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Original Article

In vitro antifungal activity of polymeric nanoparticles loaded with *Euphorbia tirucalli* extract

Atividade antifúngica *in vitro* de nanopartículas poliméricas carregadas com extrato de *Euphorbia tirucalli*

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Abstract

The therapeutic potential of medicinal plants is known as an alternative in treatment of human affections; in effect, the conventional application of these medicinal sources has several limitations like low bioavailability, solubility and stability, which affect its pharmacological efficacy. In recent decades, extraordinary advances have been made in new drug delivery systems using nanocarriers. This work consisted in determining the *in vitro* antifungal activity of the methanolic extract of *Euphorbia tirucalli* formulated in polymeric nanoparticles. The antifungal activity was determined by the microdilution method in 96-well microplates, applying nanoparticles loaded with plant extract (NP-Ext) obtained by nanoprecipitation on clinical isolates of *Trichophyton rubrum* and *T. interdigitalis*. Regarding the nanoparticles, the lots used did not present significant differences in their physicochemical characteristics, with a size of 91.885 ± 1.621nm, polydispersity index of 0.152 ± 0.025 and Z-potential of -6.047 ± 0.987. The quantification of the extract in the polymeric matrix was determined by infrared spectroscopy (FTIR), where an efficiency and encapsulation percentage of 22.15 ± 0.82 and 2.95 ± 0.11, respectively, were obtained. The *in vitro* antifungal activity of the crude and formulated extract was obtained calculating the Minimum Inhibitory Concentration (MIC) of each one; a MIC of 125 µg/mL was obtained against *T. rubrum* and *T. interdigitalis* with the crude extract, while a MIC value of 55.55 and 0.1 µg/mL was obtained with NP-Ext, respectively, against these same. Conclusions: biological activity is closely linked to the phytochemical profile of the extract; while the improvement of said potential with the NP-Ext with the dosage form was directly related to the physicochemical characteristics of the nanocarrier.

Keywords: dermatophytes, eudragit, anti-dermatophytic, nanoformulated.

Resumo

O potencial terapêutico das plantas medicinais é conhecido como alternativa para o tratamento das afecções humanas. Com efeito, a aplicação convencional dessas fontes medicinais apresenta diversas limitações, como baixa biodisponibilidade, solubilidade e estabilidade, que afetam sua eficácia farmacológica. Nas últimas décadas, avanços extraordinários foram feitos em novos sistemas de liberação de fármacos usando nanocarreadores. Este trabalho consistiu na determinação da atividade antifúngica in vitro do extrato metanólico de *Euphorbia tirucalli,* formulado em nanopartículas poliméricas. A atividade antifúngica foi determinada pelo método de microdiluição em microplacas de 96 poços, aplicando-se nanopartículas carregadas com extrato vegetal (NP-Ext) obtido por nanoprecipitação sobre isolados clínicos de *Trichophyton rubrum* e *Trichophyton interdigitalis*. Em relação às nanopartículas, os lotes utilizados não apresentaram diferenças significativas em suas características físico-químicas, com tamanho de 91,885 ± 1,621 nm, índice de polidispersão de 0,152 ± 0,025 e potencial Z de -6,047 ± 0,987. A quantificação do extrato na matriz polimérica foi determinada por espectroscopia de infravermelho (FT/IR), em que foram obtidas eficiência e porcentagem de encapsulamento de 22,15 ± 0,82 e 2,95 ± 0,11, respectivamente. A atividade antifúngica *in vitro* do extrato bruto e formulado foi calculada por meio da Concentração Inibitória Mínima (CIM) de cada um. Obteve-se uma CIM de 125 µg/mL contra *T. rubrum* e *T. interdigitalis* com o extrato bruto, enquanto o valor de CIM com NP-Ext contra estes mesmos fungos foi de, respectivamente, 55,55 e 0,1 µg/mL. Conclusões: a atividade biológica está intimamente ligada ao perfil fitoquímico do extrato, enquanto a melhora desse potencial com o NP-Ext com a forma farmacêutica esteve diretamente relacionada às características físico-químicas do nanocarreador.

Palavras-chave: dermatophytes, eudragit, antidermatófito, nanoformulado.

