IPAP18 Innovations in Pharmacy ADVANCES & PERSPECTIVES

Salamanca Congress 24-28 Sept.

#IPAP18 Posters

ETHNOPHARMACOLOGICAL STUDIES IN TRADITIONAL MARKETS FROM BOGOTÁ D.C. (COLOMBIA): ADVANCES AND PERSPECTIVES FOR THE SEARCH OF MEDICINAL PLANTS WITH THERAPEUTIC POTENTIAL

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INTRODUCTION

Colombia is one of the 14 countries that holds the highest index of biodiversity on earth, being one of the megadiverse countries of the world (Mittermaier and Gottschee, 1997). According to reports from the *Instituto de Investigación de Recursos Biológicos Alexander von Humboldt* in Colombia, the country has more than 25000 species of plants of which around 2400 are considered medicinal (Gómez *et al*, 2015; Bernal *et al*, 2011) however only about 144 species are authorized by the *Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA)* for medicinal use in phytotherapeutical products. In the country the commercialization and traditional use of plants is carried out widely in traditional markets ("*plazas de mercado*") (Proexport, 2000), where ethnobotanical studies promote the search of medicinal plants with therapeutic potential. The Giraldo´s *et al*. researches (2013, 2015, 2016) have contributed to describe the traditional use of medicinal plants in the traditional markets of Bogotá, in order to give scientific support to the ethnobotanical knowledge of the communities and to recognize native plants of the Neotropic, especially with potential antimicrobial, cytotoxic and central nervous system activity.

Figure 1: Traditional market in Bogotá ("plaza de mercado de paloquemao"). http://andandoporbogota.blogspot.com/2016/01/la-plaza-de-las-yerbas-de-bogota.html

ETHNOBOTANICAL AND ETHNOPHARMACOLOGICAL STUDIES

The ethnobotanical information was collected through a semi-structured interview according to Martin (2001). The interview was applied to 55 selling informants of medicinal plants in 20 traditional markets of Bogotá, between August 2012 and March 2013. The ethnopharmacological sampling method was used (Martin, 2001). Informant consensus techniques were applied (Albuquerque, 2007). For the ethnopharmacological study aimed at the search of medicinal plants with potential antimicrobial activity (9 plants) and on the central nervous system (4 plants), 13 markets of Bogotá were visited between 2015 and 2016. The semi-structured interview was applied on this occasion to 37 informants. The taxonomic determination of species was carried out in the *Herbario de Plantas Útiles de Colombia (Pontificia Universidad Javeriana (HPUJ)* or in the *Herbario Nacional Colombiano del Instituto de Ciencias Naturales* (Universidad Nacional de Colombia) For the statistical analysis of the results, the SPSS v.15.0 Program was used. 15.0.

PHYTOCHEMICAL STUDIES

Around 5 Kg of fresh plant material from "sanguinaria" (*Lantana camara* L.), "orozul" (*Lippia dulcis* T., synonym *Phyla dulcis* (Trevir) Moldenke), "anamú" (*Petiveria alliacea* L.) and "valeriana" (*Valeriana laurifolia* K, synonym *Valeriana pavonii* Poepp & Endl.), were purchased in the traditional market "*plaza de mercado de Paloquemao*" in the city of Bogotá. Other plants of interest were "yacón" (*Smallanthus sonchifolius*, P. & E.) and "amansaguapos" (*Hygrophila ssp.*). The raw material of each plant was dried in a forced-air oven at 40 °C for 48 h to 72 h. The ethanolic extracts and fractions in chloroform were obtained by solid-liquid extraction at atmospheric pressure and at room temperature. The solvent was removed at reduced pressure between 40 ° C to 50 ° C according to Giraldo *et al*. (2013). The preliminary phytochemical study was carried out by thin layer chromatography (TLC), using silica gel (F254, 0.25 mm) and standard substances. As eluent systems mixtures Chloroform: Methanol and / or Hexane: Ethyl Acetate were used. The spots were observed in UV light (at 254 and/or 365 nm) and visible light, by means of universal and/or specific chemical developers (CYTED, 2000). 10 mg/mL were used for the extracts and 20 mg/mL for the chloroform fractions.

