International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 01-01-2022, Accepted: 16-01-2022, Published: 01-02-2022
Volume 7, Issue 2, 2022, Page No. 29-34

Herbal medicine as an alternative for the treatment of onychomycosis

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Abstract
Onychomycosis is the most common fungal infection of fingernails or toenails that occurs in the general population. The rate of incidence has increased during the last 10 years mainly due to immuno compromised conditions. The high occurrence of fungal infections is now a growing problem of medical science due to the development of drug resistance against the antifungal activities in the pathogen. The use of medicinal plants and herbal products against fungal infection is one of the alternative treatments of synthetic drugs that are considered safe, free from adverse side effects, cheaper, and often easily available. This review aims to explore traditionally used herbal medicine and its scientific effects against onychomycosis. There is a need to take up more studies to better understand the effect of herbal plants against the emerging pathogen in the changing environment which causes various dermatological health problems.

Keywords: onychomycosis, medicinal plants, antifungal agents, plant extract

Introduction
Onychomycosis by far one of the most common superficial infections occur in the general population. The term "onychomycosis" was taken from the Greek word "onyx" meaning nail and "mykes" means fungus [1]. Fungal infections of toenails and fingernails can act as a reservoir of pathogenic microorganisms, which may spread possibly to other persons. Among immuno compromised patients, especially in diabetic feet, it can limit mobility and affect peripheral circulation and complications can lead to amputations. Oral antifungal medications, topical ointments, and other therapies like laser treatment can be a long and expensive process. However, in many cases treatment may not prove very effective. The rate of incidence of fungal infections has increased during the last 10 years mainly due to the increased incidence of immuno compromised patients. As a result, increased use of antifungal drugs or in many cases non-prescription use of topical antifungal agents will lead to increased selection pressure and risk of resistance [2]. According to Mycologists, drug selection pressure will increase over time lead to more widespread resistance [2]. Lachoria *et al*. (1999) mentioned the high occurrence of fungal infections is a growing problem of medical science due to the development of drug resistance against the antifungal activities in the pathogen [3].

Medicinal plants for the treatment of onychomycosis
The use of medicinal plants and herbal products against fungal infection is one of the alternative treatments of synthetic drugs that are considered safe, free from adverse side effects, cheaper, and often easily available. Plants secondary metabolites are an important constituent of antifungal drugs. Identifying antifungal agents against fungi is difficult as compared to bacteria because of the tough fungal cell wall, which is composed of chitin and other polysaccharides such as β-glucans [4]. Indeed, the antifungal activity of plant extracts and essential oils from plants, including their effects on dermatophytes, NDMs has been reported from time to time from various parts of the world [5]. The use of medicinal herbs in the treatment of nail infection is an age-old practice in India.
Since the beginning of human civilization, man has been using many plants and their extracts as herbal medicine all over the world. Indian traditional systems of medicine such as Ayurveda, Unani, Siddha, Allopathy all are old practices and being used by mankind from the time of immemorial [6]. Medicinal plants are being used in different healthcare systems throughout the world because of their therapeutic properties. Ethnobotany is the branch that deals with the ancient use of medicinal plants. This branch of Botany was introduced by J. W. Harshberger (1896), it deals with the collection of useful plants and plant products for curing various diseases [7]. Ayurveda documented near about 700 species 8,000 herbal remedies [8]. At present in India, nearly 960 medicinal plants are in commercial use in pharmacological industries. However, an ethnobotanical survey estimated that more than 7,000 plant species are being used by 4,539 different ethnic and tribal communities for health care in various parts of India especially N-E Indian states [7]. Medicinal plants play a key role in the pharmaceutical industry for their broad range of phytochemicals. These phytochemicals are biologically active

compounds derived from leaves, flowers, seeds, barks, roots and pulps. They serve as a raw material for making novel drugs that offer new prospects in the modern healthcare system [9].

Fungal infection of the nail is a common disease reported from every part of the world. Scientists recommended herbal medicines for the public as less toxic and more effective than synthetic drugs for various ailments [10]. Fluconazole, terbinafine and itraconazole are the most commonly used FDA approved drugs for the treatment of nail infection. They penetrate inside the nail apparatus easily and persist there several months after stopping therapy. The use of medicinal plants for the treatment of nail infection rather than the use of synthetic drugs is an old traditional practice in many parts of the world. In the past few years, many researchers have been isolated many antifungal compounds from plant extracts. These compounds are nothing but plant secondary metabolites that serve as a defence mechanism against invading micro-organisms [11].

