCÍA, Samuel MERAZ, Javier CABALLERO, Rafael LIRA

Composition and antibacterial activity of essential oil of Lippia graveolens H.B.K. (Verbenaceae)

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 8, núm. 4, julio, 2009, pp. 295-300,
Universidad de Santiago de Chile
Chile




*Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas,*
ISSN (Printed Version): 0717-7917
editor.blacpma@usach.cl
Universidad de Santiago de Chile
Chile

BLACPMA ISSN 0717 7917

**Artículo Original | Original Article**

# Composition and antibacterial activity of essential oil of *Lippia graveolens* H.B.K. (Verbenaceae)

[Composición y actividad antibacteriana del aceite esencial de *Lippia graveolens* H.B.K. (Verbenaceae)]

**Tzasna HERNÁNDEZ[1*], Margarita CANALES[1], José Guillermo AVILA[1], Ana Maria GARCÍA[1], Samuel MERAZ[1], Javier CABALLERO[2], Rafael LIRA[3]**

*[1]Laboratorio de Fitoquímica, [3]Laboratorio de Recursos Naturales, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de Mexico, México.*
*[2]Jardín Botánico Exterior, Universidad Nacional Autónoma de México. Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D. F., México*

## Abstract

The essential oil of the aerial parts of *Lippia graveolens* H.B.K. (Verbenaceae) was examined by GC and GC-MS. Nine constituents were identified. Carvacrol, α-terpinyl acetate, m-cymene, thymol and β-pinene were found to be the major components. The oil exhibited antibacterial activity against Gram-positive (*Staphylococcus aureus; Staphylococcus epidermidis, Bacillus subtilis, Sarcina lutea*) and Gram-negative bacteria (*Shigella boydii, Vibrio cholerae* No-01, *Vibrio cholerae* (clinical strain), *Vibrio cholerae* (isolated from water), *Vibrio cholerae* Tor, Esc*herichia coli, Enterobacter agglomerans, Enterobacter aerogenes; Yersinia enterocolitica, Salmonella typhi*).

**Keywords:** Antibacterial activity; Essential oil; Gastrointestinal diseases; *Lippia graveolens*.

## Resumen

El aceite esencial de la parte aérea de *Lippia graveolens* H.B.K. (Verbenaceae) fue analizado por GC y GC-MS. Se determinaron 9 componentes de los cuales carvacrol, α-terpinil acetato, m-cimeno, timol y β-pineno fueron los compuestos mayoritarios. El aceite esencial exhibió actividad antibacteriana en bacterias Gram positivas (*Staphylococcus aureus; Staphylococcus epidermidis, Bacillus subtilis, Sarcina lutea*) y Gram negativas (*Shigella boydii, Vibrio cholerae* No-01, *Vibrio cholerae* (aislado de un caso clínico), *Vibrio cholerae* (aislada de agua contaminada), *Vibrio cholerae* Tor, *Escherichia coli, Enterobacter agglomerans, Enterobacter aerogenes; Yersinia enterocolitica, Salmonella typhi*).

**Palabras Clave:** Actividad antibacteriana; Aceite esencial; Enfermedades gastrointestinales; *Lippia graveolens*.

**Recibido | Received:** May 4, 2009.
**Aceptado en Versión Corregida | Accepted in Corrected Version:** July 2, 2009.
**Publicado en Línea | Published Online:** July 22, 2009
**Declaración de intereses | Declaration of interests:** authors have no competing interests. Todos los autores contribuyeron de igual manera en el manuscrito.
**Financiación | Funding:** This work was financed by the Mexican Consejo Nacional de Ciencia y Tecnología, through grant CONACyT 400389G35-450 and UNAM PAPCA 2007-2008
**This article must be cited as:** Tzasna Hernández, Margarita Canales, José Guillermo Avila, Ana Maria García, Samuel Meraz, Javier Caballero, Rafael Lira. 2009. Composition and antibacterial activity of essential oil of *Lippia graveolens* H.B.K. (Verbenaceae). Bol Latinoam Caribe Plant Med Aromat 8(4):295 – 300. {EPub July 22, 2009}.

