Diagnosis and in vitro antidermatophytic sensitivity of the ethanolic extract of Euphorbia tirucalli

Diagnóstico e sensibilidade antidermatófita in vitro do extrato etanólico de Euphorbia tirucalli

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ABSTRACT
Dermatophytosis is the most common mycosis, accounting for 5 to 10% of all superficial mycoses. The present study aimed to evaluate the in vitro antifungal activity of the ethanolic extract of Euphorbia tirucalli on clinical isolates of dermatophytes obtained from 2 patients with signs and symptoms consistent with dermatophyte infection. Two dermatophyte etiologic agents, Trichophyton mentagrophytes and T. tonsurans, were isolated from these patients. In vitro antifungal sensitivity showed that the ethanolic extract of E. tirucalli possesses antifungal activity against these strains of interest, with a Minimum Inhibitory Concentration (MIC) of 125 and 250 g/mL, respectively. According to the phytochemical profile of this extract supported by infrared analysis, the antibiotic activity was attributed to the presence of flavonoids and tannins, phytoconstituents generally recognized for their pharmacological potential.

Keywords: dermatophytes, clinical isolates, antifungal sensitivity, phytoconstituents.

1 INTRODUCTION
In the wide range of skin diseases, there are mycoses, which are fungal infections that can be superficial, subcutaneous, deep and systemic. Superficial mycoses are the most common infections, the most representative of this group is dermatophytosis and the second most common is pityriasis versicolor, whose etiological agent is Malassezia spp. Other superficial mycoses are black stone and white stone, produced by the ascomycete Piedraia hortae and species of the genus Trichosporon, respectively. Also, black ringworm, caused by Hortaea, is reported.

From an epidemiological point of view, cutaneous mycoses caused by dermatophytes are the most common fungal infections worldwide, affects all age groups and negatively impacts the quality of life of infected patients. WHO estimates show that the global frequency of superficial mycoses is between 20 to 25% of the general population.
population, of which 5 to 10% are caused by dermatophytes \(^7,9\). Although the incidence of dermatophytosis varies between regions, Manzano-gayosso (2008) reports similar percentages for the countries of Spain (20.8%), Brazil (26.3%) and Iran (24%). Dermatophytosis are very common superficial mycoses in Mexico and currently represent 70 to 80% of all mycoses with a diagnostic incidence of 5% in dermatological consultations. Of the existing types of dermatophytosis, the most predominant clinical form is *Tinea pedis* \(^8,10-12\), whose prevalence has increased from 17.5% in 1952 to 51.3% and 51.0% in the years 1991 and 2014, respectively (Secretaría de Salud, 2008; Bonifaz et al., 2014).

On the other hand, since the prehistoric age, plants have been used as a source of medicinal agents due to the diverse properties they possess, such as antioxidant, antimicrobial, antiviral, antiprotozoal, anticancer, angiogenic, among others \(^14-18\). *Euphorbia tirucalli* is a plant extensively used in traditional medicine in Africa and other regions to treat tumors, excrecences, nodules, and abscesses. It has also been used to treat asthma, cough, ear pain, neuralgia, rheumatism, toothache, and other conditions \(^15,17\). Previous studies of isopropyl alcohol extracts of Euphorbia genus reported its antimicrobial effect \(^19\), anti-inflammatory, analgesic and angiogenic activity (Bessa, et al., 2015; Prabha et al., 2008). The aqueous extract obtained from *E. tirucalli* was also studied, showing angiogenic activity and the presence of terpenoids, triterpenes, polyphenols, serine proteases, steroids, flavonoids and isoflavonoids (Mwine and Damme, 2011), compounds recognized for their biological activities (García-Hernández et al., 2016). This is the reason for the continuous research of new properties of this plant.

The aim of this study was to obtain clinical isolates of dermatophytes and evaluate their in vitro sensitivity to the ethanolic extract of *E. tirucalli*.

### 2 METHODOLOGY

#### 2.1 COLLECTION OF DERMATOPHYTES

Diagnosis was performed during several stages, including presumptive diagnosis (clinical characteristics of the host), direct microscopic diagnosis following sample collection, isolation of the etiologic agent in growth medium and its identification from its macroscopic and microscopic characteristics \(^8,11\).

*Sample collection.* Sampling was considered in patients with signs and symptoms compatible with fungal infection (Figure 1A). First, the affected area was disinfected to reduce any environmental contamination such as microorganisms that represent the...
natural microflora of the skin and the environment. Subsequently, the dermal detritus was collected using a forceps and was placed in a petri dish.

_Direct microscopic examination / direct diagnosis._ For this analysis, 15% KOH (potassium hydroxide) was employed, as a strong base, it has the ability to dissolve cellular components, exposing some representative fungal structures (e.g., hyphae, pseudohyphae, conidia). The sample was placed between a microscope slide and a coverslip with a drop of KOH and allowed to react for 10 minutes before proceeding with microscopic observation in the search for fungal structures (Figure 1B).

_Diagnosis certainty / culture._ A sample of dermal detritus was placed in sterile Potato Dextrose Agar medium, with the addition of chloramphenicol and incubated at 28 °C for 15 days (Figure 1C), for the growth of fungal colonies.

