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Chemical composition of the *Lippia origanoides* essential oils and their antigenotoxicity against bleomycin-induced DNA damage

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ABSTRACT

The present work evaluated the chemical composition of the essential oils (EO) obtained from *Lippia origanoides* and their DNA protective effect against bleomycin-induced genotoxicity. *L. origanoides* EO chemical composition was determined by gas chromatography–mass spectrometry (GC–MS). The major compounds of the *L. origanoides* EOs were thymol (34–58%) and carvacrol (26%). The antigenotoxic effects of the EOs, major compounds and standard compound (epigallocatechin gallate) were assayed in co-incubation procedures using the SOS chromotest in *Escherichia coli*. Both EOs and their major compounds protected bacterial cells against bleomycin-induced genotoxicity indicating that these two compounds were principally responsible for the antigenotoxicity detected in the oils. Thymol and carvacrol antigenotoxicity was lower than those observed with epigallocatechin gallate. The results were discussed in relation to the chemopreventive potential of *L. origanoides* EOs and their major components, carvacrol and thymol.

1. Introduction

Lippia origanoides HBK (Vervenaceae) is an aromatic shrub or small tree (up to 3 m tall) native of Central America and northern South America. It is used as seasoning and in traditional medicine for treatments of gastrointestinal and respiratory diseases [1]. In Colombia, *L. origanoides* is commonly known as "Orégano de monte" (mountain oregano) and habits in semiarid zones of Guajira, Magdalena, Cauca, Cundinamarca, Santander and North of Santander states.

Different antimicrobial activities have been reported for *L. origanoides* EOs, supporting their use in traditional medicine. The EO of this plant shows antimicrobial activity against bacteria involved in respiratory diseases and against enteropathogens [2,3]. Recently, it has been demonstrated that *L. origanoides* EO inhibits *in vitro* replication of yellow fever virus [4].

Based on the major constituents found in *L. origanoides* EOs, different chemotypes have been reported in Brazil, Venezuela and Colombia [2,3,5,6]. Although it has been shown that extraction methods [7] and environmental factors [5] can affect secondary metabolite production in *Lippia* species; at least three different chemotypes can be differentiated for *L. origanoides*, two chemotypes whose major compounds are carvacrol and thymol respectively and a rare chemotype characterized by the absence or very low content of these compounds. Carvacrol and thymol also showed antimicrobial [8–16], antiparasitic [17] and insecticide [18,19] activities, supporting the application of these compounds in disease and pest management.

Recent works emphasize the EO use as a source of antitumor, anti-carcinogenic and chemopreventive agents [20,21]. Aromatic plants considered as "Oregano" spices (*Lippia graveolens*, *Origanum compactum*, *Origanum onites*, *Thymus spicata*, and *Thymus vulgaris*) since their EO have high

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carvacrol and/or thymol contents, have showed antigenotoxic properties against known mutagens [22–26]. Additionally, carvacrol inhibited DMBA-induced tumorigenesis in rat [27] and the growth of murine B16 melanomas [28] and of human A549 non-small lung cancer [29] cell lines, while thymol significantly increased antioxidant enzyme activities such as superoxide dismutase and glutathione peroxidase in rats [30]. Carvacrol and thymol also showed antigenotoxic activity against H_2O_2-induced genotoxicity in human colonic Caco-2, hepatoma HepG2 and leukemic K562 cell lines [31,32].

Consequently, the *L. origanoides* EO is considered as a potential source of antigenotoxic compounds. In this study, we determined the chemical composition of *L. origanoides* EO by GC–MS and then evaluated antigenotoxic activity of these oils against the clastogenic mutagen bleomycin by means of the SOS chromotest [33]. The antigenotoxic activity was related to the major constituents of the EO (carvacrol and thymol). Our work provides evidence about the chemopreventive potential of *L. origanoides* EO and its major compounds, carvacrol and thymol.

2. Materials and methods

2.1. Chemicals

Sodium sulfate and dichloromethane were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). High purity gases (helium, nitrogen, hydrogen, and air) for chromatography were obtained from AGA-Fano S.A. (Bucaramanga, Colombia). Different standard compounds (*n*-tetradecane, *n*-alkanes, C_8–C_{25}, epigallocatechin gallate (EGCG), thymol and carvacrol), Luria–Bertani (LB) media, and antibiotics (ampicillin, bleomycin and tetracycline) were obtained from Sigma-Aldrich Co. Inc. (Milwaukee, WI, USA). The substrate for β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside) and alkaline phosphatase (*p*-nitrophenylphosphate) were purchased from Merck (Darmstadt, Germany).

