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Anti-inflammatory and toxicological evaluation of *Moussonia deppeana* (Schldl. & Cham) Hanst and Verbascoside as a main active metabolite

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ABSTRACT

Ethnopharmacological relevance: Moussonia deppeana, known as Tlachichinole, is a Mexican medicinal plant used for treatment of inflammatory diseases, influenza, diarrhea, gastrointestinal disorders and arthritis.
Aim of the study: In this paper the antioxidant and anti-inflammatory activities as well as the acute and sub-acute toxicological effects were evaluated for the ethanolic extract from aerial parts of M. deppeana, also its phytochemical analysis is described.
Materials and methods: Phytochemical analysis and compound isolation were performed with thin layer chromatography. The chemical identification of the main compound was performed by ^{1}H NMR (COSY, NOESY, HSQC and HMBC) spectra. *In vitro* antioxidant capacity and total phenolic content for the ethanolic extract and its primary fractions was determined by DPPH and Folin-Ciocalteu reagent. Acute and subacute toxicity tests were evaluated on Balb/C mice. Finally acute anti-inflammatory evaluation was tested for a local (TPA) and systemic (carrageenan) murine model.
Results: The main compound isolated from the ethanolic extract of *M. deppeana* was Verbascoside, which was isolated from F3 and was identified by ^{1}H NMR and COSY data. Furthermore oleanolic and ursolic acids were isolated from primary fractions F1 and F2. Ethanolic extract showed IC_{50} = 6.71 mg/mL for DPPH test and 664.12 μg QE/mL for the total phenolic content. The LD_{50} value was > 2 g/kg by i.g. route in male and female mice. Sub-acute administration (28 days) of the ethanolic extract (1 g/kg) did not cause lethality or alter any hematological and biochemical parameters, in addition, histological analysis of the major organs exhibited no structural changes. Anti-inflammatory activity of the ethanolic extract showed an ED_{50} = 1.5 mg/ear and 450 mg/kg for TPA and carrageenan test, respectively. Primary fractions generated moderate local and systemic anti-inflammatory activity.
Conclusion: The ethanolic extract from the aerial parts of *M. deppeana* did not cause any lethality or adverse effect in either of the acute and sub-acute toxicity tests. This exhibited an important local and systemic anti-inflammatory activity and also moderate antioxidant capacity. Moreover, the primary fraction F2 was more active for the TPA model while the primary fraction F3 was most active in the carrageenan model *in vivo.* The main compound isolated from F3 was verbascoside; on the other hand also ursolic and oleanolic acids were isolated from F1 and F2.

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1. Introduction

Remedies and products prepared from herbal extracts are among the most common types of traditional medicine because these have been employed for the treatment of human diseases since ancient times (Zhang et al., 2010). World Health Organization (WHO) estimates that nearly 80% of the population uses traditional medicine because the current allopathic medicine causes several side effects along with the high cost and limited access (Debas et al., 2006; Obomsawin, 2011). Therefore is necessary that government develop policies and programs to regulate their use and the search for new drugs from natural medicinal plants with few adverse effects (Zeng and Jiang, 2010). In this context, today the toxicological studies for medicinal plants are mandatory because are consumed by much of the population, only their natural origin; however, some species produce a serious toxicity in human (Youns et al., 2010).

Mexico has over 3,000 medicinal plants that are widely used for the treatment of several diseases; however, very few species have been studied with a toxicological and pharmacological aim; hence, the risks and true benefits of these plants remain unknown (Rodríguez-Fragoso et al., 2008). Some plants such as nopal (*Opuntia ficus*) and aloe (*Aloe vera*) are employed for the treatment of diabetes; however, they generate many adverse side effects, such as diarrhea and poor platelet aggregation. Other plants, such as peppermint (*Mentha piperita*), dandelion (*Taraxacum officinale*) and chaparral (*Larrea divaricata*) are used for the treatment of gastric inflammatory diseases, but with side effects such as hepato- and nephrotoxicity (Déciga-Campos et al., 2007).

Moussonia deppeana (Schldl. & Cham) Hanst (syn. *Kohleria deppeana*, *Gesneria deppeana, M. elongata*), commonly known as *Tlachichinole*, *Tlalchichinolli*, *Cacahuatillo* and *Clachichinole* or *Valletilla*, is usually used as a medicinal plant in Mexico (Ramírez-Roa and Chiang, 2010). This shrub belongs to the *Gesneriaceae* family which is found from northern Mexico to Panama (Ramirez-Roa and Hernandez-Varela, 2011). On the ancient use of this medicinal plant in Mexico little it is known; however, in some parts of Central and South America it described that was used by American Indian tribes for blood diseases by the color of their flowers and against snake bite (Kvist and Skog, 1992). Currently, it is used for the treatment of inflammation and pain of the stomach, kidney disease, vaginal infection, tumors, gastrointestinal disorders (gastritis and diarrhea), duodenal ulcer, acne, burns, skin wounds, bleeding, cough, flu, arthritis and certain diseases related to inflammation (Kvist and Skog, 1992; Argueta and Gallardo-Vázquez, 1994; Calzada et al., 1998. Castillo-Juárez et al., 2009. Dominguez-Ortiz et al., 2010; Villavicencio Nieto and Pérez Escandón, 2010; Robles-Zepeda et al., 2011; BDMTM, 2016).

