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Medicinal plants and onychomycosis: potential and evidence of antifungal activity - an integrative review of the literature

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Onychomycosis is a fungal infection of the nails, also known as nail mycosis and *Tinea unguium*, which affects 6.9 to 23.2% of the general population. It is most commonly found in people aged 60 years and older. Onychomycosis is caused by dermatophyte fungi, non-dermatophyte filamentous fungi and yeast. Treatment includes topical or oral antifungal drugs. The use of other auxiliary, alternative and/or complementary therapies, such as those prepared from medicinal plants, are poorly explored, studied, and disseminated. Related medicinal plants and their *in vitro* antifungal activity against fungal agents of onychomycosis as well as studies that reported the clinical efficacy of these plants are presented. Integrative literature review included a search for publications from January 2011 to January 2021, in databases and/or virtual libraries: Scielo, PubMed, CAPES and Google Scholar. The main descriptors used "onychomycosis" AND "medicinal plants". Thus, a form for collecting the main information was used as a data collection instrument. Nineteen articles were selected, 15 of which included an *in vitro* evaluation of plant activity on dermatophyte fungi, 11 on yeasts and six on non-dermatophyte filamentous fungi (NDFF). Five simultaneously reported the evaluation of *in vitro* antifungal activity on the three groups of fungi, three on dermatophytes and yeasts and none on dermatophytes and NDFF or on yeast and NDFF. Most studies (16) were *in vitro*, while four described clinical studies. Most studies have shown that the medicinal plants described have antifungal activity *in vitro* against fungal agents of onychomycosis and can be promising alternatives for conventional treatment or complementary to usual antifungal therapy.

Key words: Onychomycosis – medicinal herbs - treatment - dermatophytes - *Tinea unguium.*

INTRODUCTION

Onychomycosis is a fungal infection of limited severity that affects adults' toenails and fingernails and, to a lesser extent, child's nails (Solís-Arias and García-Romero, 2017; Lipner and Scher, 2019). The word onychomycosis comes from the Greek "onyx", which means nail and "mycos", which means mycosis (Baswan

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et al., 2017), and includes all fungal infections of one or more nails. The etiological agents include dermatophyte fungi (in this case it is also called *Tinea unguium)*, yeasts (mainly *Candida* species) and non-dermatophyte molds. Mixed infections caused by the association of different groups of these fungi may occur (Baswan et al., 2017; Gupta et al., 2020).

Onychomycosis is the most frequent nail disease in dermatological clinics; the signs and symptoms included local pain, paresthesia and difficulties in performing daily activities in general. Clinical manifestations of fungal infected nails are usually discoloration, hyperkeratosis and onycholysis (Gupta et al., 2017; Lipner and Scher, 2019). Individuals affected by onychomycosis can have low self-esteem, which can impact social life and interactions and can also have a reduced quality of life and psychological damage (Lipner and Scher, 2019; Stewart et al., 2021).

The prevalence of onychomycosis is 6.9-23.2% in the North American and European populations (Elsayed, 2015), with rates increasing according to age and most infected people being aged 60 years or older (Belda et al., 2014; Lipner and Scher, 2019). Different studies show different rates, which vary according to geographic regions, gender, climate, occupation, physical activity and the presence of comorbidities (Belda et al., 2014; Gupta et al., 2017; Lipner and Scher, 2019). The factors that contribute to a high incidence of superficial mycoses, especially in tropical and subtropical regions are heat and humid (Lombardi et al., 2020; Sharma and Nonzom, 2021). According to Baswan et al. (2017), onychomycosis is responsible for 50% of all nail diseases and about 30% of superficial mycoses. Around 60-70% of infections are caused by dermatophyte fungi, whose main agent is *Trichophyton rubrum* (>50%), followed by *T. mentagrophytes* (about 20%); other cases are caused by non-dermatophyte molds (NDMs) (10-20%) and yeasts (10-20%) (Gupta et al., 2012; Lipner and Scher, 2019; Gupta et al., 2020).

The conventional therapy includes drugs such as allylamines, azoles, ciclopirox and amorolfine. This therapy commonly presents adverse effects that become more serious with prolonged use and some infectious agents are resistant to antifungals (Flores et al., 2016). Onychomycosis has shown difficulty being treated because the fungi grow inside the nail, requires prolonged time and low patient compliance to treatment, as well as the relative frequency of recurrence of the infection (Christenson et al., 2018). The mixed infections and those caused by yeast and NDMs are more difficult to treat than those caused by dermatophytes (Lipner and Scher, 2019). Alternative therapies are often used empirically by individuals with nail disease, which can be added to the treatment already prescribed by doctors, or even before seeking specialized medical attention. Thus, medicinal plants have been used as alternative and/or complementary options to treatment.

Medicinal plants are used for different purposes, such as for treating inflammatory processes and infectious diseases, as well to reduce pain and promote wound healing. The pharmacological effects of plants often come from the presence of the majority constituents, as well as the synergistic action resulting from their many constituents that belong to various chemical classes (flavonoids, phenolic acids, terpenoids, saponins and others). However, most of the plant products used are not only based on the long-term experience of the population, but also showed results of *in vitro* activity (Biabiany et al., 2013), although few clinical studies have been performed (Sipponen et al., 2013; Alessandrini et al., 2020; Romero-Cerecero et al., 2020). Thus, few plants have their effective clinically proven superficial fungal infections/ onychomycosis.

The purpose of this work was to evaluate studies that related medicinal plants and their *in vitro* antifungal activity against fungal agents of onychomycosis, as well as studies that reported the clinical efficacy of these plants.

METHODOLOGY

Type of study

This study is an integrative literature review, a method that reviews and summarizes the scientific knowledge produced on a given topic, systematizing scientific evidence and contributing to the development of more consistent conclusions in relation to existing ones. This study followed six steps: 1) establishment of the guiding question and research objectives; 2) literature search; 3) data collection; 4) critical analysis of studies and categorization of the studies; 5) discussion of results; and 6) presentation of the integrative review (Whittemore and Knafl, 2005; Mendes et al., 2008; Souza et al., 2010; Sousa et al., 2017). The study was designed based on the following questions: 1) What are the medicinal plants with potential use for the treatment of onychomycosis, including *in vitro* and clinical studies, and 2) is there evidence in the literature about the use of these plants, or their extracts/subproducts in the treatment of nail mycosis?

Selection of articles

The search for articles was done in databases and virtual libraries: Scientific Electronic Library Online (Scielo), Medical Literature Analysis and Retrieval System Online (Medline/PubMed), CAPES (Periodic Portal from Brazil) and Google Scholar. The main descriptors (Health Sciences Descriptors/Medical Subject Headings - DeCS/MESH) used are shown in Table 1. All of the identified studies were evaluated in a systematically exclusionary way (Figure 1).

