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RESEARCH ARTICLE

Effectiveness of an encecalin standardized extract of *Ageratina pichinchensis* on the treatment of onychomycosis in patients with diabetes mellitus

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Abstract

Ageratina pichinchensis is utilized in traditional medicine for the treatment of dermatomycosis and inflammation. The aim of this study was to evaluate the clinical and mycological effectiveness of the topical administration of an enecalin standardized extract of *A. pichinchensis* for treating onychomycosis in patients with type 2 diabetes mellitus (DM2). A double blind, randomized, and controlled clinical trial was carried out that included patients with DM2 and who had mild or moderate onychomycosis. Participants were administered topically, for 6 months, a lacquer containing the encecalin standardized extract of *A. pichinchensis* (experimental group) or 8% ciclopirox (control group). In a large percentage of both, the control group (77.2%) and the experimental group (78.5%), clinical efficacy was detected as a decrease in the number of affected nails and a reduction in the severity of nail involvement. Without exhibiting statistically significant differences between groups, the encecalin standardized extract of *A. pichinchensis* was clinically and mycologically effective in the treatment of mild and moderate onychomycosis in patients with DM2. The treatment of onychomycosis in patients with DM2 implies a greater challenge, while control of blood glucose levels in these patients, played a very important role in the response of patients to treatment.

KEYWORDS

Ageratina pichinchensis, dermatomycosis, inflammatory disease, onychomycosis, type 2 diabetes mellitus

1 | INTRODUCTION

In patients who have type 2 diabetes mellitus (DM2), susceptibility to develop a fungal infection is higher. This is due to changes in the immune system that compromise the body's natural defenses, such as in skin and nails. Morbidity of infections is increased by complications of diabetes (retinopathy and diabetic neuropathy), as well as by other medical conditions, especially obesity and peripheral venous insufficiency. In these patients, DM2 also generate alterations that disable the person to undertake adequate review and care of the nails, and also limits the chances of detecting an infection or following the evolution of the disease. Severity of the infection is increased when there is poor control of blood glucose levels, all this implying a greater risk for the development of complications and the treatment becoming more complicated (Gupta & Humke, 2000; Papini, Cicoletti, Fabrizi, & Landucci, 2013; Parada et al., 2013; Dogiparthi et al., 2018; Takehara et al., 2011).

Abbreviations: AcoEt, ethyl acetate; AUC, area under the curve; DM2, type 2 diabetes mellitus; Hex, n-hexane; HPLC, high-performance liquid chromatography; SCIO, the scoring clinical index for onychomycosis.

The nail is characterized by being provided with an important vascular supply that in individuals with diabetes, is diminished by the presence of chronic hyperglycemia, which in turn produces advanced and irreversible glycosylation of proteins such as type IV collagen, that renders them more susceptible to suffering from infections; even with pharmaceutical treatment, their recovery is slower (Hillson, 2016; Rich, 2002).

In order to avoid complications, treatment of onychomycosis in patients with DM2 should be immediate and based on clinical diagnosis and direct microscopy (Elewski et al., 2013; Gupta & Humke, 2000; Litz & Cavagnoli, 2010).

Ageratina pichinchensis (Kunt) R.M. King & Ho. Rob. has been used for many years in Mexican Traditional Medicine for treating skin and nails infections produced by fungus. An ethanol extract, of this plant, is empirically administered topically by spreading it with cotton on the clean and dry damaged skin (Avilés & Suárez, 1994). This plant species is also used for the treatment of swelling, "mazamorra" (fungal skin infection), and DM2 (Zurita & Zolla, 1986). When 18 plant extracts from nine Mexican medicinal plants were tested, the *A. pichinchensis* (synonymy *Eupatorium aschenbornianum*) hexane extract exhibited the strongest antifungal activity against *Trichophyton mentagrophytes* and *Trichophyton rubrum*, as well as against *Aspergillus niger* and *Candida albicans* (Navarro et al., 2003). Aguilar, Navarro, León, and Rios (2009) demonstrated that encecalin compound, isolated from the same species, was responsible of the antimicrobial activity against *T. mentagrophytes*, *T. rubrum*, *C. albicans*, and *A. niger*. Different extracts from the aerial parts of *A. pichinchensis* have been evaluated through double-blind, randomized, and controlled clinical studies during the last 10 years, in which its effectiveness was demonstrated on the treatment of diseases caused by dermatophytes such as *tinea pedis*, onychomycosis, and vulvovaginal candidiasis. In general, the results showed an important clinical efficacy of the products obtained from this species against the different strains of pathogens causing these diseases, in addition to demonstrating good tolerability and therapeutic safety (Romero et al., 2006, 2009; Romero, Islas, Zamilpa, & Tortoriello, 2017). Because ethnomedical use includes inflammatory diseases and superficial mycosis, it is important to evaluate whether the standardized extract of the plant possesses any advantage in the treatment of patients with onychomycosis and who also suffer from diabetes mellitus.