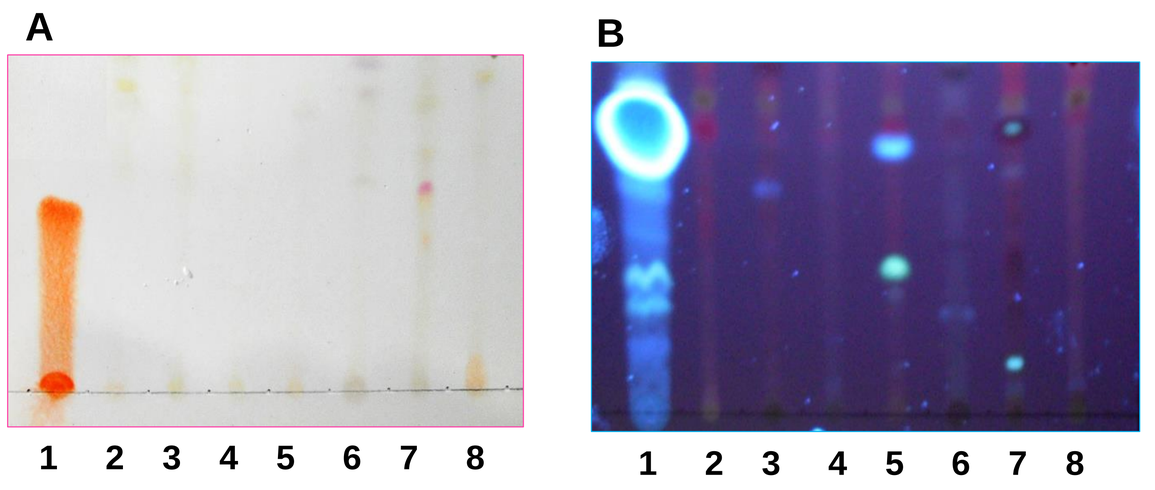

Figure 2: TLC of ethanolic extracts. 2. *P. alliacea*, 3. *L. dulcis*, 4. *L. camara*, 5. *Hygrophila* ssp., 6. *V. pavonii* (stems), 7. *V. pavonii* (leaves), 8. *S. sonchifolius*. **A. Flavonoids**. 1. Quercetin. Spray reagent: NP/PEG 4000, VIS. B. **Quinones and Courmarins**. 1. Umbelliferone. Spray reagent: Bornträger, UV, 365 nm. Eluent system: Chloroform:Methanol (90:10). Flavonoids (VIS) on *V. pavonii* (leaves); coumarins (UV) on *Hygrophila ssp;* anthraquinones (UV) (red spots possibly on all extracts)