*Contactos | Contacts: Email tzasna@servidor.unam.mx; Phone: + 52-5-623-11-36; Fax + 52-5-623-12-25.

## INTRODUCTION

Since ancient times traditional Mexican medicine has used a wide variety of plants to treat gastrointestinal and respiratory disorders which are particularly prevalent in rural areas of the country. The information obtained from oral and written means is a good option to preserve and improve human health in geographically and culturally isolated communities (Argueta & Cano, 1994; McGaw et al., 2000; Canales et al., 2005, 2006; Hernandez et al., 2005).

*Lippia graveolens* is a shrub found in America (Rzedowski, 1978). Several species of the genus *Lippia* are used in folk medicine mainly in dermatological, gastrointestinal and respiratory affections (Argueta & Cano, 1994; Pascual et al., 2001). A previous ethnobotanical study in Zapotitlán de las Salinas, Puebla, (México) showed that the local people use infusions of the aerial part of 44 different plant species to treat gastrointestinal and respiratory illnesses (mainly produced by Gram negative and Gram positive bacteria respectively). *L. graveolens* was recognized as being the most important species used (Hernandez et al., 2003). There are some publications about the chemical composition of the essential oil of *L. graveolens* (Compadre et al., 1987; Dominguez et al., 1989; Vernin et al., 2001; Salgueiro et al., 2003; Hernandez et al., 2008). However, only few pharmacological studies have been made. The aim of this work was to investigate essential oil composition of *L. graveolens* and its antibacterial activity.

## MATERIALS AND METHODS

### Plant material

Aerial parts of *L. graveolens* were collected in July 2001 in Zapotitlán de las Salinas, Puebla. Voucher specimens were deposited in the National Herbarium of Mexico (MEXU) at the Universidad Nacional Autónoma de Mexico, and the herbarium IZTA at the Facultad de Estudios Superiores Iztacala (Voucher n° 26474).

### Chemical analysis of essential oil

The essential oil was obtained by steam distillation (1 kg of fresh plant) during 4 h in a Cleavenger-type apparatus. The essential oil was analyzed in a Hewlett Packard 5890-II gas chromatograph equipped with a DB WAX column (30 m x 0.32 mm). The temperature of the column was programmed from 80 °C to 220 °C at 8 °C/min. The injector and detector temperatures were 225 °C. The gas carrier was He, at a flow rate of 1 mL/min. Peak areas were measured by electronic integration. The relative amount of the individual components was based on the peak areas. GC-MS analysis was performed on a Jeol AX50HA using a 30 m x 0.32 mm capillary column. The temperature of the column and the injector were the same as those from GC. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices and mass spectra with the NIST/EPA/NIH Mass Spectral Library.

### Antibacterial assay

Fourteen strains of bacteria were used: *Vibrio cholerae* INDRE 206 (isolated from polluted water), *Vibrio cholerae* (clinical strain pertaining to 01 group, Inaba serotype, "El Tor" biotype, and enterotoxin producer), *Vibrio cholerae* CDC V 12, *Escherichia coli* ATCC 25922, *Enterobacter agglomerans* ATCC 27155, *Salmonella typhi* ATCC 19430, *Shigella boydii* ATCC 8700 and *Staphylococcus aureus* ATCC 12398. All the strains tested were maintained in Mueller Hinton Agar and were subcultured every month. *Enterobacter aerogenes* (cephalosporin and ampicillin resistant), *Vibrio cholerae* No-01 (ampicillin resistant), *Staphylococcus epidermidis* (ampicillin, cephotaxim and dicloxacillin resistant), *Sarcina lutea* (cephotaxim and dicloxacillin resistant) and *Bacillus subtilis* (cephalothin, penicillin, cephotaxim and dicloxacillin resistant) were donated by the Laboratory of Microbiology of FES-Cuautitlán, *Yersinia enterocolitica* (ampicillin resistant) was donated by the Clinical Analysis Laboratory of the University Hospital UNAM Campus Iztacala. These strains were maintained in Mueller Hinton agar (Bioxon), submitted to sensitivity tests (multidiscs Bigaux) and were subcultured every month.