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1. Introduction

Dermatophytoses are the most common superficial mycoses in the world, representing 5 to 10% of all fungal infections at the topical level; it is reported that the most frequent etiological agents of this group of clinical manifestations are *Trichophyton rubrum* and *T. mentagrophytes* (Arenas, 2002; Pires et al., 2014). From 1958 to 2020, a limited number of antifungals (e.g., fluconazole, itraconazole, terbinafine, etc.) with therapeutic evidence against dermatophytes have been reported for the treatment of dermatophytosis; these limitations in the number of antifungals available led to their repetitive use, with low rotation. According to several authors, these conditions together induced the development of resistance of dermatophytes towards them. It is reported that the prevalence of resistance of dermatophytes towards azoles reaches 19% in some regions of the world against 1% of resistance against allylamines (i.e. terbinafine)(Ghannoum, 2016; Martinez-Rossi et al., 2008; Rudramurthy et al., 2018). Therefore, there is a growing interest in seeking alternative solutions such as those derived from medicinal plants.

Nowadays, an innumerable number of active ingredients derived from medicinal plants have been used in pharmacology (Joshi et al., 2018). Indeed, it is now known that medicinal plants have antibacterial, antidermatophytic and antifungal, anti-inflammatory, antioxidant and analgesic potential, among others (Andrea et al., 2016; Pava et al., 2017; Manso et al., 2022; Pérez-Narváez et al., 2019; Prabha et al., 2008; García-Hernández et al., 2016). Respect to *Euphorbia tirucalli*, it is a plant widely used in traditional African, Brazilian and Indian medicine, to treat tumors and excrescences on the skin (whole plant, latex), among others (Goutam et al., 2017); colloquially known as a miracle plant, previous studies have proven the biological potential of this plant and support that the methanolic and chloroform extracts of the stems of aerial parts of *E. tirucalli* have antimicrobial activity (Goutam et al., 2017; Parekh and Chanda, 2008). Additionally, a previous study confirmed its anti-dermatophyte potential (Heya et al., 2022), in México, this traditional use is not known, uses are only as ornament plant. Considering this, it is evident that secondary metabolites derived from *E. tirucalli* are promising agents for treating human affections caused by microorganisms, particularly by dermatophytes.

Due to the popularity of the phytoconstituents of medicinally important plants, a growing interest is being generated in seeking efficient and friendly ways for their application in humans, animals, among others. It is worth noting that the forms of application of assets derived from these medicinal sources are commonly done traditionally or conventionally (infusion, via organic solvents, gels, lotions, and creams, among others) (Hamishehkar et al., 2013). However, recent studies report extensive disadvantages of these forms of drug administration, including low bioavailability, absorption and stability of the active ingredient, which commonly leads to pharmacological failure due to the change in the desired therapeutic concentration and the appearance of adverse effects. On the other hand, the latest advances in the development of drug delivery systems using nanocarriers have improved therapies based on the use of green active compounds. According to Hamishehkar and his collaborators (Hamishehkar et al., 2013), the key to topical administration lies in preventing or reducing diffusion of the drug into the systemic circulation and targeting the drug to a specific layer of the skin. Therefore, it is necessary to develop more effective dosage forms from nanoparticles to improve the delivery system of the active compounds, and thus limit therapeutic failures, adverse effects, and drug invasion in unwanted sites (Kaur and Kakkar, 2010).

Eudragit polymers are widely used in the pharmaceutical industry for the development of drug delivery systems. They are widely known due to their versatility in terms of chemical composition and solubility (Kaur and Kakkar, 2010). In particular, Eudragit S100 is an anionic copolymer based on methacrylic acid and methyl methacrylate; in the framework of the present study, said polymer was selected for the encapsulation of *E. tirucalli* extract due to its desirable attributes, such as pH sensitivity, as to say, the absence of release of the drug trapped in the polymer matrix at pH of healthy skin (4.5 - 5.5). In this way, it imparts a specific nature to the fungal infections caused by dermatophytes, since the latter basicize the area of infection. Following this same order of ideas, it is reported that Eudragit S100 solubilizes or releases its content at pH ≥ 6, that is, it has a pH-dependent controlled release (Kaur and Kakkar, 2010). What turns said polymer into the suitable candidate for the dosage of the extract of interest.