_Identification._ Clinical identification of the etiologic agents was performed based on macroscopic (appearance, color, pigment produced) and microscopic characteristics (hyphal arrangement and thickness, presence of macro- and microconidia, etc.;Figure 1D). (Bonifaz et al., 2015)

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Figure 1. Stages in the diagnosis of fungal infection. (A) clinical cases of dermatomycosis, (B) positive direct microscopic examination (40x) with 15% KOH, (C) macroscopic identification of the fungal agent, (D) microscopic identification of the fungal agent (*Trichophyton tonsurans*).
2.2 IN VITRO ANTIFUNGAL ACTIVITY OF *E. TIRUCALLI* EXTRACT

**Specimen collection.** The collection of *Euphorbia tirucalli* was carried out in the north of the municipality of San Nicolás de los Garza. The collected material was washed with distilled water and dried at a temperature at 40 °C in an oven for 5 days.

*E. tirucalli extract.* The dried and ground plant was under continuous extraction in a Soxhlet system using ethanol as extraction solvent. The solvent was removed from the obtained extract under reduced pressure (Yamato rotary evaporator mod. RE200) and the recovered concentrate was completely dried from the extraction solvent in a drying oven at a temperature lower than 40 °C. Finally, the solvent-free extract was stored at 4°C until use.

**Inoculum preparation.** After 15 days of incubation, 10 mL of sterile distilled water was added over the fungal colonies and then scraped with a sterile loop; the suspensions obtained were filtered with sterile gauze to separate the hyphae and agar particles from the conidia. For the biological test, the conidia suspensions were adjusted to a concentration of 1 to 3 x 10³ CFU/mL after counting in a Neubauer chamber.

**Antifungal activity.** Determined by a modification of the microdilution method described in the M38-A protocol by the Clinical and Laboratory Standards Institute (2008), employing ketoconazole (Sigma-Aldrich) as a control. From the stock solution of the extract (40,000 g/mL), an 8,000 g/mL solution was obtained, and serial dilutions were applied in a 96-well flat-bottom microplate, using Müeller-Hinton broth as diluent. As positive controls, ketoconazole was used at a concentration of 50 g/mL.

The antifungal activity was determined by calculating the percentage inhibition (%H), according to CLSI.

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%H = \left(\frac{Control - (Treatment - Standard)}{Control}\right) \times 100
\]

2.3 PHYTOCHEMICAL CHARACTERIZATION (POLYPHENOLS)

**Infrared.** Infrared analysis of the ethanolic extract of *E. tirucalli* was performed in a conventional FTIR Bruker IFS 66 spectrophotometer with digitalization of spectra that allows to obtain electronic files of the analyses, with a standard resolution of 4 cm⁻¹ (FT-IR FRONTIER spectrometer, Perkin-Elmer, USA).
Colorimetric tests. The presence of polyphenols was evidenced by the Shinoda, ferric chloride and Liebermann-Burchard tests to determine the presence of flavonoids, tannins and terpenes, respectively. Such tests were described by Verde-Star et al. (2016).

3 RESULTS AND DISCUSSION

3.1 DERMATOPHYTE CLINICAL ISOLATES

In the present study, two dermatophyte isolates, *Trichophyton metagrophytes* and *T. tonsurans*, were isolated.

3.2 PHYTOCHEMICAL CHARACTERIZATION

Infrared analysis of the ethanolic extract of *E. tirucalli* showed the presence of phenolic compounds due to the presence of hydroxyl stretching which corresponds to the band present at 3,280.83 cm⁻¹ wavelength. On the other hand, colorimetric tests indicated the presence of flavonoids and tannins in the extract (Figure 1).

Figure 2. Infrared analysis of ethanolic extract of *E. tirucalli* showing the presence of hydroxyl groups, indicating the presence of polyphenols (flavonoids and tannins) in the extract.

3.3 ANTIFUNGAL ACTIVITY

Previous studies reported the resistance of dermatophyte strains againstazole-derived compounds (ketoconazole, clotrimazole, itraconazole, fluconazole, among others) due to their mechanisms of action consisting in the inhibition of ergosterol synthesis in the late phase; this resistance has also been reported against the allylamines (terbinafine) (Ghannoum 2016; Martínez-Rossi et al., 2008), which led to the increase in the number of cases of therapeutic failure; therefore, it is necessary to propose new alternative solutions to address this type of human conditions.
In the present study, the ethanolic extract of *E. tirucalli* was demonstrated to have moderate antifungal activity against the two clinical isolates of *T. mentagrophytes* and *T. tonsurans* with MIC of 125 and 250 g/mL. This biological activity may be attributed to the presence of polyphenols (tannins and flavonoids) and terpenes in the extract. Previous studies showed that the ethanolic extract obtained from *E. tirucalli* has antimicrobial activity against *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Candida tropicalis*, *C. albicans* and *Fusarium oxysporum* (Bhuvaneshwar Upadhyay, 2010).

Although the infrared analysis indicated the presence of the hydroxyl group, the presence of polyphenols in the extract was verified by the Liebermann-Burchard and ferric chloride color tests to evidence the presence of flavonoids and tannins, respectively. Since polyphenols have been reported as potential antimicrobials, their presence in this extract could support the antifungal activity of *E. tirucalli* extract. On the other hand, it was proved that tannins from the aerial part of *E. tirucalli* have antimicrobial activity, but they can also support collagen synthesis where tannins present in ethanolic extract can be considered as potential drugs for topical application.

4 CONCLUSION

In the present study, the antifungal potential of the ethanolic extract of *E. tirucalli* against clinical isolates of *T. mentagrophytes* and *T. tonsurans* was demonstrated. These biological activities were related to the presence of phenolic phytoconstituents, specifically flavonoids and tannins; therefore, this extract could be considered potential for treatment and/or as an adjuvant in diseases caused by dermatophytes.
REFERENCES


