International Journal of Agriculture and Biosciences 2026 15(4): 1455-1464.
https://doi.org/10.47278/journal.ijab/2025.061

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

In vitro Evaluation of the Antifungal Activity of *Vismia baccifera* (L.) Extract against the Dermatophyte *Nannizzia gypsea*

Carolina Argumedo Montes [1], Andrés José Betin Ruiz [1]*, Jorge Enrrique Arias Ríos [1], Luis Eliécer Oviedo Zumaqué [1], Dairo Enrrique Pérez Sotelo [2] and Luisa Fernanda Oviedo Paternina [1]

[1]Biotechnology Group of the Department of Chemistry and Department of Biology (GRUBIODEQ); University of Córdoba; Monteria, Córdoba-Colombia
[2]Research Group on Physical Chemistry of Liquid Mixtures; University of Córdoba; Montería, Córdoba-Colombia
*Corresponding author: abetinruiz@correo.unicordoba.edu.co

ABSTRACT

Dermatophytosis, commonly known as ringworm, is one of the most prevalent and recurrent skin infections worldwide. This superficial mycosis is caused by keratinolytic fungi belonging to the genera *Trichophyton*, *Microsporum*, *Epidermophyton*, *Arthroderma*, *Paraphyton*, *Lophophyton*, and *Nannizzia*, which invade keratin-rich tissues such as skin, hair, and nails. The aim of this study was to evaluate *in vitro* the antifungal activity of the ethanolic extract of *Vismia baccifera* (L.) against the dermatophyte *Nannizzia gypsea*. The strain was characterized phenotypically and genotypically through morphological analyses, PCR amplification of the ITS region, and phylogenetic analysis using BLAST and MEGA11. The plant extract was obtained by maceration with 96% ethanol and analyzed by gas chromatography–mass spectrometry (GC–MS). Antifungal activity was assessed using the agar diffusion method and double serial dilution assays, with clotrimazole as a positive control. Sequencing of the ITS region confirmed the strain's identity as *N. gypsea*. Inhibition zones recorded for extract concentrations of 250, 500, and 750mgL^{-1} ranged from 12.63±0.4 to 13.17±0.38mm. The minimum inhibitory concentration (MIC) was 156.25µgmL^{-1}. The results indicate that the ethanolic extract of *Vismia baccifera* exhibits *in vitro* antifungal activity against *N. gypsea*, suggesting its potential as a source of bioactive compounds for the treatment of dermatophytosis.

Keywords: Dermatophytosis, Minimum Inhibitory Concentration, Tinea.

Article History
Article # 25-713
Received: 06-Nov-25
Revised: 05-Jan-26
Accepted: 11-Jan-26
Online First: 13-Mar-26

INTRODUCTION

Dermatophyte, popularly known as tinea or ringworm, is ranked as one of the most prevalent, recurrent, and problematic infections worldwide. This disease is caused by several species of filamentous fungi belonging to the genera *Trichophyton, Microsporum, Epidermophyton, Arthroderma, Paraphyton, Lophophyton,* and *Nannizzia,* which are classified as keratinolytic fungi and are largely responsible for superficial mycoses by invading keratin-containing tissues such as the nails, hair, and skin (Cruz & Vielle, 2024). Dermatophytoses affect both animals and humans, particularly immunocompetent and immunosuppressed individuals. In the latter group, the infection can cause more severe complications (Sánchez Espinosa et al., 2022).

The World Health Organization (WHO) estimates that between 20 and 25% of the world population suffers from some type of superficial mycosis, with dermatophyte infections being the most common, accounting for approximately 70 to 80% of reported cases (Sacheli & Hayette, 2021). The broad geographic distribution of these infections is related not only to anthropological factors—such as cultural practices, population mobility, and overcrowding—which facilitate pathogen transmission, but also to environmental and biological elements that favor host colonization. These elements include high humidity and temperature, poor personal hygiene, and individual predisposition due to immunological or cutaneous barrier factors (Sánchez Espinosa et al., 2022).

Cite this Article as: Montes CA, Ruiz AJB, Ríos JEA, Zumaqué LEO, Sotelo DEP and Paternina LFO, 2026. *In vitro* evaluation of the antifungal activity of *Vismia baccifera* (L.) extract against the dermatophyte *Nannizzia gypsea*. International Journal of Agriculture and Biosciences 15(4): 1455-1464. https://doi.org/10.47278/journal.ijab/2025.061

A Publication of Unique Scientific Publishers

The species *Nannizzia gypsea*, formerly classified as *Microsporum gypseum*, is a geophilic dermatophyte of medical and veterinary significance due to its capacity to cause infections in both humans and various animal species, including dogs and cats. The primary means of transmission is through the soil, which serves as a vital reservoir for this fungus (Conceição et al., 2024). The clinical lesions caused include alopecia, resulting from the production of proteolytic and keratinolytic enzymes that degrade keratin, facilitating invasion and persistence in the *stratum corneum* of the skin (Orozco-Yee et al., 2025). Despite its prevalence and the discomfort, it causes, the treatment of dermatophytosis remains a clinical challenge; current therapeutic options, which include topical and systemic antifungals, have significant limitations, such as fungal resistance and adverse side effects (Sacheli and Hayette, 2021). Given the sustained increase of skin infections and the advent of antimicrobial-resistant strains, the World Health Organization has emphasized the need to prioritize the research and development of new antimicrobial therapies as a global public health priority (Mussin & Giusiano, 2024). In this scenario, dermatophytosis poses a mounting global health concern due to its high prevalence, recurrence, and the emergence of strains resistant to conventional treatments (Bristow & Joshi, 2023). In response to this problem, the exploration of naturally occurring compounds with antifungal activity has become a priority line of research. These secondary metabolites, particularly alkaloids, terpenoids, flavonoids, and phenolic compounds, offer alternative mechanisms of action to traditional chemical agents. This approach not only seeks to improve therapeutic efficacy but also to reduce adverse effects and contribute to the development of sustainable strategies for the control of fungal infections (Argüelles et al., 2024).

Medicinal plants constitute a relevant source of secondary metabolites with therapeutic potential, including alkaloids, flavonoids, terpenoids, and phenolic compounds. A considerable proportion of current pharmaceuticals derive from these metabolites, which highlights their value in traditional and modern medicine (Chaachouay & Zidane, 2024). These compounds have shown vasodilatory, antimicrobial, anti-inflammatory, antipyretic, and neuromodulatory properties associated with specific mechanisms of action on cellular receptors and enzymes (Elshafie et al., 2023). The scientific interest is focused on the identification of plant-derived bioactive molecules as safer and more sustainable alternatives to synthetic drugs, which often present adverse effects in long-term treatments (Lizcano et al., 2012).