The aim of the present study was to evaluate the clinical and mycological efficacy, as well as the therapeutic safety, of a pharmaceutical preparation containing the encecalin standardized (24.17 mg/ml) extract of *A. pichinchensis* in the lacquer form, for topical administration, in the treatment of mild and moderate onychomycosis in patients who also have DM2.

2 | MATERIALS AND METHODS

2.1 | Plant material and standardizing of the extract

The plant material of *A. pichinchensis* (Kunth) R.M. King & H.Rob (Asteraceae) was collected in its natural habitat in the state of Morelos, Mexico. A herbarium voucher specimen was prepared and is available at the IMSSM herbarium, with registration number IMSSM-15543. The identification of species was in charge of the Abigail Aguilar-Contreras, MSc, Herbarium Director.

Aerial parts were selected from the plant material, which were dried at room temperature and in the dark. The dried material was ground in electrical equipment until obtaining particles smaller than 5 mm, and then, it was extracted by maceration in a mixture of *n*-hexane (Hex) and Ethyl Acetate (AcoEt) (Merck, Germany) at a 7:3 ratio; later, it was filtered through cotton. The solvent was removed by evaporation with the aid of a rotaevaporator (Heidolph, Germany) with reduced pressure. The yield of the extract was 2.3%. In order to obtain a completely dry product, the extract obtained was lyophilized and, finally, a sample was analyzed by high-performance liquid chromatography (HPLC).

By means of a (HPLC) technique, we carried out the analysis of the extract utilized in the study. Waters equipment, including an Alliance 2,995 model separation module and a diode arrangement detector was used. We utilized a reverse-phase silica gel column (RP-C18 Supersphere, 120 × 4 mm, 5 μm; Merck). As mobile phase, we employed a gradient system with acidulated water (0.5% TFA Reservoir A) and acetonitrile (Reservoir B) in the following order of descending polarity: 100:0 (0–2 min); 80:20 (3–6 min); 70:30 (7–10 min); 60:40 (11–16 min); 40:60 (17–18 min); and 100:0 (19–21 min). The volume of the sample injected into the equipment was 10 μl. We utilized a 200–600-nm wavelength range, setting the analyses at a lambda of 350 nm.

With the aim of standardizing the extract employed through the previously described method, we constructed a calibration curve to quantify the concentration of encecalin, a compound previously identified in the plant and to which antimycotic activity has been attributed. We prepared increasing concentrations of the flavonoid (50, 100, 200, 400, and 500 μg/ml). These solutions were injected in triplicate (10 μl) into the equipment and the average value of the area under the curve (AUC) generated by integration of the peak corresponding to the flavonoid in the three repetitions of each concentration was utilized to generate the corresponding calibration curve, which generated the following equation: Y = 917.36X−14,761, with an R^2 value = 0.99. The extracts were analyzed employing the same injection volume (10 μl) at a 5-μg/ml concentration. The concentration of the active compound in the AcoEt extract of *A. pichinchensis* was 143.9 mg/g (Figure 1).

2.2 | Phytopharmaceutical design

In order to obtain a product that could be administered topically and to allow blinding of the treatment- administration maneuver, the plant extract was discolored. For this purpose, the extract was passed through a gravitational column packed with activated charcoal, which had previously mixed with the extract. The elution system was based on 100% methanol (Merck, Germany). For lacquer formulation, the decolorated extract was previously mixed in AcoEt and added to a