The inclusion criteria were publications about medicinal plants and onychomycoses or nail mycoses; full articles available; and published between January 2011 and January 2021. The exclusion criteria were literature reviews, dissertations, theses, and book chapters, abstracts of presentations at scientific events and studies that dealt with pure chemicals, even if they originated from plants.

A form was used to collect information such as: authors, title and year of publication, type of study (*in vivo*; *in vitro*; clinical studies), objectives, country of study, preparations and parts of the plant used, possible mechanisms of action on fungi cells, number of patients included, relevant points of discussion and conclusions of the authors.

Table 1. Association of descriptors used in the search according to the database or virtual library.

Database	Descriptor
CAPES	(onychomycos*) AND (Medicinal Plants)
Scielo	(onychomycos*) AND (treatment)
PubMed	(onychomycos* OR tinea unguium) AND (plants OR medicinal plants OR medicinal herbs OR essential oil OR oil OR extract OR plant extract)
Scholar Google	("onychomycos*" OR "tinea unguium") AND ("plants" OR "medicinal plants" OR "medicinal herbs" OR "essential oil" OR "extract" OR "plant extract")

Source: Perissato and Pedroso (2022).

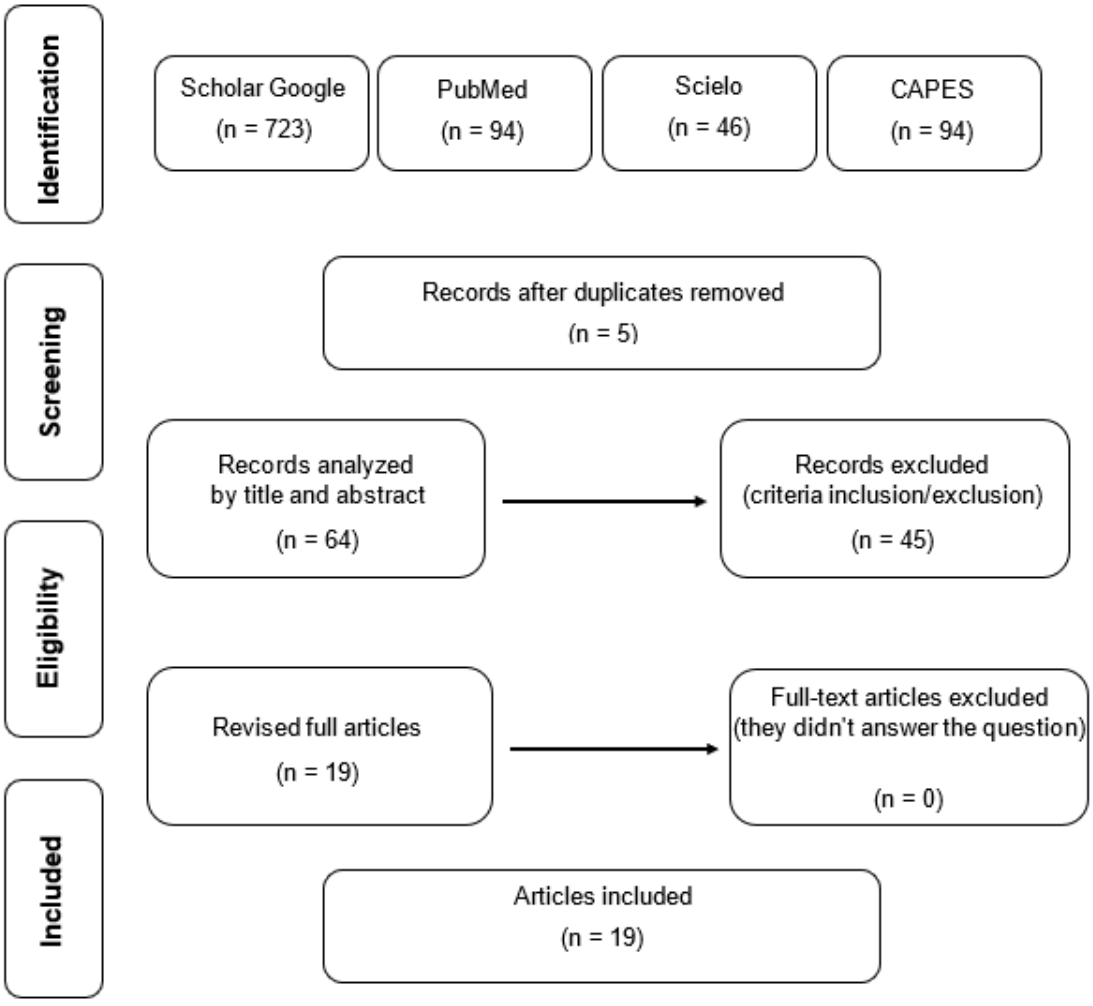

Figure 1. Flowchart of the article selection process for the integrative literature review.
Source: Perissato and Pedroso (2022).

RESULTS

The first search phase resulted in 957 articles, with 19 studies being eligible (Figure 1). Sixteen of them included the *in vitro* evaluation of plant activity on dermatophyte fungi, 11 on yeast and six on non-dermatophyte filamentous fungi (Table 2). Five simultaneously reported the evaluation of three groups of fungi and three

simultaneously on dermatophytes and yeasts, while no papers simultaneously evaluated dermatophytes and non-dermatophyte filamentous fungi (NDFF), or yeast and NDFF (Table 2). Only three articles described clinical studies.

Among the selected articles, four (21%) included studies carried out in India, three (15.7%) in Brazil, two (10.5%) in Nigeria and one (5.2%) in each of the following countries: Spain, Finland, France (Guadeloupe Islands), Italy, Mexico, Portugal, Romania, Thailand, Taiwan and Tanzania (Table 2). The number of articles found varied over the years, with four in 2012 and three each in 2016, 2019 and 2020. Figure 2 lists the chronological frequency of the articles included for evaluation in this study, according to the groups of fungi. In 2021 only January was included.

Among the plants evaluated with antifungal activity are *Angelica major, Cassia alata, Mitracarpus villosus, Lawsonia inermis, Euphorbia sanguinea, Euphorbia cotinifolia, Artemisia maritima, Sapindus saponaria L., Ageratum houstonianum, Piper betle L., Bursera simaruba, Cedrela odorata, Pluchea carolinensis, Cinnamomum zeylanicum, Evolvulus alsinoides, Enterolobium cyclocarpum, Acnistus arborescens, Pisonia aculeata and Allium sativum*. More information is described in Table 3 and the following text.