The antibacterial activity was measured by the disc-diffusion method (Van der Berghe & Vlietinck, 1991). The microorganisms were grown overnight at 37 °C in 10 ml of Mueller Hinton Broth (Bioxon). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (Lennette et al., 1987). Petri dishes containing Mueller Hinton agar (Bioxon) were inoculated with these microbial suspensions. Discs of

filter paper (Whatman no. 5) of 5 mm diameter were impregnated with 5 µL of the essential oil and placed on the agar surface. Discs with 25 µg (5 µL) of chloramphenicol (5 mg/mL Sophia Labs, Mexico) were used as positive controls. The plates were incubated overnight at 37 °C and the diameter of any resulting zones of inhibition (mm) of growth was measured. Each experiment was repeated three times.

The estimation of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out by the broth dilution method (Van der Berghe & Vlietinck, 1991). Dilutions of essential oil from 2000-7 µg/mL were used (essential oil was diluted directly into the broth). Test bacteria culture was used at the concentration of $10^5$ CFU/mL. MIC values were taken as the lowest essential oil concentration that prevents visible bacterial growth after 24 h of incubation at 37 °C, and MBC as the lowest concentration that completely inhibited bacterial growth. Chloramphenicol was used as reference and appropriate controls with no essential oil were used. Each experiment was repeated three times.

The bactericidal kinetic assay was performed by using appropriate concentrations of essential oil (corresponding to ½ MIC, MIC and MBC), in accordance with the method described by Avila et al., 1999 (Avila et al., 1999).

## RESULTS

The physicochemical data of the essential oil were as follows: $d^{25}$ = 0.93 g/mL, and 1.15% v/w of oil from fresh weight were obtained.

As shown in Table 1, nine compounds of the essential oil of *L. graveolens* were identified by GC/MS analysis representing 94.58%. The main compounds with concentrations higher than 2% as percentage peak area were the monoterpenes: carvacrol (37.84%), α-terpinyl acetate (22.35%), m-cymene (20.42%), thymol (6.72%), β-pinene (2.54%), and the sesquiterpene: isocaryophyllene (2.18%).

All the strains tested were sensitive to the essential oil. Gram-negative bacteria exhibited the biggest inhibition zones.

The results obtained in the evaluation of the antibacterial activity of the essential oil of *L. graveolens* are shown in Table 2.

**Table 1.** Composition of essential oil of *L. graveolens*.

| **Compounds** | RT | % |
|---|---|---|
| α-Thujene | 4.51 | 1.03 |
| β-Pinene | 6.13 | 2.54 |
| m-Cymene | 7.30 | 20.42 |
| α-Terpinyl acetate | 8.16 | 22.35 |
| Linalool | 8.79 | 0.26 |
| Carvacrol | 13.98 | 37.84 |
| Thymol | 15.05 | 6.72 |
| Isocaryophyllene | 15.96 | 2.18 |
| Humulene | 16.60 | 1.24 |
| Total | | 94.58 |

Compounds are listed in order of their elution from a DB WAX column.

Figs. 1 and 2 show the effect of the essential oil (in the survival curve) on a Gram-positive bacterium (*S. aureus*) and a Gram-negative bacterium (*V. cholerae* isolated of a clinical strain). Minimum inhibitory concentrations (MIC) had a bacteriostatic effect on the bacterial population of *S. aureus* and *V. cholerae,* while the minimum bactericidal concentrations (MBC) had a lethal effect on bacteria within the first eight hours.

## DISCUSSION

The essential oil of *L. graveolens* (Table 1) is constituted mainly by monoterpenes. The major components are: carvacrol (37.84%), α-terpinyl acetate (22.35%), m-cymene (20.42%), thymol (6.72%), and β-pinene (2.54%). Differences in the yield, components and concentrations compared to those previously reported in the literature for *L. graveolens* collected in other geographic areas could be attributed to some factors such as climate, time of collection, mode of extraction, etc. (Compadre et al., 1987; Dominguez et al., 1989; Vernin et al., 2001; Cimanga et al., 2002; Salgueiro et al., 2003).

