

**EVALUATION OF THE
ANTIFUNGAL ACTIVITY
OF THE ESSENTIAL OILS
OF *LEPTOSPERMUM
SCOPARIUM* (MANUKA)
AND *MELALEUCA
ALTERNOFOLIA*
(TEA TREE) IN THE
TREATMENT OF
ONYCHOMYCOSIS**

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Abstract: Introduction: Onychomycosis is a fungal infection that triggers a poor quality of life for patients and despite being considered superficial mycoses, they are difficult to diagnose and treat. Essential oils have been used as a therapeutic alternative due to their antimicrobial and antifungal action due to the presence of phenolic components in parts of the vegetable used in the therapy. **Objective:** To evaluate whether the treatment of Podiatry with essential oils, with antifungal activity, in patients with onychomycosis enables nail reconstruction opening the perspective of studies to characterize its antimicrobial principles and techniques that promote the health and beautification of the feet. **Methods:** Care for patients with onychomycosis, and the control group is using traditional topical antifungal therapy available in the pharmaceutical market. The intervention group (a) used manuka essential oil and finally the other intervention group (b) using melaleuca essential oil. All participants were submitted to direct mycological and culture examinations to identify the fungal strain. **Results:** The tests performed using essential oils in the treatment of patients with onychomycosis showed that such substances triggered improvement, even if gradual of patients and with lower cost. **Conclusion:** The essential oils studied in the present study showed antifungal activity, opening a perspective of studies to characterize their antimicrobial principles for the treatment of nail mycoses and stimulate the development of new forms of treatment containing phytopharmaceuticals. **Keywords:** Onychomycosis, essential oils, Podiatry.

INTRODUCTION

The nails have functionality to protect the fingertips, assist in the handling of small objects and even for defense mechanism (SILVA, 2014). These are structures that are commonly affected by fungal lesions such as onicopathies that affect 20 to 25% of the world population, affecting adults mainly (MENDES, 2014; MONTARIN et al, 2015). They are responsible for 50% of nail infections with a prevalence of 3% among adults, diabetic males, immunocompromised individuals with peripheral arterial diseases are also targets, being 4 to 7 times more frequent on toenails than on fingernails (ZALACAIN et al, 2018).

Nail deformities are generally considered in conjunction with dystrophy, but both have slight differences: deformities are considered as major changes in nail thickness, while dystrophy is changes in texture or composition (e.g., onychomycosis) and about 50% of nail dystrophy is caused by fungal infection (ADIGUM, 2017). The visual aspect with respect to color is yellowish or whitish. However, not all the nail that has onycholysis is affected by fungal infection. This can arise from the trauma of long nails on the shoes, and in people who run or practice impact sports. There is also nail thickening that occurs when the nails become harder, thicker and usually also darkened, and may have pain. The nail plate becomes friable, brittle with greater propensity to deformities. According to Nascimento, Oliveira (2019) report that onychomycosis is a chronic fungal lesion in which the nail plates become hypertrophic (thick), usually with discoloration, elevated, friable.

As diseases triggered by fungi has been highlighted, due to the incidence in adults and children (ARAÚJO et al. ;2003) being to this, a fungal infection of the nail lamina of the keratin and pruned, which represents

more than half of the onicopathies (PIÉRARD et al. ;2003), becomes an important fact of study and development of podiatry care for recovery of the nail system with the use of appropriate techniques, as onicoabrasion, complementary therapy with essential oils.

Treatment in some cases is discarded because it is considered only an aesthetic and non-pathological discomfort, not taking into account the professional and personal limitations that they can cause, thus affecting the quality of life of the individual and which can also be aggravated by other medical conditions especially in the elderly (GUPTA et al, 2018). With the high prevalence of these infections it is necessary to use antifungal products with lower toxicity rate that can repair the damage without harmful effects to the body (MENDES, 2013). Thus, the search for therapies where knowledge of Podiatry and aesthetics are associated with lack of toxicity in therapeutic doses and that is easy to be administered as well as obtain satisfactory results, since the pathology of a fungal nature is difficult to be treated, due to the treatment being extensive and the difficulty of adherence to the patient due to drugs administered orally causing mainly gastrointestinal discomforts (RANG. H. P. et al.; 2004).

Thus, the objective of this study is to evaluate whether the treatment of Podiatry with essential oils, with antifungal activity, in patients with onychomycosis enables nail reconstruction opening the perspective of studies to characterize its antimicrobial principles and techniques that promote the health and beautification of the feet.