Ekwealor et al. (2012) studied the *in vitro* antifungal activity of methanolic, hexane and cold aqueous extracts of *Cassia alata, Mitracarpus villosus* and *Lawsonia inermis* plants on the fungi *Aspergillus terreus, Aspergillus sclerotiorum, Aspergillus flavus, Fusarium* sp., *Chrysosporium* sp. *and Scopulariopsis* which were isolated from the nails of 135 farmers with onychomycosis. All extracts evaluated by the agar disk diffusion technique showed activity against those fungi, except for *M. villosus* and *C. alata* extracts, which did not show activity against *A. flavus* and *M. villosus* extract that did not show any activity against *A. terreus*. The authors concluded that the studied extracts could be potential antifungal drugs, as they were effective *in vitro* against non-dermatophyte fungi causing onychomycosis.

Guerrer et al. (2012) analyzed *in vitro* the antifungal activity of ozonized sunflower oil (Bioperoxoil®) in yeast isolated from nail mycosis, comparing it with the activity of the drugs amphotericin B, fluconazole, ketoconazole and itraconazole. They studied *Candida parapsilosis, Candida albicans, Trichosporon asahii, Candida tropicalis* and *Candida guilliermondii* isolates. According to the methodology used (disk diffusion in agar), they observed that the ozonized sunflower oil showed smaller inhibition halos than the traditional antifungal drugs. The researchers argued that the use of a quantitative methodology and clinical-laboratory correlation would be necessary to establish an appropriate treatment protocol, as the results obtained with ozonized sunflower oil may not mean lower efficacy, since clinical studies have not been carried out.

Chiang et al. (2013) analyzed the *in vitro* antifungal effect of *Indigo naturalis* prepared from *Strobilanthes formosanus* (Moore) on the onychomycosis fungi *Trichophyton rubrum, Microsporum gypseum, Epidermophyton floccosum, Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans and Saccharomyces cerevisiae*. They used the disk diffusion technique, in which they placed from 1 to 4 mg of *Indigo naturalis* per disk. Thus, the halo of growth inhibition around the disk was observed in *A. fumigatus* and *C. albicans* at all concentrations, in a dose-dependent manner, that is, the higher the concentration, the greater the halo formed. In *S. cerevisiae*, inhibition occurred only at the highest concentrations (3 mg/disk and 4 mg/disk) while an inhibition halo was only formed at the highest concentration (4 mg/disk) for the other fungi.

Biabiany et al. (2013) analyzed the antifungal activity of ten plants, six of which showed inhibitory activity (*Bursera simaruba*, *Cedrela odorata, Enterolobium cyclocarpum, Evolvulus alsinoides, Pluchea carolinenis* and *Pluchea odorata*) against fungi causing superficial mycoses and four which showed no activity (*Acnistus arborescens, Gliricidia sepium, Pisonia aculeata,* and *Senna bicapsularis*). The species tested were *Malassezia* sp., *Candida krusei, C. albicans, C. parapsilosis, C. glabrata, C. kefyr, Scytalidium dimidiatum, Microsporum canis, Trichophyton tonsurans, T. interdigitale, T. mentagrophytes,* and *Pneumocystis carinii*. Among these, *C. krusei* was the fungus that showed susceptibility to the highest number of extracts evaluated, while *B. simaruba* (ethanolic and ethanolic/water) and *C. odorata* (ethanolic/water) were the ones that showed activity against the highest number of strains evaluated.

The observational study carried out by Sipponen et al. (2013) evaluated the effectiveness of a nail lacquer made with *Picea abies* resin (natural conifer) for the treatment of onychomycosis in 37 patients, who used the product every day for nine months. Mycological examinations of the patients' nails were performed at the beginning and end of treatment. The authors observed that there was a trend towards complete or partial clinical cure in 14 of the 32 eligible patients, which showed favorable results for the efficacy of lacquer containing *P. abies* resin.

Two studies evaluated the essential oil (EO) activity of *Melaleuca alternifolia* (Tea tree) on dermatophyte fungi. Flores et al. (2013) analyzed the EO incorporated in emulsion, nanocapsules and nanoemulsions in an *in vitro* model of onychomycosis. Fragments nails and powdered nails were infected with *Trichophyton rubrum*. They observed that EO incorporated in nanocapsules and nanoemulsions exhibited activity against *T. rubrum*.

The study by Kumar (2014) evaluated the antifungal activity of the EO of 32 plants against *Candida albicans* and *T. rubrum*. They observed that *Ageratum houstonianum* leaf EO presented a minimum inhibitory concentration (MIC) of 400 ppm and a minimum fungicidal concentration (MFC) of 500 ppm. Thus, the authors concluded that the EO from the *A. houstonianum* leaf has

Table 2. Synthesis of selected articles for the integrative literature review.

Article reference/title	Brief objective of the paper	Country of study	Kind of study	Dermatophytes	Yeasts	Non-dermatophyte
Alessandrini et al. (2020)	To evaluate the efficacy, tolerability and patient compliance of a new topical antifungal containing vitamin E and essential oils of lemon, oregano and tea tree.	Italy	*in vivo*	*T. rubrum; T. interdigitalis; Fusarium* sp*; Scopulariopsis bevicaulis*	-	-
Romero-Cerecero et al. (2020)	To evaluate the clinical and mycological efficacy of *Ageratina pichinchensis* extract for the treatment of onychomycosis in patients with type 2 diabetes mellitus.	Mexico	*In vivo*	*Trichophyton rubrum; Trichophyton mentagrophytes; Epidermophyton floccosum*	*Candida* spp.	-
Sipponen et al. (2013)	Evaluate the efficacy of lacquer containing natural conifer resin for the treatment of onychomycosis.	Finland	*in vivo*	*Trichophyton rubrum; Trichophyton mentagrophytes*	-	-
Biabiany et al. (2013)	To evaluate the antifungal activity of ten plants from the archipelago of Guadalupe against superficial mycoses.	France (Guadeloupe Island)	*in vitro*	*Microsporum canis; Trichophyton tonsurans; Trichophyton interdigitale; Trichophyton mentagrophytes*	*Candida krusei; Candida albicans; Candida parapsilosis; Candida glabrata; Candida kefyr*	*Scytalidium dimidiatum*
Cavaleiro et al. (2015)	To Characterize the chemical composition of *Angelica major* essential oil and determine the in vitro antifungal activity of the oil and its two main constituents on species of pathogenic fungi (*Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp. and dermatophytes) and also verify the activity cytotoxic by the hemolytic activity assay.	Portugal	*in vitro*	*Trichophyton mentagrophytes; T. mentagrophytes; T. rubrum; T. verrucosum; Microsporum canis; M. gypseum; Epidermophyton floccosum*	*Candida albicans; C. krusei; C. tropicalis; C. parapsilosis; C. dubliniensis; C. glabrata*	*Aspergillus* spp., *A. flavus, A. fumigates, A. niger*
Chiang et al. (2013)	Investigate the antimicrobial effects of Indigo Naturalis on the pathogens of skin and nail infections.	Taiwan	*In vitro*	*T. rubrum, M. gypseum, E. floccosum*	*C. albicans*, *Cryptococcus neoformans* e *Saccharomyces cerevisiae*	*Aspergillus fumigatus*
Ekwealor et al. (2012)	To investigate the antifungal activity of methanol, hexane and aqueous extracts of *Cassia alata*, *Mitracarpus villosus* and *Lawsonia inermis* against non-dermatophyte fungi isolated from rice farmers with onychomycosis in Nigeria.	Nigeria	*in vitro*	-	-	*Aspergillus terreus, Aspergillus sclerotiorum, Aspergillus flavus, Fusarium* sp., *Scopulariopsis* sp., *Chrysosporium* sp.
Flores et al. (2013)	To evaluate the *in vitro* antifungal activity of nanoemulsions and suspensions of nanocapsules containing tea tree oil against *Trichophyton rubrum*.	Brazil	*in vitro*	*Trichophyton rubrum*	-	-