Based on the above, the objective of this work was to determine the antifungal activity of the methanolic extract of *Euphorbia tirucalli* formulated in polymeric nanoparticles on clinical isolates of dermatophytes.

2. Materials and Methods

2.1. Collection of plant material and obtaining the methanolic extract of E. tirucalli.

E. tirucalli was collected in the municipality of San Nicolás de los Garza, in the state of Nuevo León, Mexico, according with the current Official Mexican Standard (NOM-126-ECOL-2000, México (2000)), which establishes harvest specifications for biological materials from wild flora and fauna species and other biological resources; therefore, only the branches of said plant were collected in such a way as not to compromise its growth and/or development; accordingly, the Species Risk and Protection Standard (NOM-059-SEMARNAT-2010, México (2010)) was complied with. Subsequently, said plant was taxonomically classified by Dr. Marco Antonio Guzmán Lucio, professor of the Department of Botany of the Faculty of Biological Sciences of the Autonomous University of Nuevo León and was registered in the herbarium on folio: 029755.

2.1.1. Obtaining the extract

Initially, the biological material was deeply washed with distilled water and dried at 40°C with a 150-watt white light lamp for five days; later, it was ground by a manual crusher and conserved at 28 °C until use.

To obtain the methanolic extract of *E. tirucalli,* 40 g of plant material was ground and consecutively washed with solvents of different polarities in Soxhlet equipment using hexane, chloroform, ethanol and methanol (CTR Scientifics). Subsequently, the methanolic extract obtained was concentrated under reduced pressure (Yamato model RE200 rotary evaporator) and finally dried in an oven at a temperature below 40 °C. Once free of solvents, the extract was stored at environmental temperature until use.

2.1.2. Phytochemical characterization.

It was performed using the methods described by Verde-Star et al. (2016).

2.2. Synthesis of polymeric nanoparticles.

2.2.1. Reagents

Eudragit S100 was used as the NP forming polymer, methanolic extract of *E. tirucalli* as the active agent, analytical grade methanol as solvent (Sigma-Aldrich), 1% tween 80 as surfactant.

2.2.2. Preparation of NP-Ext

The NP-Ext were prepared by the nanoprecipitation method of Armendáriz-Barragán et al., 2016 (Armendáriz-Barragán et al., 2016). Initially, the two synthesis phases were prepared, an organic phase (OP) composed of a mixture of solvent (5 mL), Eudragit S100 (15 mg) and methanolic extract of *E. tirucalli* (10 mg), and an aqueous phase (AP) composed of 1% tween 80 (10 mL). Then, for the preparation of the NP, the OP was injected into the AP beforehand under constant magnetic agitation, which induced the aggregation of the polymer, thus trapping the active ingredient. Subsequently, the suspension of NP-Ext dispersed in the AP was subjected to evaporation at reduced pressure in a rotary evaporator to remove the solvent used in the OP.

2.2.3. Physical characterization of the NP-Ext

The physical characteristics of the NP-Ext dispersion were obtained by photon correlation spectroscopy, in terms of size and polydispersity index (PDI); the Z-potential was determined by Doopler laser microelectrophoresis in a Zetasizer Nano-ZS90 (Malvern Instruments, USA).

2.2.4. Obtaining NP-Ext tablets

The NP-Ext and NP-blank tablets were obtained by ultracentrifugation of each dispersion at 25,000 rpm for 150 min; after removing the supernatant, the tablets were stored at environmental temperature until dry.

2.2.5. Distribution of assets in the NP-Ext

The type of dispersion of the extract in the NP-Ext was determined qualitatively using the FTIR method, taking the total transmittance of the NP-Ext and NP-blank tablets, to identify the spectroscopic signals corresponding to the extract (dispersion of the active ingredient to the surface of NP-Ext) or its absence (distribution of the extract within the polymeric matrix).

2.3. Quantification of the extract encapsulated in NP by FTIR spectroscopy

The quantification of the extract of *E. tirucalli* encapsulated in the polymeric nanoparticles was carried out by the FTIR method in an FT-IR Bruker IFS 66 with spectrum digitalization, that allows obtaining electronic files of the analyses. This method was carried out in the following stages:

2.3.1. Selectivity

This stage consisted of taking the transmittance of the total extract and Eudragit S100, to identify the functional groups that are present in the extract and absent in the forming polymer.