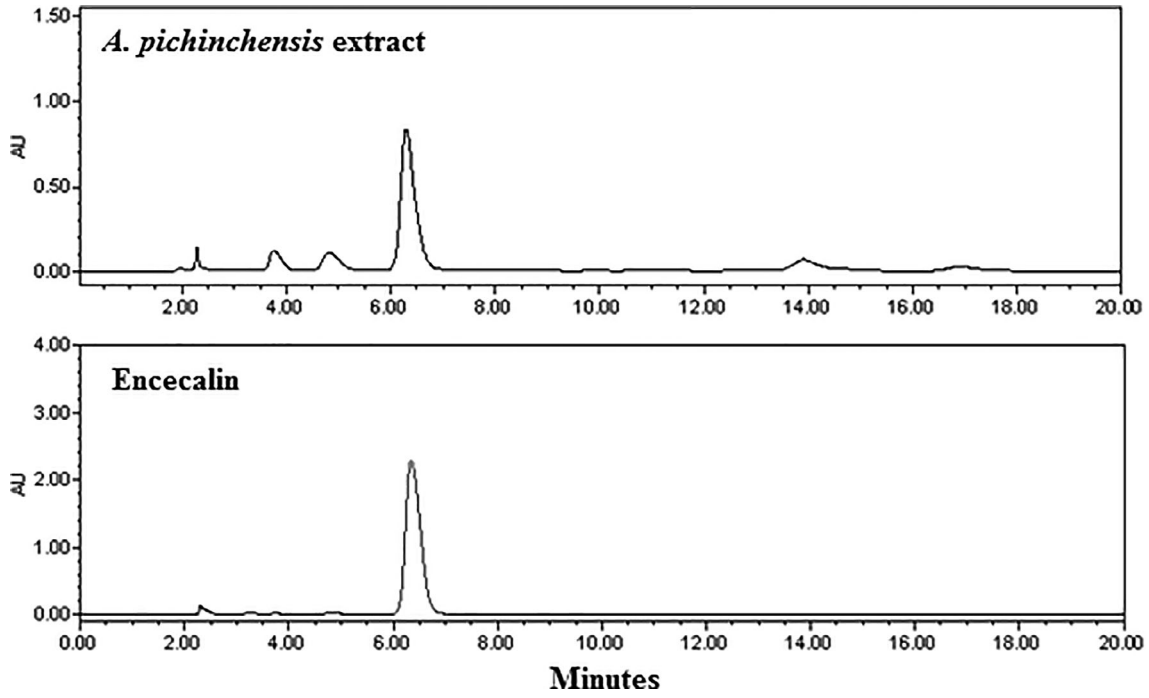


FIGURE 1 Chromatographic profile of the encecalin standardized extract of *Ageratina pichinchensis*. Fingerprint was carried out at 350 nm and encecalin displayed a retention time of 6.38 min. HPLC run conditions are described in methods section

pharmaceutical-grade lacquer at an amount necessary to obtain a clear lacquer with a final concentration of 24.17 mg/ml encecalin. The product was packed in 3-ml translucent glass bottles with a screw cap that included a small brush to facilitate administration. The bottles used were identical to those containing the control treatment. The control treatment consisted of a commercially available translucent lacquer containing 8% ciclopirox and was purchased directly at the pharmacy. This product was packed in cardboard boxes identical to those of the experimental treatment. Identification of treatments was based on a progressive folio number.

2.3 | Study description

In order to evaluate the clinical and mycological effectiveness, as well as the tolerability of a phytopharmaceutical elaborated with the encecalin standardized extract of *A. pichinchensis*, administered topically in patients with diabetes diagnosed with mild and moderate onychomycosis, we performed a randomized and double-blind clinical trial, controlled with 8% ciclopirox.

The study universe was constituted of ambulatory patients from a Hospital of the Mexican Institute of Social Security, in the state of Morelos, Mexico.

Patients of both sexes, older than 18 years of age, with a clinical diagnosis and by direct microscopy of mild or moderate onychomycosis in toenails with an evolution of no more than 5 years were included. The patients were also required to be suffering from DM2, whose last blood glucose rate was not higher than 180 mg/dl, with a disease evolution of 10 years or less, as well as their not having received a systemic or topical medication for onychomycosis for 1 month prior to inclusion in the study. Regarding the severity of the nail affectation, patients whose infection was between 1 and 15 points were included. The latter, according to The Scoring Clinical Index for Onychomycosis (SCIO), whose cut-off points are as follows: 0 points = without onychomycosis; ≤5 points = mild onychomycosis, and 6–15 = moderate onychomycosis (Carneney et al., 2011). Patients with onycholysis, diabetic foot, peripheral vascular insufficiency, or diabetic neuropathy were not included. Participants who did not adhere to the treatment were eliminated from the study; however, they all were included in the "Intention to Treat" analysis.