Table 2. Contd.

Giwanon et al. (2016)	To investigate the anti-dermatophyte activity of herbal extracts, compare the potency with the antifungal agents' econazole, miconazole, ketoconazole, metronidazole and voriconazole and also evaluate the antifungal potency of the cream of the plant with the highest activity.	Thailand	*in vitro*	*Trichophyton* mentagrophytes and *Trichophyton rubrum*	-	-
Guerrer et al. (2012)	To evaluate the antifungal activity of ozonized oil (Bioperoxoil ®) in comparison with amphotericin B, fluconazole, ketoconazole and itraconazole.	Brazil	*in vitro*	-	*Rhodotorula rubra; Candida zeylanoides; Candida krusei; C. albicans; C. parapsilosis; C. tropicalis; C. guilliermondii; Hansenula anomala; Saccharomyces cerevisiae; Trichosporon asahii; Phaeococcomyces* sp.	-
Kumar (2014)	To evaluate the antifungal activity of ozonized oil (Bioperoxoil ®) in comparison with amphotericin B, fluconazole, ketoconazole and itraconazole.	India	*in vitro*		*Candida albicans*	-
Manivannan et al. (2019)	To evaluate the antifungal activity of *Cinnamomum zeylanicum* extracted from bark, made to detect new sources of antifungal agents.	India	*in vitro*	*Trichophyton rubrum; Trichophyton mentagrophytes; Trichophyton simii; Epidermophyton floccosum*	*Candida albicans*	-
Marcos-Tejedor et al. (2021)	To evaluate the *in vitro* efficacy of tea tree essential oil to provide less harmful alternatives against the main causative agents of onychomycosis.	Spain	*in vitro*	*Trichophyton rubrum; Trichophyton mentagrophytes*	-	-
Mendes et al. (2021)	To evaluate the antifungal activity of *Sapindus saponaria* extract on dermatophyte fungi and verify its cytotoxicity and permeability through the human nail.	Brazil	*in vitro*	*Trichophyton rubrum; T. mentagrophytes; T. interdigitale*	-	-
Mishra et al. (2016)	To investigate the essential oil of *Artemisia maritima* L. a member of the Compositae family (Asteraceae) as an effective antifungal against infective nail fungus, *T. mentagrophytes* and *T. rubrum*.	India	*in vitro*	*Trichophyton rubrum; Trichophyton mentagrophytes*	-	-
Morah et al. (2016)	To determine the phytochemical composition of *Euphorbia sanguinea* sap and the antifungal activity against agents causing onychomycosis.	Nigeria	*in vitro*	*Trichophyton rubrum*	*Candida albicans, Candida glabrata*	*Aspergillus flavus*
Pandit et al. (2020)	To determine the antifungal activity of *Cissus quadrangularis* extract for the development of antifungal nail lacquer loaded for the treatment of onychomycosis	India	*in vitro and ex-vivo*	-	*Candida albicans*	-

Table 2. Contd.

Pârvu et al. (2019)	To evaluate the antifungal effect of the extract of *Allium sativum* against of the onychomycosis agents *Meyerozyma guilliermondii* e *Rhodotorula mucilaginosa*	Romania	*in vitro*	-	*Meyerozyma gulliermondii* and *Rhodotorula mucilaginosa*	-
Runyoro et al. (2017)	To investigate the antifungal activity of leaf and stem bark extracts of *Euphorbia cotinifolia* L. on onychomycosis fungi.	Tanzania	*in vitro*	*Trichophyton rubrum; Trichophyton mentagrophytes;*	*Candida albicans*	*Aspergillus niger*

Source: Perissato and Pedroso (2022)

fungicidal activity, as well as other advantages, such as low cost and availability.

Cavaleiro et al. (2015) studied the *in vitro* antifungal activity of *Angelica major* EO and two of its main components, α-pinene and cis-β-ocimene, on yeasts (*Candida* spp., *C. neoformans*), NDFF (*Aspergillus* spp.) and dermatophytes (*T. mentagrophytes, T. rubrum, T. verrucosum, Microsporum canis, M. gypseum and Epidermophyton floccosum*). EO MICs ranged from 2.5 to greater than 10 µl/mL for *Candida* spp. isolates, from 0.32 to 1.25 µL/mL for dermatophytes and from 5 µL/mL to greater than 10 µl/mL for *Aspergillus* isolates. The authors found that the MICs of the isolated compounds were lower than that of EO for dermatophytes and *Aspergillus* species. Thus, they concluded that *A. major* EO presents bioactive compounds (α-pinene and cis-β-ocimene) with antifungal activity demonstrated *in vitro*. *Artemisia maritima* EO was evaluated for its antifungal properties *in vitro* against *Trichophyton rubrum* and *Trichophyton mentagrophytes* species. The authors evaluated the EO-fungal cell contact time for the fungicidal effect. Pure EO showed fungicidal activity in 10 seconds for both isolates. The time to reach the fungicidal effect was 60 minutes for *T. rubrum* (MFC of 0.4 µL/mL) and 70 minutes for *T. mentagrophytes* (MFC 0.5 µL/mL). Thus, the EO demonstrated high fungicidal activity *in vitro* against those dermatophytes tested and those authors considered that *A. maritima* EO can be a potential alternative for the treatment of onychomycosis after appropriate clinical trials for proof (Mishra and Singh, 2016).