Colombia is home to one of the greatest plant biodiversities around the world, constituting a valuable source of natural products with applications in the treatment of diseases and a strategic reservoir of secondary metabolites with therapeutic potential (Lizcano et al., 2014). Among this biodiversity is *Vismia baccifera*, a tropical species belonging to the Hypericaceae family, which is primarily distributed in the Amazon rainforest. In traditional medicine, this plant has been utilized in macerations and decoctions of bark, leaves, stems, and roots for the treatment of various conditions (Trepiana et al., 2018). In relation to its biological properties, multiple studies have been carried out on both extracts and pure isolated compounds, and the species has been recognized as a potential source of bioactive compounds with antifungal activity, in addition to exhibiting antibacterial, antioxidant, and antitumor properties (Buitrago-Díaz et al., 2020). Previous research has reported 161 compounds isolated from different *Vismia* species, including a wide array of chemical categories such as monoterpenes, sesquiterpenes, triterpenoids, prenylated anthrones, lignans, sterols, flavonoids, flavonols, anthraones, anthraquinones, bianthraquinones, benzophenones, steroids, and xanthones (Rojas-Vera et al., 2020; Buitrago-Díaz et al., 2020). From an ethnobotanical perspective, extracts from the leaves and bark of *V. baccifera* have traditionally been used in the treatment of skin conditions, supporting their potential application against dermatophyte infections. However, research focused on this specific activity remains limited, which highlights the relevance of the present study, focused on evaluating the antifungal effect of *V. baccifera* leaf extract against *Nannizzia gypsea*, the etiological agent of dermatophytosis in humans and animals.

MATERIALS & METHODS

Study Area

The study was carried out in the facilities of the biotechnology laboratory GRUBIODEQ and at the laboratory of natural products of the University of Córdoba (8°47′037″ N; 75°50′51″ W, 15 msnm), located in Montería, Córdoba, Colombia. The area is prevailed by a bimodal rainfall regime with an annual average of 1346 mm, a relative humidity of 84%, and an average annual temperature of 27.4°C (Betin-Ruiz et al., 2025).

Phenotypic and Genotypic Characterization of a Strain of *Nannizzia gypsea* using Morphological Analysis and Molecular Techniques

Phenotypic Characterization

The *Nannizzia gypsea* strain was provided by the Department of Bacteriology at the University of Córdoba. The isolate was cultured on Sabouraud chloramphenicol agar (soy peptone 10.0g/L, dextrose 10g/L, agar 15.5g/L) (Merck, Germany), supplemented with 0.105g/L, and incubated at 28°C for seven days or until visible fungal growth was observed. Subsequently, the colonies morphological characteristics, including color, surface appearance, and pigmentation, were observed (Pérez-Rodríguez et al., 2023). Micromorphological features were analyzed using the lactophenol cotton blue staining technique (Joseph et al., 2024). The microscopic characteristics of the isolates were recorded using a digital camera.

Genotypic Characterization

DNA Extraction

Fungal biomass was obtained from cultures grown on Sabouraud agar supplemented with chloramphenicol and incubated at 28°C for seven days to obtain a fresh culture. The biomass was harvested directly from the agar plates, frozen, and ground with liquid nitrogen. Genomic DNA

was then extracted using the commercial GeneJET™ kit (Thermo Fisher Scientific Inc., USA), in strict accordance with the manufacturer's instructions.

Genomic DNA Amplification

Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) region using universal primers ITS4: 5'-TCCTCCGCTTATTGATATGC-3' and ITS3: 5'-GCATCGATGAAGAACGCAGC-3' (Sigma-Aldrich, Darmstadt, Germany). These primers amplify the ITS genetic region of fungal ribosomal DNA (Yu et al., 2022). The total reaction volume was 50µL, containing 25µL of DreamTaq Hot Start PCR Master Mix (2X) (Thermo Fisher Scientific), 1µL of each 10mM primer, 2µL of DNA (~157ng/µL), and sufficient molecular grade water to reach the desired volume. A BioRad T100 Thermocycler (BioRad Laboratories) was used under the following conditions: an initial denaturation cycle (5min at 95°C), followed by 30 cycles of denaturation (30s at 95°C), annealing (40s at 60°C), extension (1min at 72°C), and a final extension step at 72°C for 10minutes. Amplification was confirmed by 2% agarose gel electrophoresis using a horizontal electrophoresis chamber. The DNA fragments were visually analyzed using a UV transilluminator (Enduro GDS Labnet, Tewksbury, MA, USA). The fragment was purified and sequenced by the Sequencing and Molecular Analysis Service (SSiGMol) of the Institute of Genetics at the National University of Colombia.

Phylogenetic Analysis

The AB1 format sequences were read using 4Peaks v 1.8 software, which is linked to the BLAST bioinformatics tool to compare each query sequence with those in the NCBI (National Center for Biotechnology Information) GenBank database. Species assignment of the dermatophyte fungus strain was confirmed by multiple sequence alignment against standardized genomic sequences (refseq genomes) from the GenBank database. MEGA11 software (Tamura et al., 2021) was used to perform a multiple alignment with the ClustalW algorithm to construct a phylogenetic tree using the Maximum Likelihood method with 1000 bootstrap replicates, the Tamura-Nei substitution model, and automatic initial tree construction based on a maximum parsimony tree.

Evaluation of the Phytochemical Composition of *Vismia baccifera* (L.) Extract using Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

Collection of Plant Material

The plant material was gathered in the municipality of Puerto Libertador, department of Córdoba, at an altitude of 60 meters above sea level (7°53′17″ N, 75°40′18″ W) (Reina et al., 2024). The fresh material was cleaned to remove extraneous debris and dust and subsequently transported to the Natural Products Laboratory at the University of Córdoba for further processing.

Preparation of the Extract by Maceration with Ethanol

The leaves were fragmented and dried for two days in a hot air oven set at 55°C. They were then ground to a fine powder (Sasidharan et al., 2024). Subsequently, 200g of the powder was mixed with two liters of 96% ethanol (v/v) (a 1g:10mL ratio). The sample was then macerated for seven days at 25°C in the dark. The mixture was then filtered and concentrated by evaporating the solvent under reduced pressure at 40°C using a rotary evaporator (Metoui et al., 2022). The resulting extracts were stored at 4°C in an airtight amber bottle, protected from light and oxygen, until further analysis (Lfitat et al., 2021).

Phytochemical Analysis of the Extract using Gas Chromatography-mass Spectrometry (GC-MS)

The dry extract was dissolved in chloroform for purification. The solution was subsequently filtered using a CHROMAFIL Xtra cartridge with a 0.45µm pore size (Macherey-Nagel, Düren, Germany), ensuring its cleanliness prior to analysis. GC-MS characterization was performed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass spectrometry detector (Agilent Technologies, Ontario, CA, USA). The compounds in the *V. baccifera* extract were identified using 70eV electron ionization mass spectrometry, with a detection range of 50–550m/z and high-purity methane as the reagent gas. The ion source temperature was maintained at 230°C. Chromatographic separations was achieved using a DB1-MS nonpolar capillary (30m×0.25mm×0.25µm), composed of 100% dimethylpolysiloxane (Agilent Technologies, Ontario, CA, USA). The analysis was performed by injecting 1 µL of sample in splitless mode, with the injector set at 225°C. The initial oven temperature program commenced at 100°C, held for 4 minutes, followed by a ramp of 5°C min^{-1} to 193°C, held for 2minutes. The ramp continued at 3°C/min to 240°C and was held for 10minutes. The total run time was 50.27minutes. High-purity helium was used as the carrier gas, with a constant flow rate of 0.7mL/min.