In vivo and *in vitro* antifungal activities of medicinal plants have been studied in recent years. Various methods can be applied to study the antifungal activity of plant extract [4]. Handa *et al*. (2008) and Gupta *et al*. (2012) documented preparation methods of plant extracts to determine biological activities. The conventional methods of plant extraction preparation include Soxhlet extraction, thermal desorption, steam distillation, maceration, percolation, membrane process, infusion, decoction, extraction leaching, sample disruption method, surfactant mediated extraction methods etc. [12]. Few other advanced extraction methods are microwave-assisted extraction, ultrasonication assisted extraction, supercritical fluid extraction etc. [13]. Plant extraction methods are nothing but a separation process of medicinal components present in the plant by using selective solvents. During extraction, solvents diffuse into the plant material and solubilize compounds with similar polarity [14].

The determination of bioactive compounds from plants is mainly dependent on the type of solvent used for extraction. The characteristics of a good solvent used in plant extractions are defined by having low toxicity, low vaporisation temperature, rapid absorption of extract and a long period of preservation. The selection of solvent is decided by the quantity of phytochemicals to be extracted, the rate and the diversity of different compounds, should not interfere with the bioassay**,** non-toxicity along potential health hazard of the handling person**.** The choice of solvent is depending on what we need from the extracts or targeted compounds to be extracted. Common solvents that are used in the extraction process are water, methanol, ethanol, acetone, chloroform etc. [14].

Evaluation techniques for screening plant extracts for antifungal activity were reviewed by Ncube *et al*. (2008). A wide variety of methods are applied to study antifungal activity such as disc diffusion technique, agar well diffusion technique, poisoned food technique, spore germination method, dry mycelial weight method, MIC by broth microdilution and macro dilution etc. [15] The outcome of antifungal activity relies on appropriate and reliable extraction and screening methods used by the researchers only [4].

Plant-based medicines are prepared from crude plant extracts consisting of a mixture of various lifesaving phytochemicals. These phytochemicals exhibit several pharmacological activities like antibacterial, antifungal, anticancer, anti-inflammatory and antioxidant properties. Phytochemicals have unique and complex structures and are used in treating several diseases. A broad spectrum of secondary metabolites is present in plants, but merely a few numbers of compounds have been examined to be significant bioactive agents [16].

Initial screening and characterization of bioactive compounds from medicinal plants done by spectrometric and chromatographic methods, which provides the basic information on the chemical profile of the plants. In the last few years, Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography: (HPLC), Nuclear Magnetic Resonance Spectroscopy (NMR), Fourier-transform infrared (FTIR) has become the key technology for profiling of secondary metabolite profiling in plants. These technologies are commonly been applied for the detection of functional groups and identification of various bioactive compounds present in medicinal plants [17].

GC-MS is one of the best, fast and gives accurate detection of various compounds, including alcohols, alkaloids, long-chain hydrocarbons, organic acids, steroids, esters and amino acids [18]. GC-MS requires a small volume of plant extracts and can be applied to solid, liquid and gaseous samples as well. In sample processing, first, the samples are converted into a gaseous state then the analysis is carried out based on mass to charge ratio [19].

HPLC is applied for compounds soluble in solvents. Compounds are separated based on their interaction with solid particles of a tightly packed column and the solvent of the mobile phase. HPLC is useful for compounds that are not vaporised under high temperatures. Liquid chromatography-mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). Both these analysis techniques can be used to identify and quantify food compositions, pharmaceuticals, and other bioactive molecules. High-Performance Thin Layer Chromatography (HPTLC) is used for the separation, detection, qualitative and quantitative analysis of phytochemicals [19]. This chromatographic technique is a planer chromatography where separation of the sample components is done on high-performance layers with detection and data acquisition using an advanced workstation. HPTLC is a robust, rapid and efficient tool in the quantitative analysis of compounds [20].

NMR spectroscopy is an analytical technique that gives physical, chemical and biological properties of matter using simple one-dimensional techniques. These techniques are used to determine the molecular structure of complex and complicated molecules. The advantage of NMR is that it doesn't require chromatographic techniques, plant extracts are directly analysed without extensive preparative steps, short run-times (for one-dimensional (1D) 1 H NMR) [21].

