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Clinical trial to compare the effectiveness of two concentrations of the *Ageratina pichinchensis* extract in the topical treatment of onychomycosis

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ABSTRACT

The plant species *Ageratina pichinchensis* has been used, for many years, in Mexican traditional medicine for the treatment of superficial mycosis.
Aim of the study: This study compared the therapeutic effectiveness and tolerability of two concentrations of the standardized extract from *Ageratina pichinchensis* (12.6 and 16.8%) on patients with clinical and mycological diagnosis of mild and moderate onychomycosis.
Materials and methods: Two identical phytopharmaceuticals (containing the standardized extract from *Ageratina pichinchensis*) in nail lacquer solution for topical administration were evaluated in a double-blind clinical trial. Treatments were administered for 6 months to patients distributed in two groups.
Results and discussion: Of 122 patients who agreed to participate in the study, 103 (84.4%) concluded the treatment. The therapeutic effectiveness exhibited by the 12.6% *Ageratina pichinchensis* extract was 67.2%, while that of the 16.8% *Ageratina pichinchensis* extract was 79.1%. Regarding clinical evolution, analysis of results at the end of treatment evidenced that the 16.8% concentration possesses higher therapeutic effectiveness with a significant statistical difference ($p = 0.010$). No treatment produced side effects.
Conclusion: Both concentrations of phytopharmaceuticals possess high rates of effectiveness on patients with mild and moderate onychomycosis, and the formulation with a 16.8% concentration possesses higher effectiveness.

1. Introduction

Onychomycosis is a disease involving very difficult treatment mainly because it presents a poor response to available drugs; thus, treatment is frequently discontinued by patients. This disease is a therapeutic challenge determined by nail-substratum anatomical characteristics and due to disease chronicity (Gupta et al., 2004a,b; Rigopoulos et al., 2003). Onychomycosis not only represents an aesthetic problem; but also affects millions of persons who require adequate treatment to avoid serious complications and an important expenditure for public health services. Superficial mycoses have been recognized as a health problem of worldwide importance. In Mexico, dermatomycoses are among the 20 main causes for visiting a Physician. There are available drugs for treating toenail mycosis, but these are generally administered systemically; nevertheless, they present side effects (Lecha et al., 2005; Gupta and Cooper, 2008). Topical administration is utilized for mild or moderate cases in which the toenail lesion does not include the nail root or lune. Therefore, in order to select the prescription treatment, it is important to consider the severity of the lesions (Poulin et al., 2006; Gupta et al., 2005). The principal advantages of local therapy comprise safety, tolerability, and cost of treatment, which also represents greater accessibility (Gupta et al., 2006; Murdan, 2008).

The treatment of onychomycosis is complicated; the infection entertains a high index of contagion, and relapses are frequent. The drugs available have limitations, are expensive, and produce side effects; thus, different scientific works report the analysis of the cost of drugs employed for treating onychomycosis with the purpose of identifying the drugs with the best cost-effectiveness relationship (Casciano et al., 2003; Warshaw et al., 2005). Patients affected by these diseases considered onychomycosis to be an important problem that reduces the physical, mental, and social well-being of the individual due to social stigmatization (Szepietowski and Reich, in press; Nunley and Cornelius, 2008).

In Mexican traditional medicine, the use of plants for treating skin diseases is very frequent. In an ethnobotanical approach, 200 plant species were identified which, empirically, are used as a treatment of skin diseases. The pathologies most frequently

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treated by traditional practitioners included scabies, pimples, *nacidos*, swelling, cancer, *disípela*, and *mazamorra* (the latter identified as superficial mycosis) (Zurita and Zolla, 1986).

Ageratina pichinchensis (Asteraceae) plant species originating in Mexico have been utilized in Mexican traditional medicine for the treatment of dermatophytosis (Argueta et al., 1994; Avilés and Suárez, 1994; Monroy-Ortiz and Castillo-España, 2000). For this purpose, fresh or dried leaves are extracted with alcohol, and the product is administered topically onto the damaged skin.

The extract obtained from the plant showed, *in vitro*, an important capability for inhibiting the growth of different fungi, especially *Trychophyton rubrum*, which is the fungus that most frequently affects the nails (Navarro et al., 2003; Rippon, 1990). Previous studies have reported higher antifungal activity in lower polarity extracts such as hexane and ethyl acetate and the presence of encecalin in these extracts, which has been previously used for standardizing the *Ageratina pichinchensis* extract (Romero-Cerecero et al., 2008).