It is noteworthy that this plant is widely sold in Mexico as a dietary supplement in several preparations such as capsules, powders, alcoholic solutions and infusions; however, this medicinal species is not registered by Mexican Ministry of Health as a drug. Scarce phytochemical research describes the presence of β-sitosterol, ursolic and oleanolic acids, 2-methyl anthraquinone, chromones and stigmasterol in the aerial parts of *M. deppeana* (Noguera et al., 1994; Reyes-Blas, 1995; Fai and Tao, 2010); furthermore, the antioxidant potential *in vitro* and the acute anti-inflammatory effect *in vivo* of successive organic extracts (Hexanic -Hex-, Ethyl acetate -EtOAc- and Ethanolic -EtOH-) from these aerial parts have been described. The EtOAc extract showed a major antioxidant capacity and the Hex extract exhibited a major topical anti-inflammatory effect (Domínguez-Ortiz et al., 2010). In addition, from these successive extracts only EtOAc extract showed antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* with minimal inhibitory concentration (MIC) ≤ 25 μg/mL, while it did not show antiprotozoal activity *in vitro* with a median inhibitory concentration (IC_{50}) of >300 μg/mL (Jimenez-Arellanes et al., 2013).

Another study showed that the methanolic (MeOH) extract from the whole plant was active against *E. histolytica* and *G. lamblia*, with $IC_{50} = 120.92$ and 65.71 μg/mL, respectively (Calzada et al., 1998). The MeOH extract from leaves was active against *Helicobacter pylori* (MIC = 15.6 μg/mL) while the aqueous extract was inactive (MIC ≥ 1 mg/mL) (Castillo-Juárez et al., 2009). In addition, the MeOH extract from the whole plant was moderately active against clinically isolated *H. pylori* (MIC $<1{,}600$ μg/mL) (Robles-Zepeda et al., 2011). Finally, a MeOH extract from leaves demonstrated moderate activity against *Trypanosoma cruzi* (MC_{100} = 250–500 μg/mL) (Abe et al., 2005).

Despite the widespread use of *M. deppeana* in Mexican traditional medicine, there are no toxicological studies of this species to this day. In this manuscript, the acute and sub-acute toxicity of the EtOH extract of this medicinal plant is described, as well as its *in vitro* antioxidant capacity and its acute anti-inflammatory effect on two murine models *in vivo*. The isolation and identification of Verbascoside as a major compound is also described for this species for the first time.

2. Material and methods

2.1. Collection of plant material and crude extract preparation

Moussonia deppeana was collected in Veracruz State, Mexico, in February 2013. A specimen was identified by Luis Bojórquez-Galván from the Biological Research Institute of the Universidad Veracruzana and a specimen voucher was registered under reference CIB8987. The aerial parts (1,371 g) were dried at room temperature under conditions of darkness; afterward, these were macerated with EtOH (6 L) for 1 week (3 times). The extract was filtered and concentrated at 40 °C using a rotary evaporator (Buchii RE-111) coupled to a vacuum system (BuchiiVac V-153) and a cooling system (ECO 20). The extract was left under conditions of darkness until its use.

2.2. Liquid/liquid partition and phytochemical analysis

The crude EtOH extract (5 g) was subjected to liquid/liquid partition with acetonitrile (CH_3CN, F1), chloroform ($CHCl_3$, F2) and MeOH (F3). Each solvent was evaporated in a rotaevaporator coupled to a vacuum pump. In F1 and F2 primary fractions, β-sitosterol, stigmasterol, ursolic acid (**3A**) and oleanolic acid (**3B**) were detected by Thin-Layer Chromatography (TLC) using dichloromethane (CH_2Cl_2): MeOH (96:4) or Hex: EtOAc (6:4), as mobile phases, while in the F3 fraction, polyphenolic compounds such as Verbascoside (**3C**) were isolated by preparative TLC as major compound using EtOAc: EtOH: H_2O (100:13.5:10) or EtOAc: Formic acid: Acetic acid: H_20 (10:1.1:1.1:0.3), as mobile phases. Triterpenes were detected with aqueous H_2SO_4 10% and polyphenolic compounds were identified with 2-amine-ethyl ester diphenylboric acid 1% in MeOH with polyethylenglycol 5% in EtOH as a chromogenic agent. The detected and isolated compounds were compared with Sigma pure standards.

2.2.1. General experimental procedures and structural elucidation

Analytical and preparative TLC was carried out on silica gel 60 F_{254} precoated aluminum plates (0.2 mm, Merck). High-Performance Liquid Chromatography (HPLC) analysis was carried out in Waters equipment (Waters, USA) comprising a 600E multisolvent delivery system with a 486 UV detector. Equipment control, data acquisition, processing and management of the HPLC information were performed by Empower 2 software (Waters).