The *in vitro* study by Giwanon et al. (2016) analyzed the antifungal activity of the ethanol extract of eight plants (*Piper betle, Argyreia nervosa, Punica granatum, Piper sarmentosum, Rhinacanthus nasutus, Plumeria obtusa, Morinda elliptica* and *Crateva adansonii*) on the fungi *T. rubrum* and *T. mentagrophytes*. The *P. betle* extract showed a better inhibitory activity than all others. Thus, it was selected to compose a cream-based formulation, with the objective of comparing the *in vitro* efficacy with five antifungal drugs (econazole, miconazole, ketoconazole, metronidazole and voriconazole). The results obtained indicated that the cream formulated with *P. betle* presented antifungal activity *in vitro* superior to the five analyzed drugs, constituting a potential alternative to the topical treatment of infections caused by those dermatophytes.

Morah and Okoi (2016) described the phytochemical composition and physicochemical properties of the stem sap of *Euphorbia sanguinea*. In addition, they analyzed the *in vitro* antifungal and antibacterial activities against the following species: *Candida albicans*, *Candida glabrata*, *Aspergillus flavus* and *Trichophyton rubrum*, as well as *Escherichia coli*, *Staphylococcus albus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi* and *Klebsiella pneumoniae*. The *in vitro* antibacterial activity was not confirmed. However, they reported strong antifungal activity when compared to traditional antifungal drugs (fluconazole, itraconazole, posaconazole, voriconazole, amphotericin B, flucytosine, caspofungin and griseofulvin). *Candida albicans* and *Candida glabrata* were the species with the highest susceptibility.

The study by Runyoro et al. (2017) analyzed the antifungal activity of methanol extracts from leaves and stem bark of *Euphorbia cotinifolia* (dairy-red) on *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Aspergillus niger*, *Candida albicans* species. The leaf extract inhibited the growth of *T. rubrum*, forming 12 mm halos (disk diffusion technique), while *T. mentagrophytes* formed a 17 mm halo, *A. niger*, 15 mm and no halo for *C. albicans*. The stem bark extract formed a 9 mm halo for *T. mentagrophytes* and *A. niger* and no activity for *T. rubrum* and *C. albicans*. Manivannan and Bhuvaneswari (2019) analyzed the antifungal potential of the ethanolic and aqueous extract of the bark of *Cinnamomum zeylanicum* on the onychomycosis agents: *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton simii, Epidermophyton floccosum and Candida albicans*. Different concentrations of the extracts were evaluated *in vitro* by the agar disk diffusion method. When 10 mg/disk of ethanolic extract

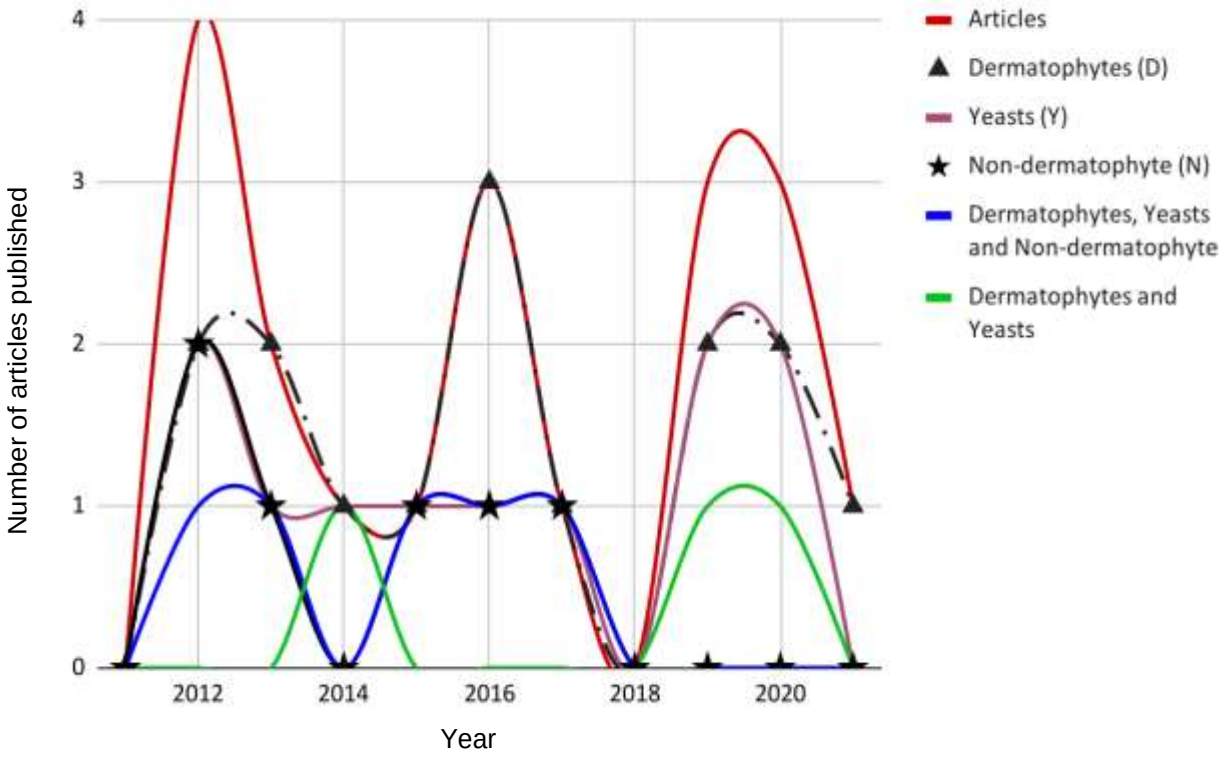

Figure 2. Chronological frequency of articles published from January 2012 to January 2021.
Source: Perissato and Pedroso (2022)

was used, all microorganisms tested were inhibited, with halo diameters ranging from 6 mm (*E. floccosum*) to 14 mm (*T. rubrum*). Using the broth dilution methodology, the authors observed that all isolates showed a MIC of 25 mg/mL. The MFC observed ranged from 50 mg/mL (*T. rubrum*) to 180 mg/mL (*T. mentagrophytes*). The aqueous extract also inhibited the growth of all tested isolates, but with less effectiveness *in vitro*. Thus, the authors concluded that extracts can be alternatives for antifungal treatment for onychomycosis caused by those agents and show promise in the development of pharmaceutical formulations.

The *in vitro* fungicidal activity of *Allium sativum* (garlic) extract was evaluated on fungi isolated from the nail of a patient with mixed infection by *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa*. The extract produced irreversible structural changes in these fungi, resulting in cell death. The *in vivo* antioxidant effect of the extract showed superior results when compared to allicin and diclofenac. Thus, the combination of antifungal and antioxidant activity makes garlic extract a potential alternative treatment for onychomycosis caused by those fungi (Pârvu et al., 2019).