***In vitro* Antifungal Activity of *Vismia baccifera* (L.) Extract Determined by the Disk Diffusion Method and Double Serial Dilutions**

***In vitro* Antifungal Activity using the Disk Diffusion Method**

The antifungal activity was evaluated using the agar diffusion method with Sabouraud medium, which was supplemented with chloramphenicol, following the guidelines of Kanagarajan et al. (2011). A stock solution of *Vismia baccifera* (L.) extract was prepared at a concentration of 1000mgL^{-1} in distilled water, from which three working concentrations were subsequently diluted: 250, 500, and 750mgL^{-1}. Sterile 5mm diameter paper discs were impregnated with each concentration and placed on the plates inoculated with the fungus. The Petri dishes were incubated at 28°C of incubation, and the inhibition zones were documented after a period of 72 to 96hours, measuring the halo without considering the disc's diameter. Clotrimazole was used as a positive control under equivalent conditions. All assays were performed in triplicate. The antifungal activity was classified into three levels according to the diameter of the inhibition halo: low (<15mm), intermediate (16–25mm), and high (>25mm), according to Davydova et al. (2024).

Minimum Inhibitory Concentration (MIC) *in vitro* of *Vismia baccifera* (L.) Extract by the Double Serial Dilution Method

The antifungal activity was determined using the double serial dilution method described by Kanagarajan et al. (2011) and Davydova et al. (2024). The extract of *Vismia baccifera* (L.) was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution at a concentration of 1000mg/L. Subsequently, an inoculum was prepared in liquid Sabouraud broth using *Nannizzia gypsea* spores obtained from slant cultures on Sabouraud agar (incubated for 1-7days). The fungal suspension was meticulously calibrated to attain a final concentration ranging from 1.1 to 1.5 × 10^2CFUmL^{-1}. This fungal suspension, after 7-14days of incubation at 28°C, was used. The quantification of colony-forming units (CFU) of the inoculum was performed using the plate count technique.

The minimum inhibitory concentration (MIC) of the extract was determined using the serial dilution method in liquid Sabouraud broth, following the technique described by Davydova et al. (2024). Ten successive dilutions were prepared by adding 1mL of the extract to 4 mL of medium and subsequently transferring 1mL of each resulting dilution to the next tube containing an equal volume of fresh medium.

Each dilution was inoculated with 100µL of a standardized suspension of *Nannizzia gypsea* and incubated at 28°C for 72–96hours. The MIC was defined as the lowest concentration without visible growth, and the Minimal Fungicidal Concentration (MFC) was defined as the concentration that completely eliminated the viable inoculum (Fitsev et al., 2022). Clotrimazole was used as a positive control (reference compound for fungi), while the culture medium and DMSO served as negative controls. All assays were performed in triplicate to ensure reproducibility.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed following Tukey's post hoc test ($p < 0.05$) to compare the effects of different concentrations of *V. baccifera* extract (250, 500, and 750mg/L) and the positive control (clotrimazole) on the diameter of the inhibition zone. A descriptive analysis (mean, standard deviation) of the effective concentrations was also performed for the minimum inhibitory concentration (MIC) test. The data analysis was conducted using SPSS software version 20, while Sigmaplot version 12.0 was employed for graphical representation. The significance level was set at 5%.

RESULTS

Phenotypic and Genotypic Characterization of a Strain of *Nannizzia gypsea* using Morphological Analysis and Molecular Techniques

Macroscopic observations of the strain revealed the development of white colonies with crateriform morphology, a powdery surface, and uniform radial growth, with defined margins and a regular outline (Fig. 1A). At the microscopic level, a septate and well-developed mycelium was evident, accompanied by abundant, thin-walled, multicellular, fusiform macroconidia with five to six septa. Furthermore, pyriform microconidia were observed arranged in a characteristic manner consistent with the diagnostic structures of the species (Fig. 1B).

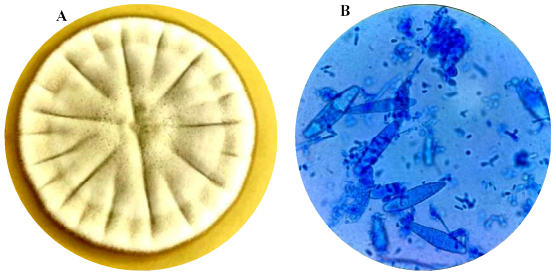

Fig. 1: (A) Macromorphological and (B) micromorphological characteristics of *Nannizzia gypsea*.

Molecular Identification

Molecular identification was performed to confirm the fungus's identity. Phylogenetic analysis revealed that the Unicor-NG strain is grouped within the clade formed by *Nannizzia gypsea* (NR131271), showing a close evolutionary relationship with this species (Fig. 2). This clade presented a bootstrap support value of 44, which indicates a moderate phylogenetic affinity between the two sequences. At higher hierarchical levels, the strain is associated with *Nannizzia lorica* (NR178109) and *Nannizzia fulva* (NR131266), while *Nannizzia praecox* (NR155489) and *Nannizzia anna* (NR155474) were located as outgroups.

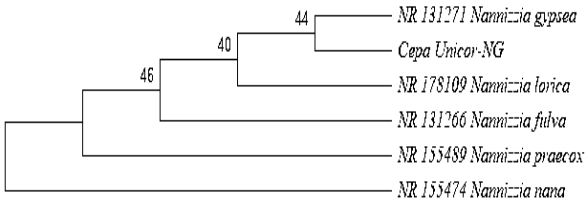

Fig. 2: Phylogenetic tree constructed using the maximum likelihood test of the fungal isolate and homologous sequences available in the GenBank database obtained using the NCBI BLAST algorithm.

Evaluation of the Phytochemical Composition of *Vismia baccifera* (L.) Extract via Gas Chromatography-Mass Spectrometry (GC-MS)

The ethanol extract of *V. baccifera* was analyzed using gas chromatography coupled with mass spectrometry (GC–MS), which enabled the qualitative identification of both major and minor compounds present. A total of 19 individual compounds were identified by comparing their mass spectra with the NIST02.L, NIST5a.L, and NIST98.L reference libraries, using relative match factors (RMF) calculated by MSDChemStation software.

The analysis revealed a complex and diverse chemical composition, consisting of metabolites belonging to different structural classes (Table 1). The predominant component was identified as a polychlorinated aryl ether, specifically 1,2,3,5-tetrachloro-4-(3,4,5-trichloro-2-methoxyphenoxy) benzene, accounting for 8.97% of the total compounds detected.