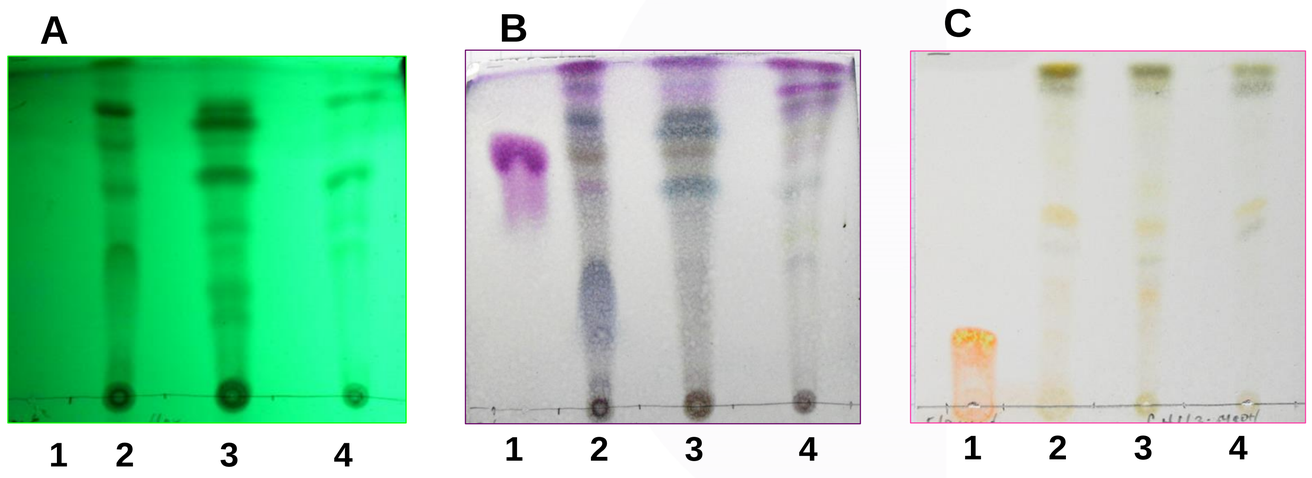

Figure 3. TLC of Fractions in chloroform. 2. *L. camara* , 3. *L. dulcis*, 4. *P. alliacea*. **A and B. Steroids and terpenoids**. 1. β-cytosterol. **A.** UV, 365 nm. B. Spray reagent: Vanillin/perchloric-acid–Sulphuric acid, VIS. **C. Flavonoids**. 1. Quercetin. Spray reagent: NP/PEG 4000, VIS. Eluent system: **A.** Hexane:Ethyl acetate (60:40). **B.** Cloroform:Methanol (95:5). Terpenoids (VIS) on all fractions; Flavonoids (VIS) on *L. camara* and *L. dulcis.*

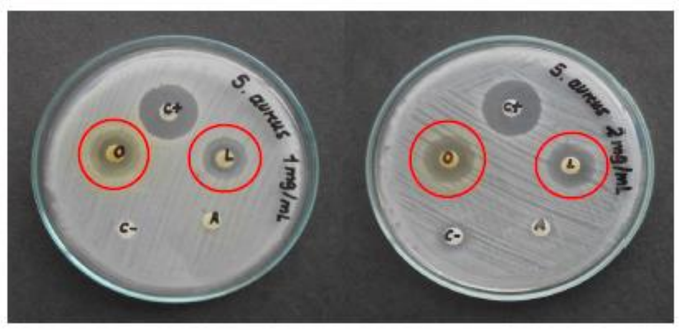

Figure 4. Antibacterial activity of ethanolic extract (1 mg/mL and 2 mg/mL) against *Staphylococcus aureus* ATCC 25923. Disk diffusion test. Inhibitory effect of *Lippia dulcis* 60,31% (1 mg/mL) and 85,48% (2 mg/mL). Inhibitory effect of *Lantana camara* 53,96% (1 mg/mL) and 58,06% (2 mg/mL). A.B. Gentamicin 10 µg/mL. Solvent: Dimethyl sulfoxide 10%

ANTIBACTERIAL ACTIVITY IN VITRO

The ethanolic extracts from L. camara, *L. dulcis* and *P. alliacea* were evaluated by diffusion techniques in agar (wells) and disk diffusion. The susceptible strains of *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 9027 were used. The extracts were evaluated in concentrations of 1mg/mL and 2mg/mL by dilution in dimethyl sulfoxide (DMSO) at 10 % (Cruz, 2010). The disk diffusion test was carried out taking into account the protocol proposed by Othman (2011) and Andrews (2001). The agar diffusion test was performed according to the protocol proposed by Othman (2011) and Cruz (2010). To determine the minimum inhibitory concentration (MIC), the broth macrodilution test was carried out (Abadie, 2014). Serial concentrations were prepared from a stock solution of the plant extract (500 mg/mL in DMSO at 10%). For each strain an inoculum was prepared (1 x 10^6 CFU/mL). The minimum bactericidal concentration (MBC) was determined (Abadie, 2014). The tests were carried out in triplicate.

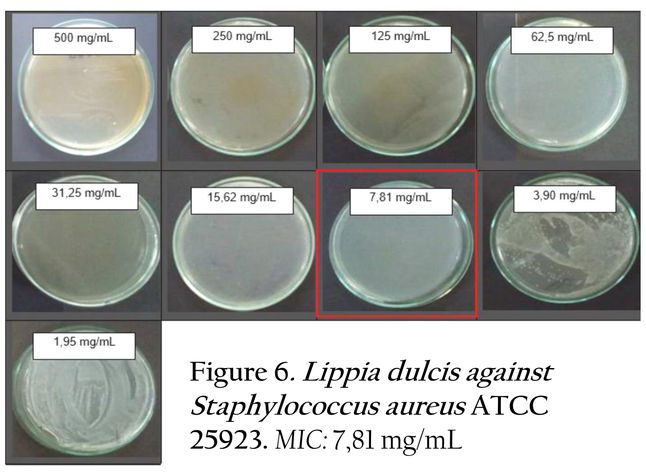

Figure 6. *Lippia dulcis against Staphylococcus aureus* ATCC 25923. *MIC:* 7,81 mg/mL