The antifungal activity of *Cissus quadrangularis* extract was studied for the development of a nail lacquer loaded for the treatment of onychomycosis, including a penetration enhancer (salicylic acid) in the nail plate. The fungicidal activity of the extract was tested *in vitro* against *Candida albicans* and showed a smaller zone of inhibition (12 ± 2 mm) with 10 mg/mL of the extract, whereas the zone of inhibition was greater (18 ± 2 mm) with 50 mg/mL. For lacquer loaded development, five formulations with different concentrations of components (salicylic acid, fluconazole and the extract) were evaluated, showing that salicylic acid increases the penetration of the extract that showed better antifungal activity than fluconazole (Pandit et al., 2020).

Alessandrini et al. (2020) carried out a clinical study of 20 patients to evaluate the antifungal activity of a topical formulation composed of the association of vitamin E and essential oils of lemon (*Citrus aurantifolia*), oregano (*Origanum vulgare*) and tea tree (*Melaleuca alternifolia*). The formulation was applied for six months on the nail plate and in the periungual area. Fifteen patients were completely cured, two showed significant improvement, one remained stable and two did not complete the treatment. The study highlighted 11 patients who showed relevant improvement within three months, which was maintained even after six months of treatment interruption.

The efficacy of the standardized *Ageratina pichinchensis* enkecalin extract was evaluated *in vivo* in a double-blind, randomized, controlled clinical trial, which included 71 patients with type 2 diabetes *mellitus*. Among

Table 3. Fungi and plant species and products with *in vitro* antifungal activity.

Onychomycosis agent fungus	Plant/Product evaluated	Preparation	Antifungal activity (quantitative*)	Reference
Aspergillus terreus	*Cassia alata*	Methanol extracts	10 mg/disk	Ekwealor (2012)
	Mitracarpus villosus		40 mg/disk	
	Lawsonia inermis		10 mg/disk	
	Cassia alata	Hexane extracts	40 mg/disk	
	Lawsonia inermis		40 mg/disk	
Aspergillus sclerotiorum	*Cassia alata*	Methanol extracts	20 mg/disk	Ekwealor (2012)
	Mitracarpus villosus		20 mg/disk	
	Lawsonia inermis		10 mg/disk	
	Cassia alata	Hexane extracts	40 mg/disk	
	Mitracarpus villosus		40 mg/disk	
	Lawsonia inermis		40 mg/disk	
Aspergillus fumigatus	*Angelica major*	Essential oil	10 µL/mL	Cavaleiro et al. (2015)
Aspergillus flavus	*Angelica major*	Essential oil	≥10 µL/mL	Cavaleiro et al. (2015)
	Lawsonia inermis	Methanol extracts	40 mg/disk	Cavaleiro et al. (2015)
	Lawsonia inermis	Hexane extracts	40 mg/disk	
	Euphorbia sanguinea	Stem sap	0.125 $mgcm^{-3}$	Morah and Okoi (2016)
Aspergillus niger	*Angelica major*	Essential oil	5.0-10 µL/mL	Cavaleiro et al. (2015)
	Euphorbia cotinifolia	Stem bark	5 mg/ml	Runyoro et al. (2017)
		Leaves	2,5 mg/ml	
Fusarium sp.	*Cassia alata*	Methanol extracts	10 mg/disk	Ekwealor (2012)
	Mitracarpus villosus		10 mg/disk	
	Lawsonia inermis		10 mg/disk	
	Cassia alata	Hexane extracts	10 mg/disk	
	Mitracarpus villosus		20 mg/disk	
	Lawsonia inermis		20 mg/disk	
Trichophyton rubrum	*Euphorbia cotinifolia*	Stem bark	5 mg/ml	Runyoro et al. (2017)
		Leaves	5 mg/ml	
	Artemisia maritima	Essential oil	CIM= 0.3µL/mL CFM=0.4 µL/mL	Mishra et al. (2016)
	Euphorbia sanguinea	Stem sap	0.125 $mgcm^{-3}$	Morah and Okoi (2016)
	Sapindus saponaria L.	Hydroalcoholic extract	781.25 µg/mL	Mendes et al. (2021)
	Ageratum houstonianum	Leaf essential oil	CIM = 400 ppm CFM = 500 ppm	Kumar (2014)
	Piper betle L.	Cream	100 µg/mL	Giwanon et al. (2016)
	Bursera simaruba	Ethanolic extract	200 µg/mL	Biabiany et al. (2013)
	Bursera simaruba	Hydro-ethanolic extract	200 µg/mL	
	Cedrela odorata		200 µg/mL	
	Pluchea carolinensis		400 µg/mL	

Table 3. Contd.

	Cinnamomum zeylanicum	Ethanol barks extract	25mg/mL	Manivannan and Bhuvaneswari (2019)
	Angelica major	Essential oil	0.32 µL/mL	Cavaleiro et al. (2015)
Trichophyton verrucosum	*Angelica major*	Essential oil	1.25 µL/mL	Cavaleiro et al. (2015)
Trichophyton mentagrophytes	*Angelica major*	Essential oil	0.32 µL/mL	Cavaleiro et al. (2015)
	Sapindus saponaria L.	Hydroalcoholic extract	390.63 µg/mL	Mendes et al. (2021)
	Cinnamomum zeylanicum	Ethanol barks extract	25mg/mL	Manivannan and Bhuvaneswari (2019)
	Artemisia maritima	Essential oil	CIM= 0.4µL/mL CFM=0.5 µL/mL	Mishra et al. (2016)
	Euphorbia cotinifolia	Stem bark	2.5 mg/mL	Runyoro et al. (2017)
		Leaves	2.5 mg/mL	
	Bursera simaruba	Ethanolic extract	200 µg/ *mL*	
	Bursera simaruba	Hydro-ethanolic extract	200 µg/ *mL*	Biabiany et al. (2013)
	Cedrela odorata		200 µg/ *mL*	
	Pluchea carolinensis		400 µg/ *mL*	
	Piper betle L.	Cream	100 µg/mL	Giwanon et al. (2015)
Trichophyton interdigitale	*Bursera simaruba*	Ethanolic extract	200 µg/mL	
	Cedrela odorata		200 µg/mL	
	Bursera simaruba	Hydro-ethanolic extract	200 µg/mL	Biabiany et al. (2013)
	Cedrela odorata		200 µg/ *mL*	
	Pluchea carolinensis		400 µg/ *mL*	
	Sapindus saponaria L.	Hydroalcoholic extract	195.31 µg/mL	Mendes et al. (2021)
Candida albicans	*Cinnamomum zeylanicum*	Ethanol barks extract	25mg/mL	Manivannan and Bhuvaneswari (2019)
	Ageratum houstonianum	Leaf essential oil	CIM = 400 ppm CFM = 500 ppm	Kumar (2014)
	Angelica major	Essential oil	2.5>MIC≥10 µL/mL	Cavaleiro et al. (2015)
	Euphorbia sanguinea	Stem sap	0.125 $mgcm^{-3}$	Morah and Okoi (2016)
Candida parapsilosis	*Bursera simaruba*	Ethanolic extract	200 µg/mL	Biabiany et al. (2013)
Candida krusei	*Cedrela odorata*	Hydro-ethanolic extract	50 µg/mL	Biabiany et al. (2013)
	Evolvulus alsinoides		50 µg/ mL	
	Pluchea odorata		200 µg/ mL	
	Pluchea carolinensis		200 µg/ *mL*	
	Bursera simaruba	Ethanolic extract	50 µg/Ml	
	Cedrela odorata		50 µg/Ml	
	Pluchea odorata		200 µg/Ml	
	Pluchea carolinensis		200 µg/Ml	
	Bursera simaruba		50 µg/Ml	