Table 1: GC-MS identification and quantification of the components in the *V. baccifera* extract (components with ω>0.04%, mass, n=3, P=0.95)

N°	tR[2],min	Component	ω[3](%)	Compound class
1	12.8604	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	0.4913	Bicyclic monoterpene
2	12.9926	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-naphthalene	3.0161	Bicyclic monoterpene
3	13.1863	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	2.3192	Bicyclic sesquiterpene
4	13.6709	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-naphthalene	0.5272	Bicyclic sesquiterpene
5	20.0133	1,1'-Bicyclopentyl	0.3031	Bicyclic alkane
6	22.6824	ethyl hexadecanoate	2.4753	Fatty acid ester
7	24.6203	Fitol	0.9368	acyclic diterpenic alcohol
8	25.237	(1R,4aS,10aR)-1,4a-dimethyl-7-propan-2-yl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carboxylic acid (Dehydroabietic acid)	1.4367	triterpenoid acid
9	25.3162	(9E , 12E , 15E) -ethyl octadeca-9,12,15-trienoate (Ethyl ester of 9,12,15-octadecatrienoic acid)	2.9383	Carboxylic acid ester
10	25.8624	ethyl octadecanoate (Ethyl stearate)	0.6533	Fatty acid ester
11	26.7257	5-(4-aminophenyl)-4-(2-methylphenyl)-1,3-thiazol-2-amine	1.4126	substituted thiazole
12	27.2366	1,2,3,5-tetrachloro-4-(3,4,5-trichloro-2-methoxyphenoxy)benzene	8.9684	Polychlorinated aryl ether
13	27.6065	1- N ,4- N -bis(5-methylhexan-2-yl)benzene-1,4-diamine (N,N'-bis(1,4-dimethylpentyl)-p-phenylenediamine)	3.5598	Aromatic amine
14	28.0734	(4 R ,4 a R ,7 R ,7 a R ,12 b S)-7-ethoxy-3-methyl-2,4,4 a ,5,6,7,7 a ,13-octahydro-1 H -4,12-methanebenzofuro[3,2-e]isoquinolin-9-ol	0.7217	Isoquinoline
15	28.5843	(1 S ,13 R ,15 R)-9-methoxy-5,7-dioxa-12-azapentacyclo[10.5.2.0 1,13 .0 2,10 .0 4,8]nonadeca-2,4(8),9,16-tetraen-15-ol (Powelline)	0.4406	Isoquinoline alkaloid
16	28.6548	N-(1,1-dimethylethyl)-3-iodobenzamide	0.5894	Aromatic amide
17	30.0642	3,6,8-trihydroxy-3-methyl-2,4-dihydrobenzo[a]anthracene-1,7,12-trione	1.3721	Anthracene Quinone derivative
18	30.2228	2,6,7-Trihydroxy-9-phenylisoxanthene-3-one	0.1153	Coumarin derivative
19	30.3725	3,5-di-tert-butyl-4-hydroxyacetophenone	2.9441	Substituted phenol

Monoterpenoids and bicyclic sesquiterpenoids collectively accounted for 6.35% of the total fraction, while substituted phenols, including 3,5-di-tert-butyl-4-hydroxyacetophenone, constituted 2.94%. Aromatic amines, particularly 1-N,4-N-bis(5-methylhexan-2-yl) benzene-1,4-diamine, represented 3.56%.

Among the carboxylic acids identified were (1R,4aS,10aR)-1,4a-dimethyl-7-propan-2-yl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carboxylic acid (1.44%) and 3,6,8-trihydroxy-3-methyl-2,4-dihydrobenzo[a]anthracene-1,7,12-trione (1.37%). Carboxylic acid esters represented 2.94% of the sample, notably the ethyl ester of (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid. Furthermore, fatty acid esters constituted 3.13%, including ethyl hexadecanoate and ethyl octadecanoate. Other metabolites of interest were detected, including the acyclic diterpene alcohol phytol (0.94%), a substituted thiazole [5-(4-aminophenyl)-4-(2-methylphenyl)-1,3-thiazol-2-amine] (1.41%), a bicyclic alkane (1,1'-bicyclopentyl) (0.30%), an isoquinoline [4R,4aR,7R,7aR,12bS)-7-ethoxy-3-methyl-2,4,4a,5,6,7,7a,13-octahydro-1H-4,12-methano-benzofuro[3,2-e]isoquinolin-9-ol] (0.72%), and an isoquinoline alkaloid (powelline) (0.44%). Finally, minor proportion compounds were identified, such as the aromatic amide N-(1,1-dimetiletil)-3-yodobenzamida (0.59%) and a coumarin derivative [2,6,7-trihidroxi-9-fenilisoxanteno-3-ona] (0.11%).

Evaluation of the Antifungal Activity of *Vismia baccifera* (L.) Extract against *Nannizzia gypsea* using *in vitro* Assays

The antifungal activity of the ethanolic extract of *V. baccifera* leaves, obtained by maceration, was evaluated against the dermatophyte *Nannizzia gypsea* using the agar disk diffusion method. The results revealed fungal growth inhibition zones ranging from 12.63±0.41mm to 13.17±0.38mm across the tested concentrations of 250, 500, and 750mg/L (Table 2). A slight dose-dependent tendency toward increased inhibition was observed; no statistically significant differences were found among the three extract concentrations (>0.05). In comparison, the positive control (1% clotrimazole) exhibited an inhibition zone of 27.7±1.29mm, which was approximately 2.1 to 2.2 times larger than the zones produced by the extract (Fig. 3). According to the classification system used, the extract demonstrates moderate antifungal activity when compared to the reference agent.

Table 2: *In vitro* antifungal activity of *V. baccifera* (L.) extract against *Nannizzia gypsea* assessed by the agar disk diffusion method

Concentration of the extract (mg/L)	Inhibition halo (mm)
250	12.63±0.41
500	13.10±0.25
750	13.17±0.38
Control (clotrimazole al 1%)*	27.70±1.29

* Reference medicine for dermatophyte fungi.

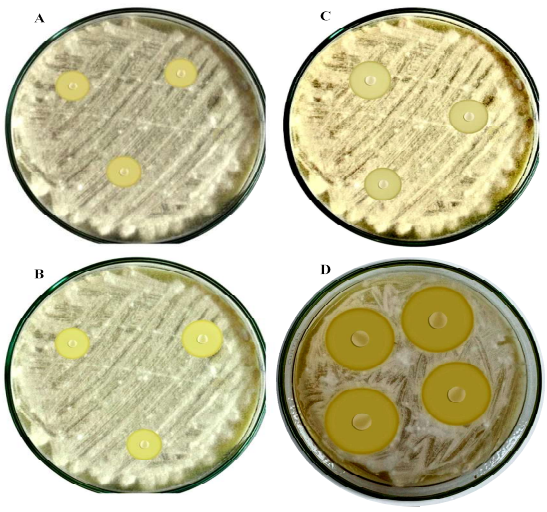

Fig. 3: Inhibition halos of *Nannizzia gypsea* on agar plates (168h) after exposure to *V. baccifera* (L.) Extract at different concentrations: (A) 250mg/L; (B) 500mg/L; (C) 750mg/L; and (D) negative control.