2.3.2. Linearity

Linearity studies were performed based on the Lambert-Beer law, which stipulates that the absorbance intensity of a substance is proportional to its concentration. Therefore, the absorbance of the methanolic extract was taken at different concentrations (100-3500 µg/mL) to determine the range of linearity of absorbance vs concentration. FTIR were recorded in the wavelength range between 4,000-400 cm^{-1}, at a resolution of 4 cm^{-1} and a sample analysis time corresponding to 64 scans per spectrum. The area under the curve was used as a quantitative variable to obtain the calibration curve.

2.3.3. Precision and exactness

In this stage, the IR analysis of the limits of the calibration curve was performed. Indeed, 6 standard solutions of each limit (100 and 3,000 µg/mL) and the theoretical concentration of extract in NP-Ext (1,000 µg/mL) were analyzed to define the final concentration in the aqueous phase after the synthesis of the nanoparticles.

2.3.4. Quantification of the extract incorporated in the nanoparticles

The five lots of NP-Ext were solubilized in 0.5 mL of methanol and analyzed by infrared spectra under the same equipment conditions described above. Finally, the encapsulation efficiency (%EE: Formula 1) and the encapsulation percentage (%E: Formula 2) were determined based on the following formulas after a numerical materialization of the previously selected spectrometric stretching.

$$\%EE = \frac{\mu g \text{ of extract in } NP - Ext \text{ lot}}{2*\mu g \text{ of extract in the OF}} *100 \quad (1)$$

$$\%E = \frac{\mu g \text{ of extract quantified in NP}}{\mu g \text{ of polymer employed}} *100 \quad (2)$$

2.4. Antifungal activity

2.4.1. Preparation of inoculate

Fungal colonies (*T. rubrum* and *T. interdigitalis*) previously inoculated in potato dextrose agar (PDA) medium

and incubated for 21 d at 28°C were covered with 10 mL of sterile distilled water to obtain a concentrate dispersion of conidia after a smooth scraping with a sterile loop and subsequent filtration to free them from hyphae and agar particles. Before carrying out the biological tests, the conidia suspensions were adjusted to a concentration of 3 x 10^4 CFU/mL in a hemacytometer (*Clinical for Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard- Second Edition. Document M38-A2. Wayne, Pa: Clinical and Laboratory Standards Institute* - CLSI (2008)).

2.4.2. Biological test

In vitro antifungal susceptibility was determined by a modification of the microdilution method described in the M38-A protocol by the Clinical and Laboratory Standards Institute (CLSI) (*Clinical for Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard- Second Edition. Document M38-A2. Wayne, Pa: Clinical and Laboratory Standards Institute* - CLSI (2008)). Therefore, from the NP-Ext lots, serial dilutions were made in 96-well flat-bottom microplates, using Müeller-Hinton broth (Sigma-Aldrich) as the diluent and the phenol red as a pH indicator; then, 100 µL of adjusted conidia dispersion were added. Clotrimazole (Sigma-Aldrich) was used as a positive control. As NP-blank, the mixture of NP-Ext with medium, without inoculum, and, as negative control, a lot of NP with inoculum. Regarding the growth control, 100 µL of each strain was inoculated in 100 µL of the medium. The mixtures obtained were incubated at 28°C for 120 h.

The antifungal activity was determined by the Minimum Inhibitory Concentration (MIC), defined according to the CLSI as that concentration capable of inhibiting the growth of the fungus (%H: Formula 3) by 80% compared to the growth control and the Minimum Fungicide Concentration (concentration capable of inhibiting 100% of the growth of the microorganism).

$$\%H = \left[\frac{\text{Control} - (\text{Tratment} - \text{Blank})}{\text{Control}}\right] * 100 \quad (3)$$

2.5. Statistics

The numerical valorization of the infrared spectrograms was carried out with the Origin.Lab program. The statistical analysis of the physical characteristics of the NP-Ext was performed using the SPSS V 20® software, with a confidence level of 95% ($\alpha = 0.05$).