Table 3. Contd.

	Angelica major	Essential oil	2.5-5 µL/mL	Cavaleiro et al. (2015)
Candida kefyr	*Pluchea carolinensis*	Hydro-ethanolic extract	200 µg/Ml	Biabiany et al. (2013)
	Bursera simaruba		50 µg/Ml	
	Bursera simaruba	Ethanolic extract	50 µg/Ml	
Candida tropicalis	*Angelica major*	Essential oil	≥10 µL/mL	Cavaleiro et al. (2015)
Candida dubliniensis	*Angelica major*	Essential oil	2,5-5 µL/mL	Cavaleiro et al. (2015)
Candida parapsilosis	*Angelica major*	Essential oil	≥10 µL/mL	Cavaleiro et al. (2015)
Candida glabrata	*Angelica major*	Essential oil	≥10 µL/mL	Cavaleiro et al. (2015)
	Euphorbia sanguinea	Stem sap	0.125 $mgcm^{-3}$	Morah and Okoi (2016)
Cryptococcus neoformans	*Angelica major*	Essential oil	0.16 µL/mL	Cavaleiro et al. (2015)
Chrysosporium sp.	*Cassia alata*	Methanol extracts	DD = 10 mg	Ekwealor (2012)
	Mitracarpus villosus		10 mg/disk	
	Lawsonia inermis		10 mg/disk	
	Cassia alata	Hexane extracts	40 mg/disk	
	Mitracarpus villosus		20 mg/disk	
	Lawsonia inermis		20 mg/disk	
Scopulariopsis sp.	*Cassia alata*	Methanol extract	10 mg/disk	
	Mitracarpus villosus		40 mg/disk	
	Lawsonia inermis		10 mg/disk	
	Cassia alata	Hexane extract	80 mg/disk	
	Mitracarpus villosus		80 mg/disk	
	Lawsonia inermis		20 mg/disk	
Epidermophyton floccosum	*Angelica major*	Essential oil	0.32 µL/mL	Cavaleiro et al. (2015)
	Cinnamomum zeylanicum	Ethanol barks extract	25mg/mL	Manivannan and Bhuvaneswari (2019)
Trichophyton simii	*Cinnamomum zeylanicum*	Ethanol barks extract	25mg/mL	Manivannan and Bhuvaneswari (2019)
Malassezia sp.	*Bursera simaruba*	Ethanolic extract	500 µg/mL	Biabiany et al. (2013)
	Pluchea carolinensis	Hydro-ethanolic extract	400 µg/mL	
Scytalidium dimidiatum	*Enterolobium cyclocarpum*	Ethanolic extract	600 µg/mL	Biabiany et al. (2013)
Microsporum gypseum	*Angelica major*	Essential oil	0.64 µL/mL	Cavaleiro et al. (2015)
Microsporum canis	*Angelica major*	Essential oil	0.32 µL/mL	Cavaleiro et al. (2015)
	Bursera simaruba	Ethanolic extract	200 µg/mL	Biabiany et al. (2013)
Trichophyton tonsurans	*Bursera simaruba*	Ethanolic extract	200 µg/mL	Biabiany et al. (2013)
	Bursera simaruba	Hydro-ethanolic extract	200 µg/Ml	
	Cedrela odorata		200 µg/Ml	
Pneumocystis carinii	*Acnistus arborescens*	Hexanic extract	100 µg/mL	Biabiany et al. (2013)

Table 3. Contd.

	Cedrela odorata		≤1 µg/mL	
	Pisonia aculeata		100 µg/mL	
	Pluchea carolinensis		100 µg/Ml	
	Acnistus arborescens	Ethanolic extract	10 µg/Ml	
	Cedrela odorata		≤1 µg/mL	
	Acnistus arborescens	Hydro-ethanolic extract	10 µg/Ml	
	Cedrela odorata		≤1 µg/mL	
Rhodotorula mucilaginosa	*Allium sativum*	Ethanolic extract	120 mg/mL	Pârvu et al. (2019)
Meyerozyma guilliermondii	*Allium sativum*	Ethanolic extract	120 mg/mL	Pârvu et al. (2019)

*DD: disk diffusion in agar; MIC: minimal inhibitory concentration expressed in µg/mL; MIC (v/v): MIC expressed as µL/mL.
Source: Perissato and Pedroso (2022)

these patients, 35 were included in the experimental group, which used enamel containing the extract, and 36 patients were in the control group, who used nail lacquer loaded containing 8% ciclopirox. The authors observed that there was a reduction in the severity of injuries and in the number of affected nails in both the control (77.2%) and experimental (78.5%) groups. Thus, they concluded that the extract showed clinical and mycological efficacy in the treatment of mild to moderate onychomycosis (Romero-Cerecero et al., 2020).

Marcos-Tejedor et al. (2021) evaluated the *in vitro* activity of EO against *T. rubrum* and *T. mentagrophytes*.

At a concentration equal to or greater than 0.05% (m/v), a fungistatic effect on *T. rubrum* was observed, while the same effect was observed at a concentration equal to or greater than 0.04% for *T. mentagrophytes*. Thus, two studies have confirmed that tea tree EO have *in vitro* activity against dermatophyte fungi and can be either an alternative or adjunctive treatment for nail mycosis (Flores et al., 2013; Marcos-Tejedor et al., 2021).