The antifungal activity of the ethanolic extract of *V. baccifera* leaves was evaluated against the dermatophyte *N. gypsea* using the serial dilution method. The Minimum Inhibitory Concentration (MIC) ascertained was 156.25µg/mL, a concentration at which total inhibition of visible fungal growth was observed. Fungal growth was evident at lower concentrations (data not shown), indicating that these doses did not reach the inhibitory threshold necessary to prevent the proliferation of the microorganism.

The positive control (1% clotrimazole) exhibited no fungal growth, thereby confirming its antifungal efficacy under the established experimental conditions. Conversely, the negative control (dimethyl sulfoxide (DMSO) solvent used in the dilutions preparation) showed visible growth of *N. gypsea*. This finding confirmed that DMSO does not exert an inhibitory effect on the pathogen under evaluation.

DISCUSSION

According to the World Health Organization (WHO), dermatophytosis is among the most prevalent superficial mycoses worldwide, affecting approximately 25% of the global population. This high prevalence is primarily associated with the environmental conditions characteristic of tropical regions, featuring elevated temperatures and humidity levels. These conditions favor the survival and spread of the etiological agents of these keratinophilic fungi (Petrucelli et al., 2020). The diversity of species responsible for dermatophyte infections exhibits marked geographical variation, influenced by ecological, climatic, and socioeconomic factors such as human migration patterns, shifts in lifestyle habits, and variations in diagnostic and therapeutic approaches across health systems, which also contribute significantly to the epidemiological dynamics of these infections (Bontems et al., 2020). Collectively, these variables influence the population susceptibility and distribution of dermatophytes, highlighting the need to strengthen surveillance and control strategies, particularly in regions with high climate vulnerability.

The term dermatophytes refer to a group of keratinophilic fungi that exhibit a wide spectrum of phenotypic variability at both the macroscopic and microscopic levels. This heterogeneity is reflected in aspects such as differential pigment production, colony texture and coloration, and the consistency and morphology of their reproductive structures, all of which are fundamental for their correct taxonomic characterization and species differentiation (Pérez-Rodríguez et al., 2023). In the present study, the *Nannizzia gypsea* strain was cultivated on Sabouraud Dextrose Agar with the addition of chloramphenicol, which permitted the documentation of its morphological behavior under controlled conditions. Vigorous and rapid colony growth was observed, with colonies having a powdery surface and beige to light brown tones, which are typical features of this geophilic species. A microscopic examination of the structures revealed thin-walled fusiform macroconidia with between three and six internal septa. This characteristic arrangement coincides with previous descriptions of the genus *Nannizzia*. These results confirm the morphological consistency of *N. gypsea* and support the usefulness of macro- and micromorphological observation as a complementary diagnostic tool in taxonomic and antifungal susceptibility studies. Additionally, the observed phenotypic stability indicates adequate physiological adaptation to the culture medium, thereby supporting its use in subsequent trials to evaluate metabolites or sensitivity to natural extracts with potential antifungal activity.

Until recently, the taxonomic identification of dermatophytes has been based on traditional phenotypic methods, relying on the evaluation of morphological, physiological, and biochemical characteristics. These approaches, while fundamental to classical mycology, present limitations in taxonomic resolution, particularly when dealing with emerging or morphologically atypical species (Hubka et al., 2014). The accuracy of species-level identification is of considerable clinical importance, given that dermatophytes can exhibit divergent antifungal susceptibility profiles, even within the same genus. Previous studies have demonstrated significant disparities in *in vitro* resistance profiles among strains of *Trichophyton rubrum*, *T. tonsurans* and *T. equinum* in response to various antifungal medications, underscoring the necessity for a more thorough and nuanced characterization of clinical isolates (Bao et al., 2013; Nyilasi et al., 2013). Conversely, conventional diagnostic techniques, predicated on morphological observations and biochemical tests, frequently exhibit variability and inconsistencies due to factors such as the culture medium, environmental conditions, or the physiological state of the fungus. Due to the aforementioned constraints, the development and implementation of molecular tools, such as the amplification and sequencing of specific genetic regions, have facilitated the refinement of taxonomic delimitation and enhanced the reliability of mycological diagnosis. These methodologies not only complement but, in many cases, supplant phenotypic approaches, providing precise identification at the genus and species levels (Hubka et al., 2014).

A body of research has evidenced that conventional methods based on the morphological characterization of dermatophytes possess inherent limitations in their discriminative capacity, which frequently leads to identification errors and taxonomic misclassification (Robert & Pihet, 2008). These restrictions have prompted the incorporation of molecular approaches, which have transformed the systematic study of the genus by allowing for more robust and accurate phylogenetic resolution. The use of specific genetic markers has proven particularly effective for elucidating evolutionary and taxonomic relationships within this group of keratinophilic fungi. The nuclear ribosomal DNA (rDNA) region is among the most widely used. Its value in phylogenetic analyses stems from its multicopy nature and the presence of both conserved genes (e.g., 18S, 5.8S, and 28S) and variable domains, represented by the ITS1, ITS2, and intergenic non-

transcribed (IGS) regions. The ITS region was the first molecular marker systematically applied for the construction of phylogenies in dermatophytes, thus enabling the redefinition of taxonomic criteria previously based solely on phenotypic characteristics. However, recent studies have demonstrated that the confirmation and precise delimitation of species requires the combination of other complementary gene loci, thus improving taxonomic resolution and phylogenetic consistency (Rezaei-Matehkolaei et al., 2014).

Advances in chromatographic techniques coupled with mass spectrometry (GC–MS) have transformed phytochemical research by enabling a comprehensive and precise characterization of secondary metabolites present within plant extracts. These analytical tools enable not only the structural identification of bioactive compounds but also the evaluation of their pharmacological and biotechnological relevance, thus establishing themselves as essential methodologies in the chemical study of species with therapeutic potential (Fitsev et al., 2021, 2022).

The existing information regarding the phytochemical composition of *V. baccifera* remains limited. Scarce previous research exists; for instance, a study by Lizcano et al. (2012). The present study reported the presence of caffeoylquinic and hydroxycinnamic acids in the leaves but lacked a comprehensive characterization of other secondary metabolite groups. In contrast, the present study provides a more extensive and intricate chemical profile, as evidenced by the results of the GC-MS analysis of the ethanolic extract. This analysis detected 19 compounds, including carboxylic acids, monoterpenoids, bicyclic sesquiterpenoids, polychlorinated aryl ethers, substituted phenols, aromatic amines, fatty acids, coumarins, and other organic compounds (Table 1).

This diversified phytochemical profile suggests a distinctive metabolic composition for *V. baccifera*, potentially associated with its biological and antimicrobial activity. Specifically, the presence of terpenoids and sesquiterpenoids has been linked to antifungal and bactericidal mechanisms of action, such as altering cell membrane permeability or inhibiting enzymes in fungal pathogens (Nikitin et al., 2023). Similarly, coumarins and their derivatives are widely recognized for their broad spectrum of bioactivities, encompassing antioxidant, antimicrobial, and antitumor properties (Davydova et al., 2024). Collectively, these findings confirm that *V. baccifera* is a promising source of metabolites with pharmacological potential. They also highlight the necessity for additional fractionation and bioactive evaluation studies to isolate the compounds responsible for the observed antifungal activity and elucidate their potential molecular mechanisms of action.