Previous works reported the development of a phytopharmaceutical formulated in a cream that contains the standardized extract of *Ageratina pichinchensis*. This product, evaluated by means of a double-blind clinical trial, showed therapeutic effectiveness of 80.3% in patients with a clinical and mycological diagnosis of *tinea pedis* (Romero-Cerecero et al., 2006). A similar extract, but depigmented and possessing a higher concentration of the active compound, was used for elaborating a phytopharmaceutical in a nail lacquer presentation for local administration in patients with a diagnosis of onychomycosis. Topical administration of this medicament employed solely one concentration of the plant extract (10%) was evaluated by means of a double-blind clinical trial to compare it with a similar drug elaborated with ciclopirox. In this study, the phytopharmaceutical demonstrated therapeutic effectiveness of 59.1%, achieving a very similar effect in patients and without differences from that produced by 8% ciclopirox (Romero-Cerecero et al., 2008).

The present study shows the results of the double-blind clinical trial to compare the therapeutic effectiveness and tolerability of two concentrations (12.6 and 16.8%) of *Ageratina pichinchensis* standardized extract (in a nail lacquer solution) for topical treatment in patients with mild and moderate onychomycosis.

2. Materials and methods

2.1. Plant material

Ageratina pichinchensis (Kunt) R.M. King & Ho. Rob (Asteraceae) was collected by local healers in Cuernavaca, Morelos, Mexico (2007, July). A voucher sample was submitted for identification and storage at the Herbarium of the Instituto de Antropología e Historia (INAHM) and was registered with the number INAH-2050.

2.2. Extract preparation

Aerial parts of the plant were dried at environmental temperature under dark conditions. Dried material was extracted by maceration with a mixture of hexane and ethyl acetate (7:3). The extract was concentrated and then submitted to a chemical depigmentation procedure by means of a gravitational chromatography column with activated charcoal as support. The extract obtained was analyzed by HPLC with the aim of standardizing the content of the active compound. Microbiological tests were used to evaluate biological activity. By means of the agar dilution method the Minimal Inhibitory Concentration (MIC) was determined for the extract against *Trychophyton rubrum* (125 μg/mL) and *Trychophyton mentagrophytes* (250 μg/mL).

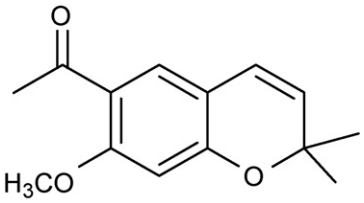

Fig. 1. Chemical structure of encecalin.

2.3. Quantification of the active compound in the extract

In order to standardize the phytopharmaceuticals, encecalin (Fig. 1) was used as marker compound. Identity of the standard was confirmed by comparison with spectroscopy data and by co-chromatography with an authentic sample. HPLC methodology was utilized for measuring the encecalin concentration in the extract and in the phytopharmaceuticals. The method was developed and implemented in a 2695 separation equipment (Waters) with a 2996 diode array detector and the Empower Chromatography Manager version 1.0 operative system (Waters). Analysis was developed with a Lichrospher column (Merck) with a stainless steel cartridge and the following characteristics: Si 60 (5 μm), 250 mm × 4 mm. Elution system consisted of an isocratic solution of n-hexane/ethyl acetate, 80:20 and a flow rate of 1 mL/min. Analysis was performed employing $\lambda = 254$ nm; the total time run was 20 min. A control curve was built with ascending concentrations (50, 100, 200, and 400 μg/mL) of encecalin. Each concentration was injected three times (20 μL, $R^2 = 0.99$). The retention time of the standard was 6.76 min. This method was used in all cases, i.e., for the production of extracts that were employed as raw material for the elaboration of phytopharmaceuticals and quality control of the final product. The encecalin concentration in the extract was 143.9 mg/g.

2.4. Phytopharmaceutical production

A cosmetic nail lacquer (Perlamex 59, Mexico) was used as the base of the medicament design. As described by Romero-Cerecero et al. (2008), *Ageratina pichinchensis* extract (previously diluted in ethyl acetate) was added until obtaining, in one case, a 12.6% concentration, and in the remaining other case, one of 16.8%.

With the aim of blinding the experimental procedure, the medicaments (in a 3-mL flask presentation with a spiral cover that contained a brush for administration on nails) were packed and labeled in identical form.

2.5. Subjects

The clinical trial followed the guidelines of the Declaration of Helsinki and Tokyo for research in humans and was approved by the Institutional Ethics Committee. All patients received information concerning the study and were required to sign an informed consent letter to be included in the study. In this study, patients who were pregnant or breastfeeding, with diabetes mellitus or another serious disease, as well as patients who received treatment for this disease at least 1 month prior to the date of study inclusion, or with a history of sensitivity to topically administrated drugs, were not accepted for participation in the study.

In the study, 122 patients with a diagnosis of mild or moderate onychomycosis aged 19–65 years were included. Based on a table with randomized numbers, two groups were organized: patients of group 1 were treated with the 12.6% *Ageratina pichinchensis* extract, and group 2 received treatment with the 16.8% *Ageratina pichinchensis* extract.