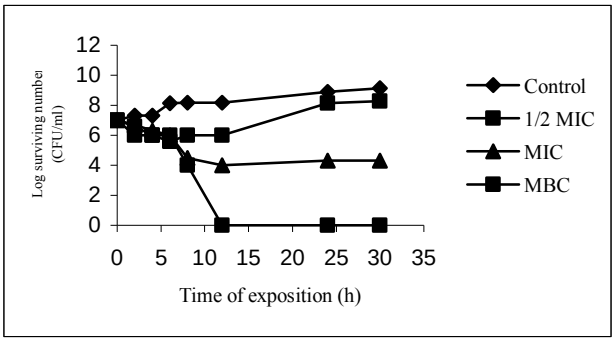
The essential oil of *L. graveolens* presented antibacterial activity against the tested strains (Table 2), showing the biggest inhibition zones in the four strains of *V. cholerae, S. typhi* and *Y. enterocolitica* (these strains commonly cause gastrointestinal disease).

**Table 2**. Antibacterial activity of the essential oil of *L. graveolens*.

| Microorganism | Inhibition zone (mm) Chloramphenicol 25µg | Essential oil | Essential oil MIC (µg/mL) | MBC (µg/mL) |
|---|---|---|---|---|
| Sa | 18.67 ± 0.58 | 23.00 ± 0.05 | 125 | 250 |
| Se | 19.67 ± 2.08 | 24.67 ± 0.58 | 62 | 125 |
| Sl | 22.67 ± 0.58 | 23.67 ± 0.58 | 7 | 15 |
| Bs | 25.00 ± 1.00 | 28.67 ± 3.00 | 125 | 250 |
| Sb | 25.00 ± 1.00 | 25.00 ± 0.50 | 7 | 15 |
| St | 27.00 ± 1.00 | 30.00 ± 0.55 | 125 | 250 |
| Ye | 15.67 ± 0.58 | 30.00 ± 0.50 | 31 | 62 |
| Eag | 25.33 ± 0.58 | 25.00 ± 0.50 | 125 | 250 |
| Vch Indre | 24.33 ± 0.58 | 30.00 ± 0.50 | 62 | 125 |
| Vch No 01 | 24.33 ± 0.58 | 30.00 ± 0.50 | 62 | 125 |
| Vch Tor | 22.67 ± 1.53 | 30.00 ± 0.50 | 62 | 125 |
| Vch cc | 19.33 ± 0.58 | 30.00 ± 0.50 | 62 | 125 |
| Eae | 24.00 ± 1.00 | 21.00 ± 2.65 | 125 | 250 |
| Ec | 18.33 ± 1.15 | 20.33 ± 1.15 | 125 | 250 |

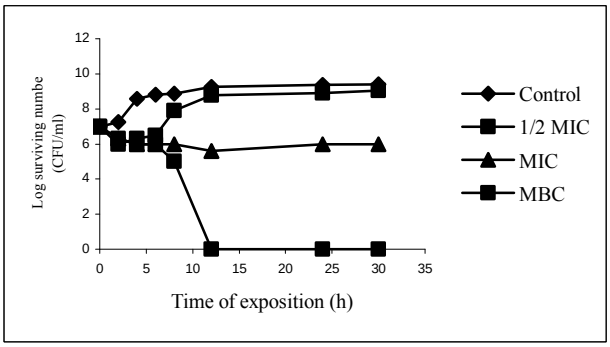
Sa, *Staphylococcus aureus*; Se, *Staphylococcus epidermidis*; Sl, *Sarcina lutea*; Bs, *Bacillus subtilis*; Sb, *Shigella boydii*; St, *Salmonella typhi*; Ye, *Yersinia enterocolitica*; Eag, *Enterobacter agglomerans*; Vch Indre, *Vibrio cholerae* (isolated from water); Vch No-01, *Vibrio cholerae* No-01; Vch Tor, *Vibrio cholerae* CDC V12; Vch cc, *Vibrio cholerae* (clinical strain); Eae, *Enterobacter aerogenes*; Ec, *Escherichia coli*. Chloramphenicol was a positive control

**Figure 1.** Survival curve of *S. aureus* exposed to essential oil of *L. graveolens.*




The essential oil was added to each experimental culture in zero time. The concentrations used for *S. aureus* were: 62 µg/ml (½ MIC), 125 µg/mL (MIC)) and 250 µg/mL (MBC), the control tube did not contain essential oil.