3. Results and Discussion

3.1. Characterization of extract and polymeric nanoparticles

Phytochemical characterization. The methanolic extract of *E. tirucalli* exhibited phytoconstituents: flavonoids (tirucallin B, euphorbine A, euphorbine F), sterols (campesterol, stigmasterol, beta-sitosterol, isofucosterol, and cycloartenol), sesquiterpenes (tirucadalenone), and amino groups (García-Hernández et al., 2024).

Features of polymeric nanoparticles. Particle size is one of the crucial characteristics of nanocarrier systems, since it influences cellular uptake (Armendáriz-Barragán et al., 2016; Rafiee et al., 2019). In the present study, the NP-Ext dispersions obtained (5 lots) showed an average particle size of 91.885 ± 1.621 nm, while the NP without active, NP-blank showed a particle size of 53.6 ± 0.389 nm; said the difference in size could indicate a possible encapsulation of the extract in the polymer. On the other hand, PDI (polydispersibility index) average values of NP-Ext of 0.152 ± 0.025 were obtained, which is less than 0.2, indicating good homogeneity in the size distribution and dispersion of NP-Ext (Kesente et al., 2017).

Finally, the Z-potential (surface charge) of the NP-Ext, as well as its target, was determined. According to some authors, it provides information on the dispersion stability of nanoparticles (Kesente et al., 2017; Roussaki et al., 2014). In the present study, NP-Ext and NP presented a negative charge; according to Subudhi and his collaborators (Subudhi et al., 2015) charge is due to the free acrylic acid groups of Eudragit S100, as an anionic polymer. No statistically significant difference was obtained between the size, PDI and Z-potential of the five lots of NP-Ext ($p \leq 0.05$); which reveals homogeneity in the lots population of NP-Ext and reproducibility in the synthesis method employed.

Type of distribution of the extract in the NP-Ext. As seen in Figure 1, the NP-Ext and NP spectrograms behaved in the same form; so, the NP-Ext are a system of nanospheres with a dispersion of the active within the polymeric matrix.

3.2. Quantification of the methanolic extract of E. tirucalli encapsulated in NP-Ext by FTIR method

3.2.1. Study of equipment conditions

Instrumental parameters, such as the nominal resolution and the number of scans, are relevant to establish the best analytical conditions using the FTIR for quantitative purposes, since they influence the magnitude of the signals and the sample analysis time (Robaina et al., 2013). However, in this study, only the scan number was used as the analytical variant, considering that the equipment has a standard resolution of 4 cm^{-1} (FRONTIER FT-IR Spectrometer, Perkin-Elmer, USA), and the visual behavior of the IR spectrum

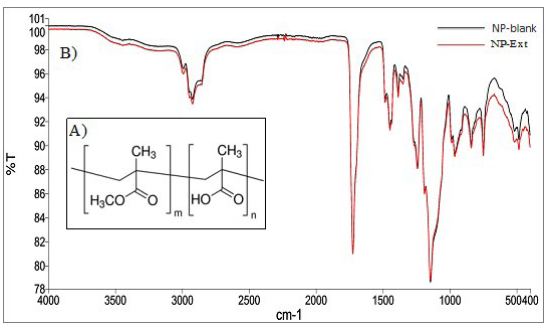

Figure 1. A) Chemical structure of Eudragit S100; B) FT-IR spectrograms of NP-Ext nanospheres and Eudragit S100 (NP-blank).

located between 1640-1560 cm^{-1} (1670-1550 cm^{-1}, as adjusted value), corresponding to the amino group (N-H).

3.2.2. Selectivity

In the first stage of the development of the analytical method, the total transmittance of the extract and the Eudragit S100 were taken (Figure 2). Therefore, the intensity band between 1640-1560 cm^{-1} was selected, which is attributed to the stretching of the amino group (N-H).

3.2.3. Linearity

To determine the range of linearity of absorbance versus concentration of the extract, solutions of different concentrations of extract dissolved in methanol were prepared and analyzed by FTIR (Figure 3).

The linearity range of the amino group was determined between 1640 and 1560 cm^{-1} adjusted to 1670-1550 cm^{-1}, calculating the area under the curve. The results indicated a linearity range between 100-3000 µg/mL with an r^2 of 0.982 for the area under the curve (Figure 4).