Monoterpenes and sesquiterpenes were, as previously indicated, the primary chemical constituents identified in the ethanolic extract of *V. baccifera*. This finding aligns with reports on various plant extracts and essential oils, where these metabolites predominate due to their high volatility and structural diversity. These characteristics associate them with important biological properties, including antimicrobial, antioxidant, and anti-inflammatory activities (Perricone et al., 2015). However, the results obtained in this study reveal notable differences in chemical composition when compared to the profiles reported for other parts of the plant. For instance, Vizcaya et al. (2014) examined the essential oil extracted from the bark of *V. baccifera* and described a set of compounds entirely distinct from those identified in the leaf extract evaluated here. Notably, none of the major compounds detected in the leaf extract were present in the essential oil of the bark, suggesting a significant degree of tissue specificity in the biosynthesis and accumulation of secondary metabolites within this species. These phytochemical variations can be attributed to physiological and ecological factors, including differences in enzyme expression, tissue metabolic function, or environmental conditions that modulate the production of volatile and non-volatile compounds. This phenomenon underscores the necessity of taking into account the plant's provenance and the specific organ or tissue utilized when assessing the bioactive potential of *V. baccifera*. Furthermore, it emphasizes the importance of conducting additional intraspecific comparative studies to elucidate the metabolic regulatory mechanisms that underpin the observed chemical diversity.

According to the criteria proposed by Álvarez-Martínez et al. (2021), plant extracts are classified as follows: highly active if the minimum inhibitory concentration (MIC) is less than 100µg/mL; significantly active if the MIC is between 100 and 512µg/mL; moderately active if the MIC is between 512 and 2048µg/mL; and inactive if the MIC is above 2048µg/mL. Van Vuuren and Holl (2017) have indicated that medicinal plant extracts with minimal inhibitory concentration (MIC) values approximating 160µg/mL are promising candidates for the development of natural antimicrobial agents. In this context, the ethanolic extract obtained by maceration in the present study recorded an MIC value of 156.25µg/mL, falling within the range of significant activity, with a clear trend towards promising activity. This result suggests that the extract possesses bioactive compounds with relevant antimicrobial potential, possibly attributable to the synergy between secondary metabolites of a terpenoid or phenolic nature, which are often responsible for inhibiting microbial growth in various phytotherapeutic species.

Numerous reports have indicated a direct correlation between the antifungal activity of plant extracts and the presence of terpenoid secondary metabolites, particularly mono- and sesquiterpenes (Costa et al., 2017). These metabolites have also been detected in the chemical profile of the extract examined in this work. These compounds have been shown to exert a fungicidal effect via multiple mechanisms of cellular interference, primarily compromising the structural integrity of the plasma membrane, which leads to cellular death. In addition, it has been documented that terpenoids have the capacity to influence critical metabolic processes, including membrane component synthesis, mitochondrial respiration, and spore germination, thereby impeding mycelial development and proliferation (Sachikonye & Mukanganyama, 2016).

In this investigation, the *in vitro* antifungal activity of

the ethanolic extract obtained from *Vismia baccifera* leaves against the dermatophyte *Nannizzia gypsea* was determined. The results demonstrate a significant inhibition of mycelial growth, indicating the presence of metabolites with biocidal potential. However, given that the crude extract is a complex mixture of secondary metabolites, it is not possible to attribute the observed antifungal activity to a specific compound within the chemical profile identified by GC-MS (gas chromatography-mass spectrometry).

The phytochemical complexity of plant extracts poses a significant challenge in elucidating mechanisms of action and assigning biological activity to individual molecules. Consequently, there is an imperative to progress towards the isolation, purification, and structural characterization of the primary bioactive constituents to facilitate independent evaluation of their antifungal properties and determination of their minimum inhibitory concentrations (MIC). It would also enable the identification of synergistic or antagonistic interactions between metabolites, which could enhance or modulate the observed activity of the total extract.

While there is background information on the general biological activity of *Vismia baccifera* extracts, specific information on their antifungal action remains limited. Studies targeting dermatophytes are scarce, and those directed against *N. gypsea*—a keratinophilic fungus frequently associated with dermatophytosis in humans and animals—are virtually nonexistent. In this context, the results obtained herein constitute preliminary but relevant evidence of the antifungal potential of *V. baccifera*, contributing to knowledge about its mycotoxic properties and its possible application in the search for new natural agents for the control of superficial fungal infections.

Conclusion

The ethanolic extract of *Vismia baccifera* leaves exhibited significant in vitro antifungal activity against the dermatophyte *Nannizzia gypsea*, as evidenced by mycelial growth inhibition and a Minimum Inhibitory Concentration (MIC) value within the range reported as relevant for crude plant extracts. Phytochemical analysis revealed a diverse chemical profile composed of secondary metabolites of recognized biological relevance, including terpenoids, phenolic compounds, fatty acids, and esters. These metabolites may act individually or synergistically to inhibit the evaluated dermatophyte. Such chemical complexity suggests that the observed activity cannot be attributed to a single compound but rather to the interaction of multiple metabolites. These findings position *V. baccifera* as a promising species for the development of natural antifungal agents. However, further studies —including bioassay-guided fractionation, structural identification of active metabolites, evaluation of synergistic effects, and toxicity and safety assessments— are required to validate its therapeutic potential and future applicability in the treatment of dermatophytosis.

DECLARATIONS

Funding: This research was supported by the University of Córdoba (Colombia) through its institutional research funding programs.

Acknowledgement: The authors would like to thank the University of Córdoba, the Biotechnology group of the Department of Chemistry and Department of Biology-GRUBIODEQ and to the bacteriology program at the University of Cordoba for supplying the fungus, to significantly contribute to the development of this manuscript.

Conflict of Interest: The authors declare that they have no competing financial interests or known personal relationships that could have influenced the work presented in this article.

Data Availability: All the data is available in the article.

Ethics Statement: This study did not require approval by an institutional research ethics committee, as it did not involve human participants or the use of vertebrate animals. All experimental activities were conducted exclusively using microbial cultures, which are not considered sensitive organisms according to current international ethical standards for scientific research. Experimental procedures were performed under controlled laboratory conditions, complying with standard biosafety protocols for microbiological research and adhering to Good Laboratory Practices (GLP). No interventions involving ethical, environmental, or animal welfare risks were conducted.

Author's Contribution: First author (C.A.M): methodology, research, data analysis, conceptualization, writing, revising and editing. Second author (A.J.B.R): research, conceptualization, data analysis, writing, review and editing. Third author (J.E.A.R): visualization and revision. Fourth author (L.E.O.Z): review and editing. Fifth author (D.E.P.S): review and editing. Sixth author (L.F.O.P). All authors have read and accepted the published version of the manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Argüelles, J.C., Sánchez-Fresneda, R., Argüelles, A., & Solano, F. (2024). Natural substances as valuable alternative for improving conventional antifungal chemotherapy: lights and shadows. *Journal of Fungi*, *10*(5), 334. https://doi.org/10.3390/jof10050334

Álvarez-Martínez, F.J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V.