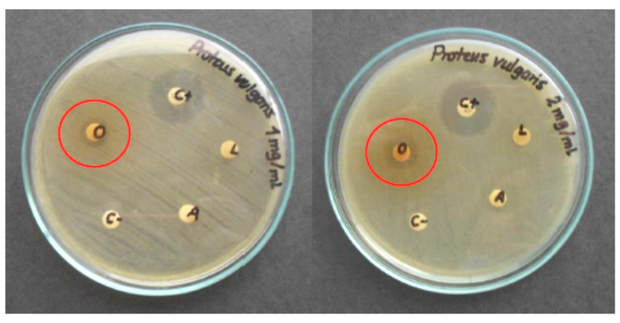

Figure 5. Antibacterial activity of ethanolic extract (1 mg/mL and 2 mg/mL) against *Proteus vulgaris* ATCC 6380. Disk diffusion test. Inhibitory effect of *Lippia dulcis* 52,23% (1 mg/mL) and 59,09% (2 mg/mL). A.B. Gentamicin 10 µg/mL. Solvent: Dimethyl sulfoxide 10%

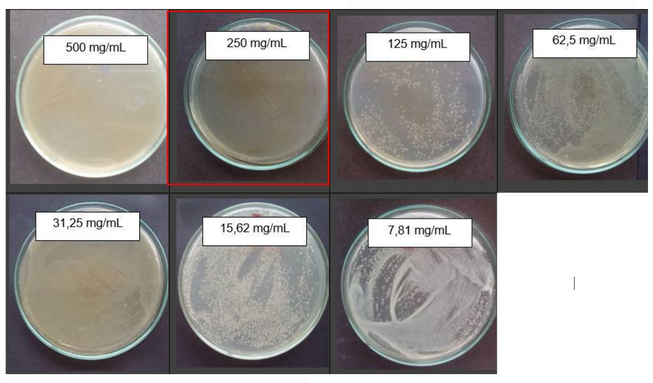

Figure 7. Lippia dulcis against Proteus vulgaris ATCC 6380. *MIC:* 250 mg/mL

PERSPECTIVES

The results obtained contribute to the phytochemical and pharmacological knowledge of plant species not yet included in the Vademecum Colombiano de Plantas Medicinales such as *L. camara* and L. *dulcis*. It is proposed to carry out bioguided fractionation studies to identify the metabolites responsible for the antibacterial activity from *L. camara* and *L. dulcis*. It is proposed to study the antifungal activity of the ethanolic extracts from *L. camara*, *L. dulcis* and *P. alliacea* against yeast fungi strains (*Candida albicans* ATCC 10231, *Candida tropical* ATCC 22019 and *Sacharomices cerviceae* ATCC 2609) and fungal filamentous strains(*Aspergillus niger* ATCC 16404). *L. camara*, *L. dulcis*, *P. alliacea* and *V, pavonii* have shown *in vitro* cytotoxicity effects in different reports. It has been proposed to evaluate the effects of *in vitro* cytotoxic activity of these species on neoplastic cell lines MCF-7 (breast adenocarcinoma), SiHa (cervical carcinoma) and a healthy macrophage cell line (J774). *V. pavonii* and *Hygrophila ssp*. have demonstrated anticonvulsant and anxiolytic activity in *vivo* tests. It is necessary to continue carrying out both *in vivo* and *in vitro* tests aimed at evaluating the anxiolytic, antidepressant and migraine activity of both fractions and compounds isolated from these species and from *Smallanthus sonchifolius*. Scientific reports have shown neuroprotective effects of *Petiveria alliacea* and of species of the genus *Valeriana*, these species have not yet been studied for this purpose in Colombia. It is proposed to carry out studies of neuroprotective activity, initially in a model of *C. elegans* which is used to evaluate natural products with potential activity in neurodegenerative diseases such as Alzheimer's and Parkinson's.

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Financial support and acknowledgments

The authors wish to thank Pharmacy Department, School of Sciences, Universidad Nacional de Colombia-Bogotá (DIB, Projects: 8485, 9334, 8035, 5698) and to thank Universidad Nacional Abierta y a Distancia (SIGI-call for research projects 002 and 005) for providing financial support. Thanks to the Colombian National Herbarium at Universidad Nacional de Colombia. The authors especially want to thank Dr. Henry Bernal (Herbarium HPUJ, Pontificia Universidad Javeriana), professor Adriana Morales (statistical analysis) and to the students Erika Pita and Alejandra Navarrete (Universidad Colegio Mayor de Cundinamarca, antibacterial activity). The authors have no financial or personal conflicts of interest related to this work.