3.2.4. Precision and accuracy

For precision and accuracy, the analysis of the FTIR spectrum of standardized solutions of the plant extract corresponding to the limits of the calibration curve (100, 1000 and 3000 µg/mL) was performed; then, the NP-Ext pills were treated (extraction of the active ingredient trapped in the NP) and analyzed by FTIR. Finally, the numerical evaluation of the infrared spectra was carried out (Table 1).

The relative standard deviation (RSD%) was determined to assess precision and reproducibility of the method used. The RSD% results indicate that the calibration curve was obtained using the area below the curve, with good precision and reproducibility; with values of -3.925, such result is very similar to the results obtained by Pandey et al., 2007 (Pandey, 2007). A value of 0.001 µg/mL was obtained in terms of detection limit, while 0.003 µg/mL as the quantification limit of the extract encapsulated; these results justify that the method of quantification using FTIR is reliable for quantification of nanoformulated extract in NP-Ext.

3.2.5. Encapsulation percentage

Among the important parameters in determining the pharmacological effectiveness of nanoformulations is the amount of active ingredient trapped in the nanocarrier; in this work, this parameter was determined by the FTIR method. Indeed, five lots of NP-Ext prepared in the same condition were used, based on a theoretical concentration of extract equal to 1,000 µg/mL. The results obtained are listed in Table 2, using the area under the curve of the spectrogram band corresponding to the amino group. Encapsulation efficiencies of 22.15 ± 0.82% were obtained. This value is similar to that reported for other biodegradable NP formulations using the same polymer with others with plant extracts and evaluated under HPLC (El-Maghawry et al., 2020; Ramzy et al., 2020; Tuncay Tanriverdi and Algin, 2017. Regarding the percentage of encapsulation (%E), considerably low values were obtained (2.95 ± 0.11); this result could be related in the first stay to the size of the NP-Ext, given that a reduced size could imply a lower load capacity of the nanoparticles. Although methanol (a highly hydrophilic solvent) has been used as a dissolution solvent in the organic phase, previous studies report the difficulty that plant extracts have in dissolving completely in a single solvent, due to the variety of compounds they contain (i.e. terpenes, sterols, flavonoids, sesquiterpenes); this limitation could highly affect the incorporation of this type of asset in the NP system (Nguyen et al., 2014).

3.3. In vitro antifungal activity of extract formulated in polymeric nanoparticles

The *in vitro* antifungal activity was performed by determining the MIC and the CMF applying the NP-Ext

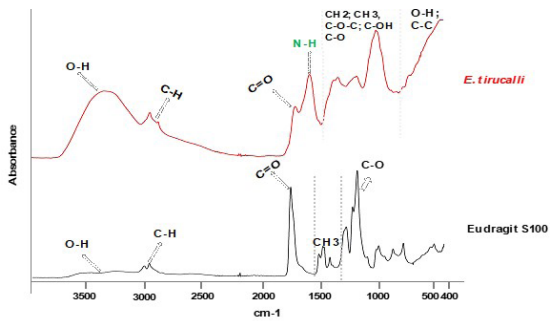

Figure 2. Comparative infrared spectrum of the extract and eudragit S100.

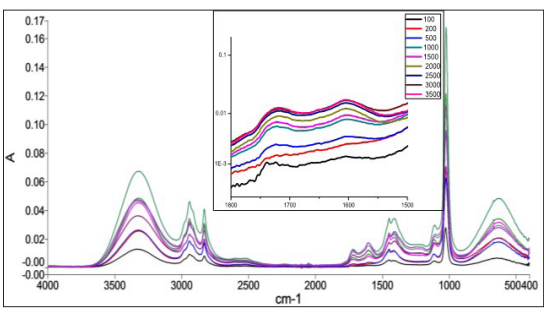

Figure 3. IR spectrogram of solutions with extract at different concentrations.