(2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine*, *90*, 153626. https://doi.org/10.1016/j.phymed.2021.153626

Bao, Y., Wan, Z., & Li, R. (2013). In vitro antifungal activity of micafungin and caspofungin against dermatophytes isolated from China. *Mycopathologia*, *175*(1–2), 141–145. https://doi.org/10.1007/s11046-012-9571-6

Betin-Ruiz, A.J., Cantero -Guevara, M.E., Oviedo -Zumaqué, L.E., & Pardo-Plaza, Y.J. (2025). Effect of an enterobacter cloacae-based biofertilizer on the growth and performance of cassava plants (manihot esculenta crantz) MCol 2066 variety (Chirosa). *International Journal of Agriculture and Biosciences*, *14*(5), 917–930.

Bontems, O., Fratti, M., Salamin, K., Guenova, E., & Monod, M. (2020). Epidemiology of dermatophytoses in Switzerland according to a survey of dermatophytes isolated in Lausanne between 2001 and 2018. *Journal of Fungi*, *6*(2), 95. https://doi.org/10.3390/jof6020095

Buitrago-Díaz, A.A., Rojas-Vera, J., & Velasco-Carrillo, J. (2020). Analisis fitoquimico preliminar y evaluacion de la actividad antibacteriana de fracciones de diferentes polaridades obtenidas de Vismia baccifera (L.) Triana & Planch y Vismia macrophylla Kunth. *Revista de la Facultad de Farmacia*, *62*(1), 15-23. https://doi.org/10.53766/refa/2021.62.01.02

Bristow, I.R., & Joshi, L.T. (2023). Dermatophyte resistance – on the rise. *Journal of Foot and Ankle Research*, *16*(1), 69. https://doi.org/10.1186/s13047-023-00665-5

Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates*, *3*(1), 184–207. https://doi.org/10.3390/ddc3010011

Conceição, A.B. da S., Chueiri, M.C., Silva, L. de O., Schneider, L. de L., Gomes, J. de F., Oliveira, Á.A. de, Campos, S.G. de, Baroni, F. de A., & Bonci, M.M. (2024). Avaliação da presença de fungos dermatófitos e resistência antimicrobiana a partir de amostras ambientais de comunidades ribeirinhas da Região Amazônica: Uma questão de vigilância em saúde. *Research, Society and Development*, *13*(4), Article 4. https://doi.org/10.33448/rsd-v13i4.45590

Costa, M., Silva, A., Silva, A., Lima, V., Bezerra-Silva, P., Rocha, S., Navarro, D., Correia, M., Napoleão, T., Silva, M., & Paiva, P. (2017). Essential oils from leaves of medicinal plants of Brazilian flora: chemical composition and activity against candida species. *Medicines*, *4*(2), 27. https://doi.org/10.3390/medicines4020027

Cruz, C.R., & Vielle O.P. (2024). Actualización en taxonomía, diagnóstico y tratamiento de las dermatofitosis. *Revista Chilena de Infectología*, *41*(2), 218–224. https://doi.org/10.4067/s0716-10182024000200218

Davydova, L., Menshova, A., Shumatbaev, G., Babaev, V., & Nikitin, E. (2024). Phytochemical study of ethanol extract of *Gnaphalium uliginosum* l. and evaluation of its antimicrobial activity. *Antibiotics*, *13*(8), 785. https://doi.org/10.3390/antibiotics13080785

Elshafie, H.S., Camele, I., & Mohamed, A.A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *International Journal of Molecular Sciences*, *24*(4), 3266. https://doi.org/10.3390/ijms24043266

Fitsev, I.M., Nikitin, E.N., Rakhmaeva, A.M., Terenzhev, D.A., Sakhno, T.M., & Nasybullina, Z.R. (2022). Chemical composition of *Cupressus sempervirens* L. and *Thuja occidentalis* L. essential oils and their activity against phytopathogenic fungi. *Uchenye Zapiski Kazanskogo Universiteta. Seriya Estestvennye Nauki*, *164*(3), 392–407. https://doi.org/10.26907/2542-064X.2022.3.392-407

Fitsev, I.M., Rakhmetova, E.R., Mukhammetshina, A.G., Burkin, K.E., & Shlyamina, O.V. (2021). Gas chromatography-mass spectrometry determination of deltamethrin in food. *Uchenye Zapiski Kazanskogo Universiteta. Seriya Estestvennye Nauki*, *163*(4), 569–580. https://doi.org/10.26907/2542-064x.2021.4.569-580

Hubka, V., Cmokova, A., Skorepova, M., Mikula, P., & Kolarik, M. (2014). *Trichophyton onychocola* sp. Nov. isolated from human nail. *Medical Mycology*, *52*(3), 285–292. https://doi.org/10.1093/mmy/myt010

Joseph, R., Darrisaw, C., Lloyd, A., Hoel, D., & Keyhani, N.O. (2024). Isolation of a Novel *Pythium* sp., *P. thermoculicivorax*, and *Trichoderma* sp. from natural enzootic mosquito larval infections. *Journal of Fungi*, *10*(3), 199. https://doi.org/10.3390/jof10030199

Kanagarajan, V., Ezhilarasi, M.R., & Gopalakrishnan, M. (2011). In vitro microbiological evaluation of 1,1′-(5,5′-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones, novel bis acetylated pyrazoles. *Organic and Medicinal Chemistry Letters*, *1*(1), 8. https://doi.org/10.1186/2191-2858-1-8

Lfitat, A., Zejli, H., Bousraf, F.Z., Bousselham, A., El Atki, Y., Gourch, A., Lyoussi, B., & Abdellaoui, A. (2021). Comparative assessment of total phenolics content and in vitro antioxidant capacity variations of macerated leaf extracts of *Olea europaea* L. and *Argania spinosa* (L.) Skeels. *Materials Today: Proceedings*, *45*, 7271–7277. https://doi.org/10.1016/j.matpr.2020.12.990

Lizcano, L.J., Viloria-Bernal, M., Vicente, F., Berrueta, L.A., Gallo, B., Martínez-Cañamero, M., Ruiz-Larrea, M.B., & Ruiz-Sanz, J.I. (2012). Lipid oxidation inhibitory effects and phenolic composition of aqueous extracts from medicinal plants of colombian amazonia. *International Journal of Molecular Sciences*, *13*(5), 5454–5467. https://doi.org/10.3390/ijms13055454

Lizcano, L., Siles, M., Trepiana, J., Hernández, M., Navarro, R., Ruiz-Larrea, M., & Ruiz-Sanz, J. (2014). Piper and vismia species from colombian amazonia differentially affect cell proliferation of hepatocarcinoma cells. *Nutrients*, *7*(1), 179–195. https://doi.org/10.3390/nu7010179