METHODS

And clinical study of blind nature with the objective of serving the public with indicative or diagnosis of onychomycosis consisted of studies of acceptability (real

conditions of use) of podiatry techniques such as onicoabrasion, podoprophylaxis and administration of essential oils of *Melaleuca alternifolia* and *Leptospermum scoparium*, had 15 volunteers of both sexes, over 18 years of age. The representatives of research participants were recruited among the clients with diagnosis and/or clinical signs of onychomycosis from the Discipline of Podiatry of the Technological Course of Aesthetics and Cosmetics of a higher education institution of the Federal District where the study was conducted. This project was presented and approved to the Ethics and Research Committee, by the CEP/CONEP system, where the research participants did not have any type of identification (BRASIL, 2012) under the CAAE number: 52041321.2.0000.5058.

These participants were randomly allocated in the first consultation in the intervention (a) or intervention (b) and control group (c) groups. A group consisted of 05 people, control group, using the traditional treatments available in the pharmaceutical market, topical antifungal drugs (cyclopirox olamine solution). The other groups, intervention, were composed of 05 people each. One group (a) used the essential oil of *Melaleuca alternifolia* and the other intervention group (b) used the essential oil of *Leptospermum scoparium* (Manuka), both used in the practice of Podiatry in patients with onychomycosis, and were applied after podoprophylaxis procedures. Both intervention groups were treated for the removal of the fungal lesion using sterile instruments. Each follow-up was performed by means of photography and evaluation of nail characteristics, control of skin structure and for analysis with podoprophylaxis procedure with fortnightly monitoring of nail growth.

In the first care, biological material was

collected for direct mycological and culture examinations. Adverse reactions were also analysed during the use of essential oils by the research participants. In order to characterize the people involved in the study, an anamnesis form containing questions about the characterization of the patient, the foot injury and acceptance of podiatry therapy with the use of essential oils was filled out by signing the terms of free and informed consent and term of use of imaging. The analysis of the data that occurred through obtaining the results in the field, was inserted in spreadsheets by excel software, for statistical analysis.

LITERATURE REVIEW

Onychomycosis encompasses all fungal infection caused by nail pathogens that invade the healthy nail plate, are caused by dermatophytes of the genera *Trichophyton*, *Microsporum* and *Epidermophyton*, yeasts mainly of the genus *Candida* and non-dermatophyte filamentous fungi such as *Aspergillus* and *Fusarium*. (MONTARIN et al, 2015). They are more frequent lesions on toenails than on fingernails, the risk of involvement is related to several factors such as age, with adults being more affected, male individuals, advanced age, smoking, diabetes, peripheral arterial disease and immunodeficiency and predisposing genetic factors (ABDULLAH and ABBAS, 2011). They mainly affect toenails involving several nails, differ according to the invasion modality (PIRACCINI and ALESSANDRINI, 2015).

These fungal infections can be divided into white superficial onychomycosis or mycotic leuconychia where fungi invade the surface of the nail plate form colonies and thus occurs the formation of opaque white spots caused mainly by the genus *Trichophyton rubrum* (WOLLINA et al, 2016). On the other hand, proximal subungual Onychomycosis

comes from eponychium occurs particularly in immunosuppressed patients, mainly caused by molds, but may also originate from *Trychophyton rubrum*, *Trychophyton magnigii* and *Aspergillus sp*, in this case nail plate acquires white coloration (WOLLINA et al, 2016; SILVA, 2014). There is also the iii) Distal subnugour onychomycosis that can be caused by any of the pathogens, is characterized by a yellow/brownish color that is a characteristic of the color of the colonies, where the nails have longitudinal striae and irregular edge. As symptoms are present, onycholysis and nail hyperkeratosis, which consists of a hypertrophy of the nail bed (SILVA, 2014; PIRACCINI et al, 2013).

There is also candida onychomycosis which is less frequent than infection by dermatophytes, caused by *Candida albicans* which mainly affect the fingernails and total dystrophic onychomycosis that can occur by an evolution of a distal submychomycosis or a long-lasting proximal submychomycosis, the nail plate is completely taken by fungi, acquires yellowish coloration and undoes easily (MOBIN et al, 2018; PIRACCINI and ALESSANDRINI, 2015). Finally Paronychia is an inflammation of the proximal nail fold that occurs as a result of the mechanical aggression of the eponychium by instruments (pliers, toothpick and spatula), contact with aggressive agents (alkaline water) and with chemical agents, which cause the loss of the cuticle thus leaving the nail susceptible to external attacks (microorganisms and allergens) being thus classified as acute, usually comes from *Staphylococcus aureus* infection, when trauma is direct or indirect and as chronic when it is due to inflammation by irritating agents or allergens (BEBER et al, 2012).