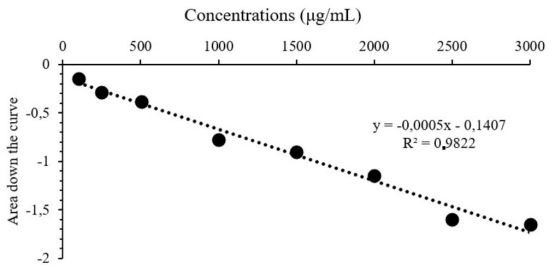

Figure 4. Range of linearity of the concentration vs area under the curve.

Table 1. Numerical valorization of infrared spectra for quantification of extract formulated in NP.

Active	Measuring method	Band	Correction interval	Linearity[a]	R^2	RSD[b] (%)	LoD[c] (µg/mL)	LoQ[d] (µg/mL)
Extract	Area under curve	1640-1560	1670-1550	100-3000	0.982	3.925	0.001	0.003

[a] Concentration range (µg/mL) in which it was obtained R^2. [b] The RSD was obtained from the area under the curve of the theoretical concentration of the formulated extract (1000 µg/mL). [c] Detection limit. [d] Limit of quantification. $\boldsymbol{LoD} = 3.3*\frac{\sigma}{b}$ $\boldsymbol{LoQ} = 10*\frac{\sigma}{b}$
Note: $\boldsymbol{b}$ is the slope.

Table 2. Percentage and efficiency of extract encapsulation in NP-Ext.

Measuring method	interval	Correction interval	Lots	CO[a]	CT[b]	%EE*	%E**
Area under the curve	1670-1620	1670-1550	n=5	442.9±16.5	1000	22.15±0.82	2.95±0.11

[a] Concentration value of encapsulated extract expressed in µg/mL. [b] Theoretical concentration in µg/mL. %EE* (encapsulation efficiency): is the amount of the compound that was loaded, related to the initial concentration used. %E** (percentage of encapsulation): is the amount of encapsulated compound compared to the amount of polymer used.

Table 3. Anti-dermatophytic activity of crude and formulated extract of *E. tirucalli* against clinical isolates of dermatophytes.

Dermatophyte strain	Methanolic extract		NP-Ext		Clotrimazole	
	100%	80%	100%	80%	100%	80%
T. rubrum	500	125	250	55.55	0.4	0.1
T. interdigitalis	250	125	71.43	0.1	0.1	< 0.0004

n= 3. Results expressed in µg/mL

and the crude extract on clinical isolates of *T. rubrum* and *T. interdigitalis*. A MIC of 55.55 and 0.1 µg/mL was obtained with the NP-Ext, while it was 125 µg/mL with the crude extract against *T. rubrum* and *T. interdigitalis*, respectively (Table 3). For the first time, the biological activity of the crude extract could be related to its phytochemical profile; since previous studies have reported that the antimicrobial activity of plant extracts is directly related to their phytoconstituents (Le et al., 2021; Olea et al., 2019; Popova et al., 2009; Veloza et al., 2014). However, the improvement in antidermatophytic activity with NP-Ext could be related to its physicochemical properties; The average size of the NP-Ext ranges between 91.885 ± 1.621 nm; this small size provides the NP-Ext with a larger contact surface with the microorganism, where the improvement of the pharmacological potential with the NP-Ext compared to the crude extract (Roduner, 2014). According to Roduner and his collaborators smaller nanoparticles have a larger surface-volume; so, the number of particles per unit mass increases compared to diameter of particles, where a significant change in pharmacological behavior. So a larger contact surface leads to greater pharmacological reactivity or effectiveness (Roduner, 2014). Also, it has been verified that nanoparticles with a size less than or equal to 200 nm accumulate easily in the intracellular medium, since they are easily absorbed (passive absorption); which increases the concentration of nanoparticles in the cell cytoplasm, and consequently increases the toxicity and pharmacological efficacy of the encapsulated active (Roduner, 2014). In addition to the aforementioned, nanoparticles with this size (≤ 200 nm) have a longer circulation or lifetime, which increases the bioavailability of the active ingredient in the desired site.