Antifungal activity of medicinal plants against onychomycosis

The herbal antifungal remedy is an alternative therapy treatment against onychomycosis which is cheaper, safer and often easily available. Antifungal activity of plant extracts from native plants, including their effects against dermatophytes, NDM, yeast has been reported across the globe [5]. In recent years, medicinal plants have gained attention because of their disease cure properties and fewer side effects. Researchers established that use of plant-based products to fight fungal infections has also been considered an effective approach. The antifungal activity of some plants, such as Ginger, Neem, Cilantro, Garlic, Tulsi, Henna, Aloe vera etc. on fungal infections has already been reported. Flavonoids, alkaloids, tannins, citronellol, geraniol, thymoquinone, and phenolic compounds are some of the bioactive compounds found in these plants [22, 23].

Antifungal activity of *Euphorbia cotinifolia* methanolic leaf and stem bark extracts was reported from Tanzania. In the Kilimanjaro regions of Tanzania, this ornamental plant is used to treat onychomycosis (Figure 1a) The methanolic extracts of both leaves and stem bark of *E. cotinifolia* inhibited *Trichophyton rubrum, T. mentagrophytes* and *Aspergillus niger* but standard drug fluconazole was unable to inhibit the growth of these tested fungi. Methanolic extract from the latex of *Euphorbia antiquorum* was also reported antifungal activity against *C. albicans, A. flavus, A. fumigatus* [24]. Morah and Okoi (2016) reported stem sap of *Euphorbia sanguinea* is locally used as a traditional remedy against onychomycosis. The sap showed high antifungal activity compared with the antifungal drugs. *E. sanguinea* has antimycotic activity against *Candida albicans* and is responsible for causing onychomycosis [25].

Antifungal activity of 61 traditional medicinal plants were screened against dermatophytes fungi *Trichophyton rubrum* causing onychomycosis. The results showed that the crude extracts of *Allium sativam* Linn., *Annona reticulata* L., *Annona squamosa* L., *Argemone mexicana* L., *Butea monosperma*, *Ceasalpinia bonducella*, *Citrus medica* L., *Corchorus oleterius* L., *Emblica officinalis*, *Euphorbia tirucalli* L., *Ficus racemosa* L., *Gymnosporia montana*, *Lawsonia inermis* L., *Solanum nigrum* L., *Sterculia foetida* L., *Tribulus terrestris* L., *Vitex negundo* L., (Figure 1.d) and *Zingiber officinale* exhibited significant antidermatophytic activity against *T. rubrum*. Shinkafi and Manga (2011) reported the aqueous and organic leaf extracts of *Mitracarpus scaber* and *Pergularia tomentosa* exhibited anti-fungal activities against major dermatophytes *T. mentagrophytes, T. rubrum and M. gypseum* causing onychomycosis [26].

Phytochemicals of plants and their bioactivity

The plant contains various non-nutrient chemical compounds which are medically bioactive in nature called "phytochemicals". Phytochemicals produce by plants such as alkaloids, flavonoids, glycosides, phenolics, saponins, terpenes, essential oils and steroids [27] attract the attention of researchers and pharmaceutical industries. Isolation of antifungal compounds having antifungal properties now become the natural blueprint for the development of new drugs for the treatment of fungal infections [28]. Rural communities from various parts of the world used medicinal plants for the treatment of onychomycosis which is not well documented or reported without any scientific validation of antifungal activity [4]. Few works had been reported on antifungal susceptibility tests of medicinal plants against dermatophytes causing onychomycosis, but very rare scientific evidence is reported on the antifungal activity of plants against NDMs [29].

Koroishi *et al*. (2010) from Brazil reported *Piper regnellii* has great antifungal potential to treat onychomycosis caused by *T. rubrum*. Although isolated neolignans such as eupomatenoid-3 and eupomatenoid-5 showed low activity when compared to the dichloromethane extract against *T. rubrum* [30].