All patients, regardless of the study group, had to clean and file the surface of the nail, and by means of the brush to spread the medication topically from the proximal to the distal toenail surface, avoiding covering the adjacent skin. During the first 2 months, the medication was administered once a day, every third day. Subsequently, the medication was administered twice a week, until the end of 6 months of treatment. Patients were asked to keep their nails trimmed and file the nail lightly once a month.

All patients included in the study signed an informed consent letter according to the Declaration of Helsinki and Tokio for humans, and treatments were assigned by means of a random number table. In compliance with the ethical standards, the project was evaluated by the National Scientific and Ethical Committee and received the authorization number R-2016-785-029. The development of the project followed the rules established in the Mexican General Law of Health.

2.4 | Study groups

2.4.1 | Experimental group

This consisted of patients who met the inclusion criteria, who were topically treated, for 6 months, with a product elaborated with the lacquer containing the encecalin standardized (24.17 mg/ml) extract of *A. pichinchensis*.

2.4.2 | Control group

This was made up of patients who met the inclusion criteria, who received a pharmaceutical product containing 8% ciclopirox as a treatment, administered in the same manner and during the same time as the extract. The presentation of the medication was identical to that of the experimental group. In order to blind administration of the treatment, both control and experimental treatments had the same presentation and were packed in identical cardboard boxes identified only by a folio number.

2.5 | Direct observation of the nail sample and mycological culture

In all cases, and in order to perform the mycological diagnosis, all patients underwent direct observation under a microscope before being included in the study. Once the affected nail was cleaned, small scales were taken from it, which were deposited on a sterile slide. A drop of a 40% potassium hydroxide (KOH) dilution was added to the sample, covered with a coverslip, and proceeded to be observed under a phase contrast microscope in order to identify filaments and confirm or reject the mycological diagnosis of onychomycosis. After 6 months of treatment, once the medication administration has finished (control or experimental), direct microscope observation was again carried out, this time in order to evaluate the mycological efficacy of the treatments.

A culture was carried out of the nail scales obtained from the participants, both, at baseline and after 6 months of treatment. The culture was performed on Sabouraud agar and microbiox. The culture cages were maintained at room temperature and observations by direct microscopy were made 7 and 15 days after the sample were cultured; nevertheless, a final observation was carried out after 6 weeks. In order to facilitate identification of the pathogen, Clonazole blue was used.

2.6 | Outcome variables

2.6.1 | Efficacy or clinical improvement

This was considered when the clinical condition remitted or evolved to a less severe stage, and the following scale was considered: (a) Complete cure, when there was a total remission of the number of nails affected by the infection; (b) significant improvement, when there was at least an 80% decrease in the number of affected nails; (c) moderate improvement, when the decrease was between 79 and 40%; (d) slight improvement, when it was less than 40%, and (e) no improvement, when there was no change in the number of affected nails after 6 months of treatment.

2.6.2 | Mycological efficacy

This was considered when, at the end of the treatment, direct microscopy was negative to hyphae or yeast, and the culture was negative.

2.7 | Data analysis

Once the clinical phase was done, a database was built, and the analysis was carried out through the "intention to treat" method. For the analysis of continuous and categorical variables, descriptive statistics were used. Identification of differences in proportions was performed by the X^2 test, while the analysis of variance test was employed for differences of means. Values of $p \leq .05$ were considered to define significant differences between groups.

3 | RESULTS

3.1 | Baseline characteristics of the population-under study

A total of 71 patients were admitted into the study; 36 were included in the control group, who were treated topically with 8% ciclopirox, and 35 were included in the experimental group, who were administered with the encecalin standardized extract of *A. pichinchensis* (Figure 2).

All patients were included in the intention-to-treat analysis. The number of patients was reduced throughout the study development, and statistical analysis was performed with the total number of patients who attended the monthly evaluation in each of the study groups.

A total of 97.2% (35) of patients who were included in the control group completed the 6 months of treatment administration, while in the experimental group, this value was 80% (28). The patients who left the study included five (14.2%) of the experimental group and one (2.7%) of the control group, all of these patients withdrew due to personal reasons. Also, in the experimental group, there were two withdrawals (5.7%); of these, one was because of pregnancy and another, due to lack of treatment adherence.

In the study, female patients predominated with 83.1% (59), while men constituted 16.9% (12). The median age of the total sample was 57 ± 6.7 years.