Metoui, R., Mighri, H., Bouajila, J., Znati, M., El-Jani, H., & Akrout, A. (2022). Artemisia campestris dried leaf extracts: Effects of different extraction methods and solvents on phenolic composition and biological activities. *South African Journal of Botany*, *151*, 288–294. https://doi.org/10.1016/j.sajb.2022.10.002

Mussin, J., & Giusiano, G. (2024). Synergistic antimicrobial activity of biogenic silver nanoparticles and *Acanthospermum australe* essential oil against skin infection pathogens. *Antibiotics*, *13*(7), 674. https://doi.org/10.3390/antibiotics13070674

Nikitin, E., Fitsev, I., Egorova, A., Logvinenko, L., Terenzhev, D., Bekmuratova, F., Rakhmaeva, A., Shumatbaev, G., Gatiyatullina, A., Shevchuk, O., & Kalinnikova, T. (2023). Five different artemisia l. species ethanol extracts' phytochemical composition and their antimicrobial and nematocide activity. *International Journal of Molecular Sciences*, *24*(18), 14372. https://doi.org/10.3390/ijms241814372

Nyilasi, I., Kocsube, S., Krizsan, K., Galgoczy, L., Papp, T., Pesti, M., … & Vágvölgyi, C. (2013). Susceptibility of clinically important dermatophytes against statins and different statin-antifungal combinations. *Sabouraudia*, *52*(2), 140-148. https://doi.org/10.3109/13693786.2013.828160

Orozco-Yee, E.A., Rojas-Castañeda, R.G., Guevara-Gutiérrez, E., Mayorga-Rodríguez, J., & Tlacuilo-Parra, A. (2025). Dermatofitosis causadas por *Nannizzia gypsea:* informe de 155 casos en el oeste de México. *Enfermedades Infecciosas y Microbiología Clínica*, *43*(4), 205–209. https://doi.org/10.1016/j.eimc.2024.08.011

Pérez-Rodríguez, A., Duarte-Escalante, E., Frías-De-León, M.G., Acosta Altamirano, G., Meraz-Ríos, B., Martínez-Herrera, E., Arenas, R., & Reyes-Montes, M.D.R. (2023). Phenotypic and genotypic identification of dermatophytes from Mexico and central american countries. *Journal of Fungi*, *9*(4), 462. https://doi.org/10.3390/jof9040462

Perricone, M., Arace, E., Corbo, M.R., Sinigaglia, M., & Bevilacqua, A. (2015). Bioactivity of essential oils: a review on their interaction with food components. *Frontiers in Microbiology*, *6*, 76. https://doi.org/10.3389/fmicb.2015.00076

Petrucelli, M.F., Abreu, M.H.D., Cantelli, B.A.M., Segura, G.G., Nishimura, F.G., Bitencourt, T.A., Marins, M., & Fachin, A.L. (2020). Epidemiology and diagnostic perspectives of dermatophytoses. *Journal of Fungi*, *6*(4), 310. https://doi.org/10.3390/jof6040310

Reina, A.M.M., Zumaqué, L.T., Martínez, L.M.G., Pinto, M.D.V.R., Cabadiaz, A.D., & Leal, H.A.B. (2024). Eficiencia técnica del cultivo de cacao (*Theobroma cacao* L) en el sur de Córdoba, Colombia. *Ciencia y Tecnología*, *17*(2), 1-8. https://doi.org/10.18779/cyt.v17i2.712

Rezaei-Matehkolaei, A., Mirhendi, H., Makimura, K., De Hoog, G.S., Satoh, K., Najafzadeh, M.J., & Shidfar, M.R. (2014). Nucleotide sequence analysis of beta tubulin gene in a wide range of dermatophytes. *Medical Mycology*, *52*(7), 674–688. https://doi.org/10.1093/mmy/myu033

Robert, R., & Pihet, M. (2008). Conventional methods for the diagnosis of dermatophytosis. *Mycopathologia*, *166*(5), 295-306. https://doi.org/10.1007/s11046-008-9106-3

Rojas-Vera, J., Buitrago-Díaz, A., Arvelo, F.A., Sojo, F.J., Suarez, A.I., & Rojas, L. (2020). Essential oil composition and cytotoxic activity in two species of the plant genus Vismia (Hypericaceae) from the Venezuelan Andes. *Revista de Biología Tropical*, *68*(3), 884-891. https://doi.org/10.15517/rbt.v68i3.39503

Sacheli, R., & Hayette, M.-P. (2021). Antifungal Resistance in Dermatophytes: Genetic Considerations, Clinical Presentations and Alternative Therapies. *Journal of Fungi*, *7*(11), 983. https://doi.org/10.3390/jof7110983

Sachikonye, M., & Mukanganyama, S. (2016). Antifungal and drug efflux inhibitory activity of selected flavonoids against *Candida albicans* and *Candida krusei*. *Journal of Biologically Active Products from Nature*, *6*(3), 223-236. https://doi.org/10.1080/22311866.2016.1231078

Sánchez Espinosa, K.C., Díaz Vázquez, L., Almaguer, M., & Fernández Andreu, C.M. (2022). Dermatofitosis: Un desafío creciente de salud internacional. *Revista Salud y Desarrollo*, *6*(1), 149–177. https://doi.org/10.55717/jrzr1886

Sasidharan, S., Nair, R.V., Kumar, N., Nair, K., & Gomathi, S. (2024). Ultrasonication assisted percolation extraction, isolation, identification and in silico studies of secondary metabolites from Caesalpinia bonduc (L.) Roxb. seeds with special reference to the target proteins involved in Benign Prostatic Hyperplasia. *South African Journal of Botany*, *168*, 89-105. https://doi.org/10.1016/j.sajb.2024.03.008

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Trepiana, J., Ruiz-Larrea, M.B., & Ruiz-Sanz, J.I. (2018). Unraveling the in vitro antitumor activity of *Vismia baccifera* against HepG2: Role of hydrogen peroxide. *Heliyon*, *4*(6), e00675. https://doi.org/10.1016/j.heliyon.2018.e00675

Van Vuuren, S., & Holl, D. (2017). Antimicrobial natural product research: A review from a South African perspective for the years 2009–2016. *Journal of Ethnopharmacology*, *208*, 236–252. https://doi.org/10.1016/j.jep.2017.07.011

Vizcaya, M., Pérez, C., Rojas, J., Rojas-Fermín, L., Plaza, C., Morales, A., & Pérez, P. (2014). Composición química y evaluación de la actividad antifúngica del aceite esencial de corteza de Vismia baccifera var. Dealbata. *Revista de la Sociedad Venezolana de Microbiología*, *34*(2), 86–90.

Yu, Y., Yang, Q., Petropoulos, E., & Zhu, T. (2022). ITS3/ITS4 outperforms other ITS region and 18S rRNA gene primer sets for amplicon sequencing of soil fungi. *European Journal of Soil Science*, *73*(6), e13329. https://doi.org/10.1111/ejss.13329