**Figure 2.** Survival curve of *V. cholerae* isolated of a clinical sample exposed to essential oil of *L. graveolens.*




The essential oil was added to each experimental culture in zero time. The concentrations used for *V. cholerae* isolated of a clinical sample were: 31 µg/mL (½ MIC), 62 µg/mL (MIC)) and 125 µg/mL (MBC), the control tube did not contain essential oil.

It was observed that *S. lutea* and *S. boydii* presented the lowest values of MIC and MBC. The strains which presented the biggest inhibition zones (diffusion method) are not always the most sensitive (values of MIC and MBC were lower) because the size of the inhibition zone does not reflect the

antibacterial effectiveness of a compound, since it is affected by the solubility of the oil, the diffusion range in the agar, the evaporation (it can affect the dose), etc. (Kim et al., 1995; Cimanga et al., 2002).

*L. graveolens* has been used traditionally to treat various gastrointestinal and respiratory ailments, which may be either Gram negative or Gram positive bacteria, the survivals curves were performed on a gastrointestinal (*V. cholerae* isolated of a clinical strain) and a respiratory (*S. aureus*) common pathogens. Comparison of the survivals curves for the organisms studied showed that the effect of the essential oil on the bacterial population of *S. aureus* and *V. cholerae* (Figs. 1 and 2) was bacteriostatic at MIC dose, but a bactericidal effect is observed at MBC dose within the first eight hours. This confirms the use of *L. graveolens* to treat Gram negative and Gram positive gastrointestinal and respiratory ailments respectively.

The results suggest that the antibacterial activity showed by the essential oil can be attributed, to a considerable degree, to the existence mostly of carvacrol (37.84%), α-terpinyl acetate (22.35%), thymol (6.72%), and β-pinene (2.54%)), which appears to possess similar activities against all of the tested microorganisms. Essential oils rich in phenolic compounds such as carvacrol, thymol, etc. are reported to possess high levels of antimicrobial activity (Knobloch et al., 1985; Juven et al., 1994; Harborne et al., 1995; Kim et al., 1995; Cimanga et al., 2002). In general, the essential oils in bacteria produce membrane damage. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool (Knobloch et al., 1985; Helander et al., 1998). The essential oils can coagulate the cytoplasm (Ultee et al., 2002). Damage on the cell wall and membrane can lead to the leakage of macromolecules and to lysis (Juven et al., 1994).

## CONCLUSION

The present study tends to validate the use in the folk medicine of *L. graveolens* in gastrointestinal and respiratory diseases.

## ACKNOWLEDGEMENTS

This research could not have been done without the cooperation of many people in Zapotitlan de las Salinas, Puebla. The authors are grateful to Roberto Garcilazo, Martin Paredes and Dr. Edith López Villafranco for their technical assistance. To Javier Pérez and Luis Velasco for their support on gas-mass measurement.

## REFERENCES

Argueta VA, Cano AJ. 1994. Atlas de las Plantas de la Medicina Tradicional Mexicana. México, Instituto Nacional Indigenista pp 15-34, 804, 1073-1074.

Avila JG, De Liverant J, Martínez A, Martínez G, Muñoz JL, Arciniegas A, Romo de Vivar A. 1999. Mode of action of *Buddleja cordata* verbascoside against *Staphylococcus aureus*. J Ethnopharmacol 66:75-78.

Canales M, Hernández T, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R. 2005. Informant consensos factor and antibacterial activity of the medicine plants used by the people of San Rafael Coxcatlán, Puebla, Mexico. J Ethnopharmacol 97:429-439.