Mendes et al. (2021) analyzed the *in vitro* antifungal activity on the fungi *Trichophyton rubrum*, *T. mentagrophytes* and *T. interdigitale*, the cytotoxicity on *HeLa* cells and the permeability of the hydroalcoholic extract of *Sapindus saponaria* L. in nail fragments *in vitro*. For the *in vitro* evaluation of the antifungal activity, they used the broth microdilution method and found equivalent MIC and MFC, which ranged from 195.31 to 781.25 µg/mL. The extract showed low cytotoxicity and excellent permeability in the nails; in one hour, it fully penetrated the nail layers and remained detectable for at least 24 hours. Thus, the authors concluded that the extract of *Sapindus saponaria* L has potential in the topical treatment of onychomycosis.

DISCUSSION

Fungal infections in nails start when the fungus cell comes into contact with its surface or with the periungual region, followed by adhesion, invasion and multiplication. These mycoses are a major health problem, since they occur worldwide, are persistent, difficult to treat and negatively affect the quality of life of the infected individual, especially in women (Stewart et al., 2021).

The traditional treatment of onychomycosis is usually very long (over six months) when compared to other superficial mycoses. It is made with traditional antifungal drugs such as itraconazole, clotrimazole and terbinafine, among others, in pharmaceutical preparations for oral or topical use. The use of alternative natural therapies has been poorly studied. Plants are sources of various bioactive compounds with potential for antifungal biological activity. Many of these plants and their derived products, such as extracts, essential oils and resins, are known for their medicinal properties and have also been evaluated for antifungal effects, either through *in vitro* or *in vivo* studies (Biabiany et al., 2013; Flores et al., 2013). In this literature review, published articles that addressed the antifungal activity of products extracted from plants on fungi that cause onychomycosis were analyzed.

The included articles were mostly from researchers from India and Brazil, but also from other countries to a lesser extent. Most studies reported *in vitro* results, which mainly included plant evaluations of dermatophyte fungi and/or yeast. Figure 1 shows the list of studies published by year, with four in 2012 and three in each of the

years 2016, 2019 and 2020.

Currently, the search for new therapeutic strategies and new drugs from natural sources, especially plants, as well as from different extracts and pharmaceutical formulations, has increased the interest in research involving antifungal activity and it's potentializing through different strategies. New products can contribute to the conventional therapy for mycoses, considering the increase in fungal agents responsible for infections described in the literature, as well as emerging fungi with resistance to conventional drugs.

The nail has a dense constitution of cells, which makes it difficult for different substances to penetrate. Thus, in addition to considering evidence of antifungal activity in pharmaceutical formulations, it is necessary to seek ways to increase the penetration of compounds into the nail plate. The nail keratin structure and its pH can interfere with the antifungal activity in clinical tests (Davies-Strickleton et al., 2020). According to Pandit et al. (2020), as the salicylic acid concentration increased, so did the penetration of the extract of *Cissus quadrangularis*. Nanocapsules and nanoparticles can also facilitate the penetration of active substances into the nail layer (Flores et al., 2013), hence the importance of not only researching antifungal compounds, but also formulations that improve the penetration efficiency of substances. On the other hand, Davis-Strickleton et al. (2020) showed *in vitro* the binding of the antifungal drug to keratin, reducing its activity. Thus, it would be necessary to increase the concentration of the drug to maintain efficacy. They concluded that there needs to be a balance between drug efficacy and permeation through the nail, which is a requirement for the development of effective topical therapies.

Thus, studies involving strategies for incorporating the active ingredient (whether plant extracts, essential oils, or others) in pharmaceutical formulations, which constitute the vehicle to take the drug to the agent causing the infection, can contribute to the effectiveness of the therapy (Biabiany et al., 2013). Formulations such as emulsions, nanoemulsions, nanocapsules and loaded lacquers, which can be used topically in clinical studies, can confirm the *in vivo* antifungal biological activity observed in the *in vitro* studies, in addition to confirming their safety (Pandit et al., 2020). Therefore, checking *in vitro* antifungal activity is one of the first steps in a long process prior to the drug becoming available over the counter. In addition, the antifungal potential of a new drug, as well as natural products, is evaluated primarily by *in vitro* studies, which confirm the antifungal activity. From there, several other studies are needed, such as cytotoxicity and effectiveness, for example, in animal models or other alternatives followed by clinical studies.

In vitro studies on antifungal activity also present technical aspects that need to be controlled to obtain effective and comparable results between different studies. The lack of standardization of methods for evaluating *in vitro* tests was one of the limitations that prevented more detailed conclusions, as well as the comparison between different studies. The type of substance used to obtain the extracts (the extractors) influence the results of *in vitro* antifungal activity tests, as the active constituents can be selectively solubilized in different solvents, whether organic or inorganic (Biabiany et al., 2013; Runyoro et al., 2017; Manivannan and Bhuvaneswari, 2019). In addition, the methodological conditions for carrying out the tests and the microorganisms used in the study, genera and species (and even different strains), can lead to variable results and conclusions due to the intrinsic sensitivity of each technique.

Conclusion

In this study, it was observed that there are few publications on the antifungal activity of plants in animal models (or alternatives), as well as clinical studies. Three clinical studies were found, one double-blind, randomized and controlled and two others which were observational. All patients included in those studies had laboratory evaluations of nail fragments (direct examination and culture for fungi), so that clinical and laboratory diagnosis for onychomycosis was confirmed. The treatments performed with the essential oil (one study) and with the nail lacquer loaded containing the extract (two studies) for a period of 6 to 9 months; clinical and mycological cures were confirmed for most patients.

Onychomycosis presents varied manifestations, of limited severity, but with significant epidemiological importance and impact on the quality of life of affected individuals. Treatment is time-consuming and requires persistence of infected individuals undergoing treatment, complying with the recommendations and guidelines of the health professional. As we can see in this study, the use of preparations from plants, such as extracts, their fractions, essential oils and resins, needs to be more extensively studied for use as alternatives to conventional treatment; this includes rational and safe use, since natural products from plants can present toxicity and also need to have clinical evaluation demonstrated by controlled studies, which seems to exist for few plants.

The effectiveness of products originating from the plants used in the studies was dependent on the plant used, drug concentration and fungal species; that is, the *in vitro* antifungal activity observed was fungus-specific, which means that the evaluated plant may have an antifungal effect on an infectious agent but no activity on another. We reinforce the importance of the correct diagnosis of onychomycosis and laboratory identification of the fungal species involved in the infection process.

In conclusion, this study shows that many medicinal plants have *in vitro* activity against fungal agents of onychomycosis; however, few studies have a clinical approach that proved the usefulness and effectiveness of some plants to treat nail infections. The authors emphasize

the need for clinical studies, using different strategies and the development of different pharmaceutical formulations, to establish the effectiveness, dosage, and best therapeutic regimen. This will ensure that greater clinical efficacy with fewer undesirable effects can be obtained, for these products to be added to clinical practice for the treatment of onychomycosis.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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