It was observed that 5.5% (2) of the patients in the control group and 8.5% (3) of the experimental group experienced adverse effects

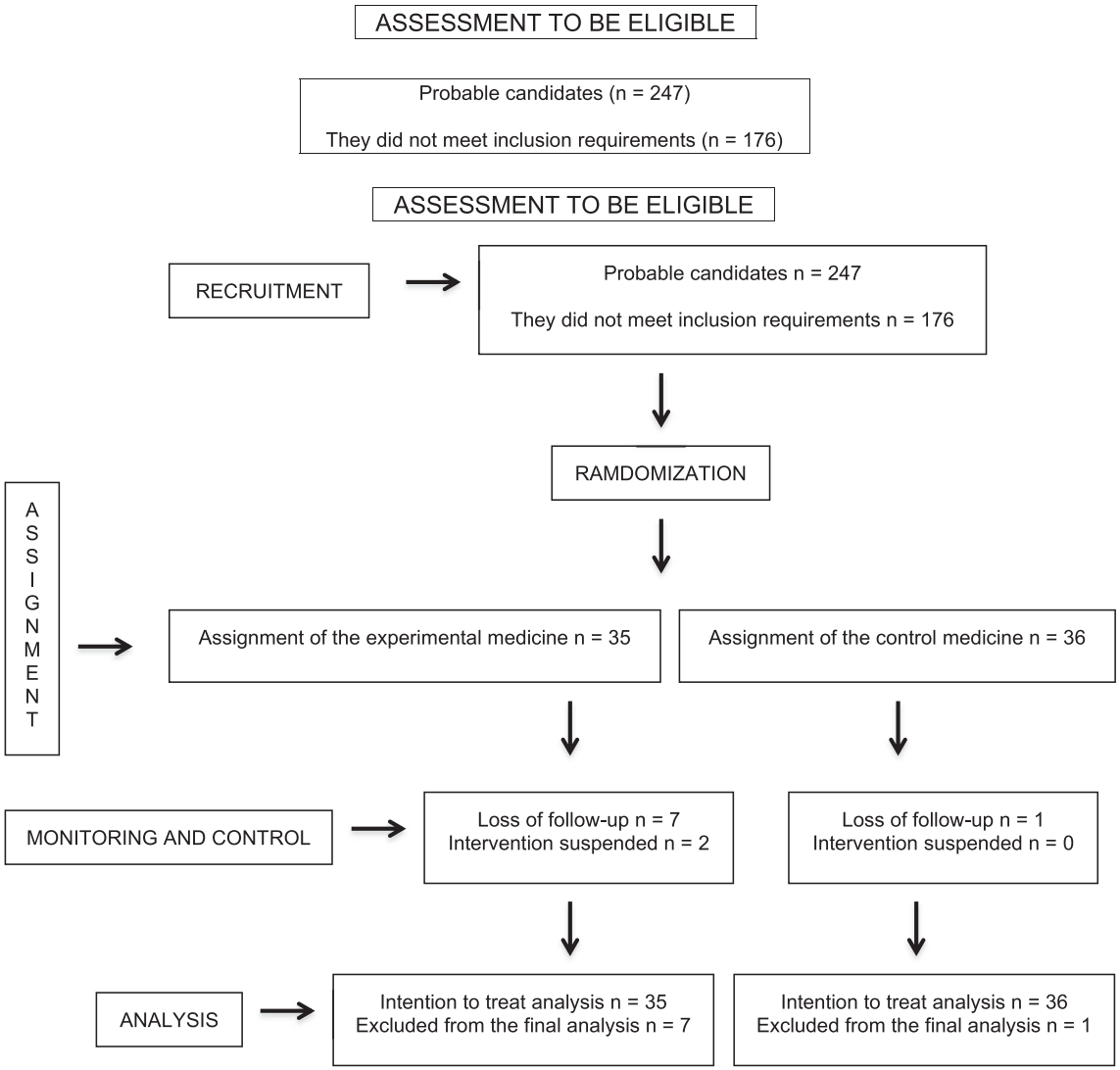


FIGURE 2 Diagram illustrating the study procedure

(p = .84). In all cases, the adverse effect comprised irritation in the skin surrounding the nail, which always presented temporarily (for less than 7 days) and there was no need to suspend administration of the treatments.