In vitro antifungal activity of methanol, hexane and cold-water extracts of *Cassia alata, Mitracarpus villosus and Lawsonia inermis* were investigated against NDMs onychomycosis among rice farmers in Anambra State, Nigeria. Methanol extracts of *L. inermis* showed significant antifungal activity against *Aspergillus terrus, A. sclerotiorum, Fusarium* sp., *Chrysosporium* sp. and *Scopulariopsis* sp. at MIC range of 10-40mg/disc and *A. flavus* at a concentration of 40mg/disc respectively. Results of this study were supported by the work of Babu and Subharsree (2009) and Sharma and Sharma (2011), who reported the antimycotic potential of *L. inermis* against fungal pathogens due to the presence of Lawsome, 2 hydroxy-1,4-naphthoquinones compounds in the leaves [29, 31, 32].

Biasi-Garbin *et al*. (2016) published the first report of antifungal activity of *Schinus terebinthifolius, Piptadenia colubrina, Parapiptadenia rigida, Mimosa ophthalmocentra* and *Persea americana* plant spp. from semiarid vegetation (caatinga) of Northeast Brazil against *T. rubrum* and *T. mentagrophytes* [5]. Giwanon *et al*. (2016) reported leave extract of *Piper betle* L. to have a strong antifungal effect against dermatophytes associated with nail infection. They compared antifungal effect of the *P. betle* leave extract with five antifungal drugs (Econazole: (10 µg/disc), Miconazole: (10 µg/disc), Ketoconazole: (15 µg/disc), Metronidazole: (50 µg/disc), and Voriconazole: (1 µg/disc)) against *Trichophyton mentagrophytes* (n=2), *T. rubrum* (n=1). The Betle leaf extract showed antifungal activity against dermatophytes much more than tested antifungal drugs. Bhalerao *et al*. 2013 demonstrated Betle leaf extract consists of a wide variety of phenolic compounds having an antifungal effect against dermatophytes [33].

Fig 1: Photograph of some of the medicinal plants reported antifungal activity

a. *Euphorbia cotinifolia* L. [24]
b. *Croton tiglium* L.
c. *Lawsonia inermis* L.
d. *Vitex negundo* L.

Mansour-Djaalab *et al*. (2012) reported the hydroalcoholic extracts of *Lawsonia inermis*, *Juglans regia* and *Pistacia lentiscus* possess antifungal activities against *T. rubrum*, *T. mentagrophyte*, *M. canis* and *C. albicans*. Phytochemical analysis of *L. inermis* (Figure 1.c) revealed high dominancy of phenolic compounds *viz*. coumarins, flavonoids, and naphthalene derivatives of gallic acid and other compounds such as triterpenoids, steroids and aliphatic carbohydrates [34]. Abulyazid *et al*. (2010) reported the richness of the plant due to natural naphthoquinones molecules, they inhibit the growth of fungal strains and bacterial pathogens [35]. Li *et al*. (2008) reported *J. regia* and *P. lentiscus* plants to contain phenolic compounds like naphthoquinones and flavonoids. These molecules are already known for their antifungal power [35, 36].
Lin *et al*. (2016) reported antifungal activities ethanolic extracts of *Croton tiglium* (Figure 1.b) against three dermatophytes *T. rubrum*, *T. mentagrophytes* and *E. floccosum*. Results of this study showed the greatest inhibitory activities against *T. mentagrophytes* and *E. floccosum* by *C. tiglium* stem extract and had a lower activity against *T. rubrum*. Four major compounds in the stem extract were oleic acid, ethyl ester (20.87%), hexadecanoic acid (palmitic acid, 20.77%), hexadecanoic acid, ethyl ester (14.11%), and oleic acid (14.04%). The authors mentioned that two high content compounds oleic acid and hexadecanoic acid were mainly responsible for inhibition of tested dermatophytes [37]. Medicinal plant extracts showed strong antifungal potency against onychomycosis and can be considered further as a good source of antimycotic guide for the herbal treatment. Moreover, bioactive compounds present in plants have been found to interfere with pathogenic fungi. Naphthoquinones and flavonoids have been shown to work as antifungal compounds to treat nail infection [38].

Conclusion

Published reports from different parts of the world done by several scientists, research workers and pharmaceutical companies as well are mainly looking for successful nontoxic nature plant-based antifungal agents. Having known the fact of antifungal resistivity; azoles are generally given to cure fungal infection. The traditional practice of using herbal medicines for fungal infections is an alternate nontoxic old-age practice carried out mainly by rural Indian populations. There is a need to take up more studies to better understand the effect of herbal plants against the emerging pathogen in the changing environment which causes various dermatological health problems.

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