2.2. Plant material

L. origanoides plants were collected from the Chicamocha river canyon (Santander, Colombia). The taxonomic identification was performed by Dr. José Luis Fernández Alonso (National University, Bogotá, Colombia). Two *L. origanoides* specimens (COL519799 and COL516290) were stored at the Colombian National Herbarium (Bogotá). Propagation cuttings from these specimens were used in establishing experimental gardens at CENIVAM Agroindustrial Pilot Complex located at the Universidad Industrial de Santander campus (Bucaramanga, Colombia). Plant growing conditions were as indicated by Stashenko et al. [6].

2.3. EO extraction and chromatographic analysis

Fresh leaves and flowers from *L. origanoides* plants were employed for EO extraction. Microwave-assisted hydrodistillation was used as described by Stashenko et al. [7].

Compound identification was based on chromatographic (retention times, retention indices, standard compounds) and mass spectroscopic (spectral interpretation, comparison with databases and standard) criteria [34]. Gas chromatography (GC) analyses were performed with a 6890 Plus gas chromatograph (*Agilent Technologies*, Palo Alto, CA, USA) equipped with a mass selective detector MSD 5975 (Electron impact ionization, EI, 70 eV, *Agilent Technologies*, Palo Alto, CA, USA), a split/splitless injector (1:30 split ratio), a 7863 automatic injector and a MS-ChemStation G1701-DA data system, that included the spectral libraries WILEY, NIST and QUADLIB 2007. A fused-silica capillary column DB-5MS (*J&W Scientific*, Folsom, CA, USA) of 60 m × 0.25 mm i.d., coated with 5%-phenyl poly (methysiloxane) (0.25 μm film thickness) was used. Samples were prepared mixing aliquots of dehydrated EO (25 μL) with *n*-tetradecane (25 μL) and dichloromethane (974 μL). Chromatographic conditions were as follows: The GC oven temperature was programmed from 45 °C (5 min) to 150 °C (2 min) at 4 °C/min, then to 250 °C (5 min) at 5 °C/min, and finally, to 275 °C (15 min) at 10 °C/min. The temperatures of the injection port, ionization chamber and of the transfer line were set at 250, 230 and 285 °C, respectively. Helium (99.99%, AGA-Fano, Bucaramanga, Colombia) was used as carrier gas, with 155 kPa column head pressure and 27 cm s^{-1} linear velocity (1 mL min^{-1}, at constant flow). A standard solution of *n*-alkanes (C_8–C_{25}) was used to obtain the retention indices. Mass spectra and reconstructed (total) ion chromatograms were obtained by automatic scanning in the mass range *m/z* 30–300 at 5.1 scan $^{-1}$. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms for the characteristic fragment ions and with the help of the peak purity program.

2.4. Bacterial strains and culture

Escherichia coli PQ37 strain [F^- *thr leu his-4 pyrD thi galE galK or galT lacΔU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc*$^+$ *sfiA::Mud(Ap,lac)ts*] proposed to detect genotoxic carcinogens [33] was used. This strain carried the *sulA::lacZ* gene fusion on the chromosome as a reporter of the primary DNA damage induced during SOS response. The cells were grown overnight at 37 °C and shaken at 100 rpm in Luria–Bertani (LB) media (10 g tryptone/L, 5 g yeast extract/L, 10 g sodium chloride/L, pH 7.4) supplemented with 50 μg/mL ampicillin and 17 μg/mL tetracycline.

2.5. Genotoxicity assay

Genotoxicity assays were performed using the SOS chromotest as described by Quillardet et al. [33]. Briefly, overnight cultures were grown in fresh LB medium as described above until an optical density $OD_{600nm} = 0.4$, diluted 10-fold in double force LB medium (20 g tryptone/L, 10 g yeast extract/L, 20 g sodium chloride/L, pH 7.4), and mixed (v/v) with test substance (essential oils). Negative (distilled water) and positive (1 μg/mL of Bleomycin) controls were always included in each assay. Cells were exposed to substances during 30 min at 8 °C and then cultured during 2 h at 37 °C. To assay β-galactosidase activity, cell membranes were disrupted mixing 1.42 mL of Z buffer (Na_2HPO_4 60 mM, NaH_2PO_4 40 mM, KCl 10 mM, Mg_2SO_4 1 mM, SDS 0.1%, β-mercaptoethanol 40 mM, pH 7.0) with 0.15 ml of cell culture for 20 min at room temperature. The enzyme reaction was started by the addition of 0.3 mL of