Table 1 describes the antecedents related to DM2 and the onychomycosis of the patients included in the study. It can be observed that the majority of patients, included in both groups, presented an evolution of DM2 of longer than 5 years. Similarly, the majority of patients lived with people affected with the same infection. However, no significant differences were identified between the two study groups.

Color changes and thickening were the clinical manifestations most frequently identified among the patients included in the two study groups (Table 2). These clinical manifestations allowed an analysis of the clinical evolution of the disease.

According to the SCIO, it was observed that, at the baseline condition, the highest proportion of patients included in the two study groups had a score of between 6 and 15 points-of-involvement of the nail plate.

At baseline, in the experimental group, a minimal nail affectation score of 2.8 and a maximal score of 13.5 was identified, with a mean of 7.8 ± 2.9. In the control group, the minimal score was 1.6 and the maximal score was 13 with a mean of 7.0 ± 2.7. At the end of the treatment, the minimal score in the experimental group was 0 and the maximal score was 11, with a mean of 2.7 ± 2.4, while in the control group, the minimal value was 0 and the maximal value was 6.5, with an average of 1.9 ± 1.2.

3.2 | Evolution of symptomatology among the participants

Clinical affectation of the nail was modified with relation to a longer administration time of the drugs. Table 3 illustrates the evolution toward the improvement of the affected nails among the patients included in the two study groups, with the data corresponding to the baseline, intermediate, and final moment of the treatment

TABLE 1 Backgrounds related to the presence of type 2 diabetes mellitus (DM2) and onychomycosis in the patients included in the study, organized by treatment group as follows: experimental group (treated topically with the encecalin standardized extract of *Ageratina pichinchensis*), and the control group (which received 8% ciclopirox, applied topically)

Variable	Experimental group *n* = 35 % (frequency)	Control group *n* = 36 % (frequency)	*p*
Evolution time of DM2			
<1 year	11.4 (4)	13.8 (5)	.55
1–5 years	37.1 (13)	27.7 (10)	
6–10 years	51.4 (18)	58.3 (21)	
Most recent glycemia values			
≤120 mg/dl	37.1 (13)	41.6 (15)	.06
130–160 mg/dl	57.1 (20)	41.6 (15)	
161–180 mg/dl	5.7 (2)	16.6 (6)	
DM2 complications			
Yes	5.7 (2)	5.5 (2)	.97
No	94.2 (33)	94.4 (34)	
Living with people suffering from onychomycosis			
Yes	65.7 (23)	75 (27)	.39
No	34.2 (12)	25 (9)	
Tinea pedis comorbidity			
Yes	48.5 (17)	55.5 (20)	.63
No	51.4 (18)	44.5 (16)	
Start of symptoms compatible with onychomycosis			
Less than 1 year	5.7 (2)	16.6 (6)	.34
1–5 years	94.2 (33)	83.3 (30)	

administration. Figure 3 shows the clinical evolution of a patient treated with the encecalin standardized extract of *A. pichinchensis*.

The pathogens identified in the majority of the patients included in the two study groups were *T. rubrum* (32.4%) and *T. mentagrophytes* (28.2%). Other pathogens identified were *Epidermophyton floccosum* (14.1%) and *Candida* spp (25.3%).

Of the total cultures that were identified as negative at the end of the administration of the treatments, initially 60% had identified *T. rubrum* and 40% *T. mentagrophytes*.

At baseline, the average number of affected nails in the experimental group was 4.14 ± 2.28, and in the control group, 3.38 ± 2.25. The values of *p* (.31) revealed no statistically significant difference between the study groups.

After 6 months of treatment, the average number of affected nails in the experimental group was 0.89 ± 1.03, and in the control group, 1.0 ± 1.01. In this case, the results also demonstrated no statistically significant difference ($p = .38$).

TABLE 2 Proportion of patients who were included in the two study groups who presented changes in coloration and thickening of the nails

	Baseline condition *n* = 71 % (frequency)	Final condition *n* = 63 % (frequency)	*p* baseline /end
Nail color changes			
Experimental group			
No	0 (0)	7.1 (2)	1.0/0.11
Yes	100 (35)	92.8 (26)	
Control group			
No	0 (0)	8.5 (3)	
Yes	100 (36)	91.4 (32)	
Nail thickening			
Experimental group			
No	21.4 (6)	46.4 (13)	.13/0.38
Yes	82.8 (29)	53.5 (15)	
Control group			
No	8.3 (3)	34.2 (12)	
Yes	91.6 (33)	65.7 (23)	