Canales M, Hernández T, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R. 2006. Análisis cuantitativo del conocimiento tradicional de las plantas medicinales en San Rafael Coxcatlán, Valle de Tehuacán-Cuicatlán, Puebla, México. Acta Bot Mex 75:21-43.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 79:213-220.

Compadre CM, Hussain RA, Leon I, Enriquez RG. 1987. Volatile constituents of *Montanoa tomentosa* and *Lippia graveolens*. Planta Med 53:495-496.

Dominguez XA, Sanchez VH, Suárez M, Baldas JH, González MR. 1989. Chemical constituents of *Lippia graveolens*. Planta Med 55:208.

Harborne JB, Williams CA. 1995. Anthocyanins and other flavonoids. Nat Prod Rep 7:639-657.

Helander IM, Alakomi HL, Latva-Kala K, Mattilia-Sandholm T, Pol I, Smid EJ, Gorris LGM, Von Wright A. 1998. Characterization of the action of selected essential oil components on Gram negative bacteria. J Agric Food Chem 46:3590-3595.

Hernández T, Canales M, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R. 2003. Ethnobotany and antibacterial activity of some plants used in tradicional medicine of Zapotitlán de las Salinas, Puebla (México). J Ethnopharmacol 88:181-188.

Hernández T, Canales M, Caballero J, Duran A, Lira R. 2005. Análisis cuantitativo del conocimiento tradicional sobre plantas utilizadas para el tratamiento de enfermedades gastroiuntestinales en Zapotitlán de las Salinas, Puebla, México. Interciencia 30(9):529-535.

Hernández T, Canales M, Avila JG, García AM, Meraz S, Duran A. 2008. Antifungal activity of the essential oils of two Verbenaceae: *Lantana achyranthifolia* Desf. And *Lippia graveolens* H.B.K. of Zapotitlán de las Salinas, Puebla (México). Bol Latinoamer Caribe Plant Med Aromat 7(4):203-207**.**

Juven BJ, Kanner J, Schved F, Weisslowicz H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Applied Bacteriol 76:626-631.

Kim J, Marshall MR, Wei C. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. J Agric Food Chem 43:2839-2845.

Knobloch L, Weigand H, Weis N, Schwarn HM, Vigenschow H. 1985. Action of terpenoids on energy metabolism. In: Progress in Essential Oil Research. USA, Eds.: Ernst-Joachim Brunke Editor, Walter de Gruyter. pp 429-448.

Lennette HE, Balows A, Hausler JW, Shadomy HJ. Manual de microbiología clínica. 4 edición. Argentina. Médica Panamericana, 1987. pp. 336-352, 358-382, 1192, 1200-1245.

McGaw LJ, Jager AC, van Staden J. 2000. Antibacterial, anthelmintic and antiamoebic activity in South African medicinal plants. J Ethnopharmacol 72:247-263.

Pascual ME, Slowing K, Carretero E, Sánchez MD, Villar A. 2001. *Lippia*: Traditional uses, chemistry and pharmacology: A review. J Ethnopharmacol 76:201-214.

Rzedowski J. Vegetación de México. México. Limusa, 1978. p 432

Salgueiro LR, Cavaleiro C, Goncalves MJ, Proenca da Cunha A. 2003. Antimicrobial activity and chemical composition of the essential oil of *Lippia graveolens* from Guatemala. Planta Med 69:80-83.

Ultee A, Bennik MH, Moezelaar R. 2002. The phenolyc hydroxyl group of carvacrol is essential for the action against the food-borne pathogen *Bacillus cereus.* Appl Environ Microbiol 68:1561-1568.

Van der Berghe DA, Vlietinck AJ. 1991. Screening methods for antibacterial agents from higher plants. In: Dey, P. M., Harborne, J. B., Hostettman, K. (Eds.), Methods in plant Biochemistry. Assay for Bioactivity, vol. 6. London. Academic Press, pp 47-69.

Vernin G, Lageot C, Gaydou EM, Parkanyi C. 2001. Analysis of the essential oil of *Lippia graveolens* HBK from El Salvador. Flav Frag J 16:219-226.