The incidence of infections, as well as the increasing costs of treatment, has driven to conduct research in an attempt to obtain alternative therapeutic compounds, in this

sense medicinal plants have an economical alternative and easy access for the treatment of onychomycosis (GIORDANI et al., 2004). Medicinal plants have been widely used by mankind since the earliest times, and are an important source of new biologically active compounds (OLIVEIRA et al., 2006). Many plants have been used due to their antimicrobial characteristics, due to the compounds synthesized in their secondary metabolism, these products are known for their active substances, for example, phenolic compounds that are present in essential oils (RIBEIRO et al., 2012).

The essential oils originate from secondary metabolism, extracted from various parts of plants by distillation by drag with water vapor, and those obtained by expression of citrus pericarps, are composed of numerous volatile compounds, odoriferous, insoluble in water, but soluble in alcohol and various solvents immiscible in water (SIMÕES et al., 2003). According to Oliveira et al. (2006) essential oils have about 60% that have antifungal properties and 35% exhibit antibacterial properties. These substances may interact with drugs that trigger histamine release, but it is recommended not to administer it concomitantly and the oil should not be administered in open wounds, in the ocular region, over the cracked dermis or in areas expressing a *skin rash*. The same may alter the result of laboratory tests for neutrophil counts (ITF 2008).

According to the ITF (2008) *apud* Buck et al. (1994), clinical trials were conducted in 117 patients who had proven distal subnugueal onychomycosis and who underwent applications of *pure melaleuca alternifolia* cheel oil, *Eugenia caryophyllata*, *Rosmarinus Officinalis* and *Thymus vulgaris* as a solution of clotrimazole at a concentration of 1%. They were analyzed after six months and observed that the clotrimazole group had negatively

negative 11% of the cultures and the other 18%. And the appearance of the clotrimazole group had improved by 61%, and the avia oil group improved by 60%. After another three months, the results remained positive for both groups with a percentage of 56% for the oil and 55% for clotrimazole.

The *melaleuca alternifolia* Cheel plant belongs to the *Myrtaceae* family, which is composed of approximately one hundred and thirty genera and four thousand species of tropical and subtropical distribution, which is considered one of the largest families of the Brazilian flora (SILVA, 2007). It can reach in natural conditions a tree size, up to 8 meters high, has thin and soft bark, the leaves are simple and tapered, about 2 cm long (BIASI and DESCHAMPS, 2009) Its flowers are sessile, with thorns and white, yellowish or purplish (ITF, 2013).

The genus *Melaleuca* is rich in volatile oils, its main constituents being terpinen-4-ol, 1,8-cineol, α -terpineno, γ -terpineno, α -pineno, β -pineno, α -terpineol, responsible for its medicinal properties is terpinen-4-ol (VIEIRA et al., 2004), p-cymene and sesquiterpeneic alcohols, which represent about 90% of oil (SILVA et al., 2003). It presents fungicide activity, acaricide, applicability for insect bites and skin infections, viral treatment such as herpes, antiseptic, expectorant, gastrointestinal disorders, used in perfume industries, and as the oil has no toxic characteristics and does not promote skin irritations in therapeutic doses, is used as germicides and pesticides and presents scar action (SILVA, 2007; ITF, 2013). According to Enshaieh et al. (2007), *Melaleuca alternifolia* oil has proven antimicrobial activity in vitro (ITF, 2013), since it can smooth bacteria in the exponential phase period as much as in the stationary phase. This essential oil is a known antiseptic because it has activity on a wide spectrum of pathogens, including methicillin-

resistant *Staphylococcus aureus* and *Candida albicans* (WOOLLARD, 2007).

Leptospermum scoparium, manuka, is a tree of the Family Myrtaceae, characteristic of New Zealand. It presents high concentration in glucose and fructose, low water content, phenolic compounds and a compound with antibacterial activity that makes it unique, methylglyoxal, dihydroxyacetone, which is present in the nectar of vegetable flowers (DURATE, 2017). It is a leafy shrub of green leaves with strong fragrance and small flowers of pink to white color. It can be isolated or in clusters and can reach eight meters high and adapts to different altitude and weather conditions (VAN VUUREN, DOCRAT et al. 2014). Its leaves when used in infusion have a bitter taste (LIS-BALCHIN, DEANS et al. 1996) and manuka oil is obtained by steam distillation of leaves and branches (MADDOCKS JENNINGS, WILKINSON et al.; 2005) and is quite aromatic, of light yellow color.