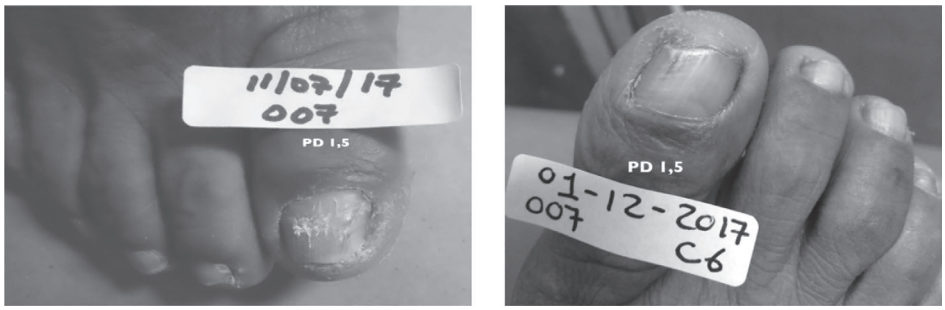
Note: The baseline situation is shown and that of the end of administration of the treatments. Experimental group (treated topically with the encecalin standardized extract of *Ageratina pichinchensis*), and control group (who received 8% ciclopirox, applied topically).

TABLE 3 Evolution of the clinical involvement of the nails in patients with mild and moderate onychomycosis who also have type 2 diabetes mellitus (DM2), treated topically with the encecalin standardized extract of *Ageratina pichinchensis* (experimental group) or 8% ciclopirox (control group)

Evolution time/study group	Experimental group % (frequency)	Control group %(frequency)	*p*
Baseline condition			
≤5 points	25.7 (9)	22.2 (8)	.33
6–15 points	74.2 (26)	77.7 (28)	
Intermediate			
0 points	0 (0)	2.9 (1)	.36
≤5 points	56.6 (17)	76.4 (26)	
6–15 points	43.3 (13)	20.5 (7)	
Final			
0 points	7.1 (2)	8.6 (3)	.47
≤5 points	85.7 (24)	88.5 (31)	
6–15 points	7.1 (2)	2.8 (1)	

Note: Affectation of the nail was measured by means of the SCIO scale, where 0 = least affectation and 15 = greatest affectation.
Intermediate = 3 months of treatment; final = 6 months of treatment.

In 22.8% (8) of the patients included in the control group and in 21.4% (6) of those in the experimental group, no decrease in the number of affected nails was observed at the end-of-treatment administration; however, there was an improvement in severity.



Before (July 11th,2017) After (December 1rst, 2017)

FIGURE 3 Photography showing the clinical evolution of a patient, before and after being treated topically with the encecalin standardized extract of *Ageratina pichinchensis*

TABLE 4 The clinical effectiveness observed in patients with mild or moderate onychomycosis who also suffered from type 2 diabetes mellitus (DM2) treated topically with the encecalin standardized extract of *Ageratina pichinchensis* (experimental group) and with 8% ciclopirox (control group)

	Experimental group	Control group	
	n = 28	*n* = 35	
Variable	% (frequency)	% (frequency)	*p*
Clinical effectiveness			
Complete cure of all affected nails	7.1 (2)	8.6 (3)	
Significant improvement, decrease in the number of affected nails of higher than 80%	7.1 (2)	2.8 (1)	
Moderate improvement, decrease of affected nails of between 79 and 40%	42.9 (12)	42.9 (15)	.76
Mild improvement, decrease of affected nails of less than 40%	21.4 (6)	22.8 (8)	
No improvement, no change in the number of affected nails	21.4 (6)	22.8 (8)	

With the results obtained, a complete remission of the clinical manifestation was detected in 7.1% (2) of the patients in the experimental group (who received the phytopharmaceutical) and in 8.6% (3) of the control group (treated with 8% ciclopirox). In the remainder of the participants, there was a partial decrease in the number of affected nails. In all cases, the severity of the condition changed to a lower stage of severity.

With the results obtained, clinical effectiveness could be identified in 78.5% of the patients in the experimental group and in 77.2% of the patients in the control group, information described in detail in Table 4.

Both, in the patients included in the experimental group and in those of the control group, the cultures carried out after 6 months of treatment exhibited mycological improvement. A total of 7.1% of the cultures of patients administered the pharmaceutical product elaborated with the encecalin standardized extract of *A. pichinchensis* were negative, and as were 8.5% of those who received 8% ciclopirox.