Another factor that actively influences the efficiency of the bioabsorption of nanoformulated drugs is the electrostatic charge. The surface charge of the synthesized NP-Ext is negative. This charge, in addition to reflecting the stability of the NP-Ext due to the homogeneity of Z-potential in the lots of NP-Ext, allows a greater electrostatic attraction with the cell membrane of the microorganisms; this interaction could constitute a crucial point in the antifungal activity, since it potentiates the pharmaceutical absorption of the extract (electrostatic absorption), thus increasing its bioavailability in the intracellular medium, which increases the toxicity of the active ingredient (Kesente et al., 2017; Zillich et al., 2015). In similar studies, it has been found that plant extracts formulated in polymeric NPs with a negative charge allowed the penetration of the NPs and a controlled release in the skin. Other studies confirmed that negatively charged NPs potentiate the pharmaceutical activity of plant extracts on microorganisms that affect the skin (Krausz et al., 2015; Tamargo et al., 2017). Finally, Feng et al., 2015, found that negatively charged NPs have a lower affinity with the plasma membrane of eukaryotic cells compared to positively charged NPs; which greatly boosts its pharmaco-targeting and selectivity (Feng et al., 2015).

On the other hand, the NP-Ext obtained in the present study, being colloidal in nature with a dispersion of

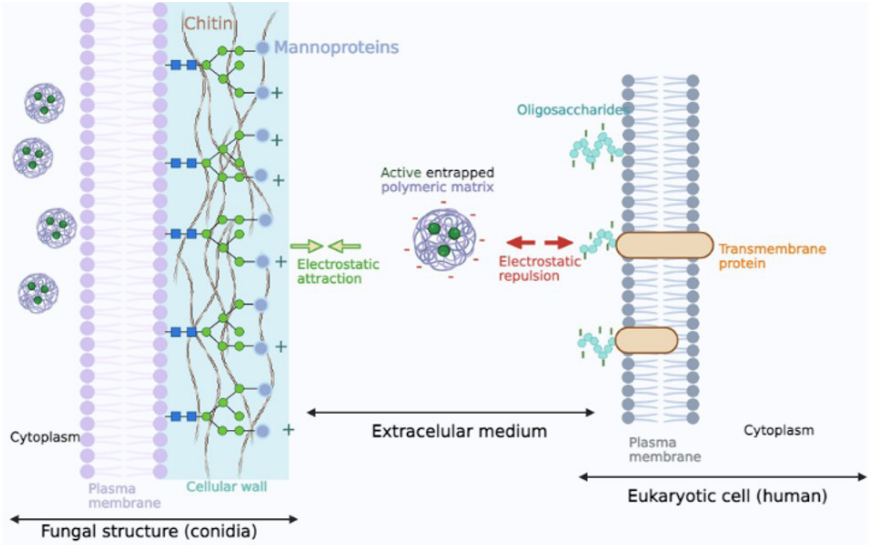

Figure 5. Pharmacological selectivity induced by the electrostatic charge of the nanoparticles.

the active within the polymeric matrix; in addition to having a negative charge, could have greater selectivity in biological systems and a more targeted destination (Figure 5), which would considerably limit the possible adverse effects of the active ingredient (Armendáriz-Barragán et al., 2016).

Another factor that could justify the improvement of NP-Ext over the raw partition of *E. tirucalli* is the nature of the solvent used in the biological test, which can considerably affect the hydration and interaction of the dosage form and the microorganism. According to Upadhyay and his collaborators, aqueous dosage forms facilitate drug absorption compared to other application forms, such as organic solvents, etc. (Upadhyay et al., 2019; Akhtar et al., 2013).

4. Conclusion

It was found that the methanolic extract of *E. tirucalli* has *in vitro* antifungal activity against clinical isolates of *T. rubrum* and *T. interdigitalis*, due to its secondary metabolites, which can be considered promising antimicrobial agents for the treatment or coadjuvant in the control of dermatophytosis infections. On the other hand, the formulation of said extract in polymeric nanoparticles led to the improvement of its antifungal potential against the strains of interest; this improvement was related to the physicochemical characteristics of the NP-Ext like its size, which provides an elderly contact surface with the microorganism, the Z-potential, which, in addition to allowing pharmacological selectivity, allows electrostatic attraction, and finally, the amount of active ingredient trapped in the NP-Ext that is responsible for the therapeutic efficacy. Then, nanocarrier systems can be considered as a promising dosage form for the possible application of active ingredients of plants in human infections caused by microorganisms.

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