4 | DISCUSSION

Severity of nail involvement and the presence of diabetes mellitus are the factors that exert a significant impact on the incidence and recurrence of onychomycosis (Chang et al., 2008; Tosti & Eleweski, 2016), while the patient's age is another predictive factor that renders the prognosis of the infection worse (Szepietowski, Reich, Garlowska, Kulig, & Baran, 2006). In the present study, median age was 57 years (among the patients included in the two study groups) and, in 100% of the participants, the severity of onychomycosis was mild or moderate.

It has been reported that monotherapy in onychomycosis with topical medications achieves a complete cure in approximately 40% of patients (Gupta & Josrph, 2000). A phytopharmaceutical elaborated with the standardized *A. pichichensis* extract, evaluated in patients with mild or moderate onychomycosis, but who did not have a history of having diabetes, demonstrated clinical efficacy of up to 79.1%. In addition, in the aforementioned study, clinical effectiveness was defined as total remission of the clinical manifestations (Romero et al., 2009), while in the present study, clinical effectiveness was categorized, and all patients included in the study had diabetes mellitus. In this case, it was observed that in all patients, disease severity evolved into a less severe stage in terms of the involvement of the nail plate area and, in a great majority of the participants of the experimental group (78.5%) and the control group (77.2%), there was a decrease in the number of nails affected by the infection.

Administration time (ranging from 6–12 months) has been considered a significant inconvenience of topical therapy for onychomycosis because it requires willingness and commitment on the part of the patient to adequately carry out the posology and time of drug administration (Mehra et al., 2015; Singal & Khanna, 2011). With the development of the present study, we identified that 88% (31) of the patients included in the experimental group and 80.5% (29) of those of the control group had had previous treatment without presenting improvement or remission of the disease, even in patients with oral administration. In addition, in this study, the duration of administration of the drugs was 6 months and average adherence to treatment reached 91% in the experimental group (5 dropouts), revealing outstanding treatment adherence, and evidencing commitment in the majority of participants.

It has been shown that developing novel drugs for topical administration in patients with onychomycosis is a challenge (Elkeeb, Hui, Murthy, & Maibach, 2014; Gupta & Simpson, 2014). With the results obtained in the present study and, in comparison with previous results in which the clinical and mycological efficacy of a phytopharmaceutical elaborated with the species *A. pichinchensis* was shown, we identified that the challenge is greater when, in addition to onychomycosis, the patient is suffering from DM2.

There are proposals for early diagnosis and treatment of onychomycosis in patients with DM2 who continue to be asymptomatic, thus preventing complications (Elbeldary, El Tawdy, Zaki, Alfishawy, & Rateb, 2015). With the present project, we detected that the majority of patients included in the experimental group (94.2%) and in the control group (83.3%) had an evolution greater than 1 year. Among these patients, 7.1% (2) had total remission and belonged to the experimental group, unlike the control group, in which there was remission in 8.5% (3) in patients who had an evolution of less than 1 year. It is also possible to confirm that onychomycosis is a widely distributed infection with high prevalence. It is a chronic disease, very difficult to eradicate, and usually caused by dermatophytes, non-dermatophyte molds, and yeasts (Gupta & Stec, 2019). However, *T. rubrum* and *T. mentagrophytes* are the most frequently isolated fungi (Narayan-Kayarkatte, Singal, Pandhi, & Das, 2019). In our work, *T. rubrum* and *T. mentagrophytes* were also the pathogens identified in the majority of the patients included in the two study groups.

Thickening of the nails has been correlated with patients who present high levels of glycosylated hemoglobin, and good control has been recommended of glucose in patients with diabetes to prevent these signs associated with onychomycosis (Takehara et al., 2011). It is very important to emphasize that, in the present study it was possible to appreciate that 100% of patients who had complete disease remission (in the two study groups) had glycemic rates lower than 160 mg/dl. This shows the importance of glycemic control in the evolution and response to treatment in patients with DM2.

5 | CONCLUSION

A pharmaceutical formulation elaborated with the encecalin standardized extract of *A. pichinchensis* was clinically and mycologically effective in the treatment of patients with mild and moderate onychomycosis who also suffered from diabetes mellitus. It was observed that the treatment for onychomycosis in patients with DM2 implied a greater challenge, and that the control of blood glucose levels in these patients played a very important role in the response of patients to treatment.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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