The chemical composition of manuka oil presents a great variability which hinders its characterization (LIS-BALCHIN, Hart et al. 2000, MADDOCKS JENNINGS, WILKINSON et al. 2005). In New Zealand, at least four distinct chemotypes were characterized: a) one rich in tricetans, b) another rich in linalool and eudesmol, c) another rich in pineno and d) another with deficit in tricetans, linalool and eudesmol (PORTER; WILKINS, “. 1998). Manuka oil will be all the more interesting the higher its content of tricetans (CHRISTOPH, KAULFERS et al.; 2000) which are the molecules responsible for most of antimicrobial activity (PORTER; WILKINS, “. 1998), highlighting leptospermone (LIS-BALCHIN, DEANS et al. 1996). The antimicrobial efficacy of manuka essential oil in pathogenic microorganisms such as *Candida albicans*, *Staphylococcus* spp.,

Escherichia coli and *Pseudomonas aeruginosa* has been investigated, but still with limited clinical evidence (CHEN, YAN et al.; 2016). In not-so-rich trietane-rich chemotypes sesquiterpenes may play an important role in antiseptic, antibacterial, analgesic and anti-inflammatory activity (MADDOCKS-JENNINGS, WILKINSON et al.; 2005).

RESULTS AND DISCUSSION

Of the 15 patients with onychopathies with clinical characteristics of nail fungal infections, submitted to direct mycological examination and culture, it was diagnosed, after confirmation of the etiological agent by direct mycological examination and growth in culture, onychomycosis in the toenails in 100% of the cases studied. We found yeasts of the genus *Candida* (33.34%) as the most frequent etiological agents of onychomycosis. In contrast, dermatophytes were more isolated from the nails resulting in a total of 4 patients with *Trichophyton rubrum* (26.67%). Onychomycosis by emerging fungi accounted for 49% of all onychomycosis. In the onychomycosis by emerging fungi, infections were found by *Fusarium* sp., *Aspergillus* sp. and *Trichosporon beigelii*.

The most frequent location was the nail of the first toe in 12 patients (80%). In this work, the fungi *Aspergillus* sp. and *Trichosporon beigelii* were the most frequent fungal strains responsible for the onychomycosis caused by emerging fungi, and 6 patients from the group of 15 people tested were tested, representing 40% of the total onychomycosis by emerging fungi. In all cases that had as agent the emerging fungi, distal and lateral submychomycosis was the predominant one, occurring more in the toenails and hallux. Onychomycosis by *Fusarium* sp was observed in only one patient, which represented 6.67% of the total onychomycosis identified by mycological examinations performed.

In the first care, the patients answered the questions and they were randomly divided into three groups, which presented similar characteristics (Table 1).

It is possible to verify in our results that with the administration of essential oils has suggested improvement in fungal infection. Onychomycosis is considered the most difficult superficial mycosis to treat and the difficulty of establishing a clinical diagnosis, added to the fact that the treatment requires the use of antifungals for long periods and with potential side effects, so using products of natural origin represents an alternative treatment, so essential oils obtained from plants are considered promising sources for the treatment of mycoses (CASTRO and LIMA, 2011).

Our results indicate each Podology care that was performed in all groups with removal of the fraction of the injured nail and also by photographic follow-up and evaluation of the characteristics of the nail had improvement in the visual aspect of the nail parade. In the intervention group (a), using Melaleuca essential oil, which had as treatment in addition to the removal of the fungal lesion, the use of this essential oil showed improvement in clinical characteristics associated with fungal infection (Figure 1). In the intervention group (b) in complementary use of Manuka essential oil applied after podiatry care, the clinical evolution of patient treatment is also verified, showing improvement in signs indicative of onychomycosis (Figure 2). In the control group (c) (figure 3) it is possible to clinically observe the clinical evolution of the patient using topical medication during podiatry visits.

The first reports show that in topical formulations this plant was used for the treatment of wounds, tissue injuries and skin diseases in addition to inward use for colds (MATHEW et al., 2020). It is

Variable	Category	Total(n=15)		Group a (n=5)		Group b (n=5)		Group c (n=5)	
		n	%	n	%	n	%	n	%
Age	18 years	1	6,7						
	19 to 24 years	1	6,7			1	20,0		
	35 to 44 years	2	13,3			1	20,0	1	20,0
	45 to 54 years	3	20,0	2	40,0			1	20,0
	55 to 64 yers	2	13,3					2	40,0
	65 or more	6	40,0	3	60,0	3	60,0	1	20,0
Sex	Male	5	33,3	3	60,0	2	40,0		
	Feminine	10	66,7	2	40,0	3	60,0	5	100,0
Color	White	3	20,0			1	20,0	1	20,0
	Black	1	6,7			1	20,0		
	Yellow	3	20,0			1	20,0	1	20,0
	mixed	8	53,3	5	100,0	2	40,0	3	60,0
	Indigenous	0	0,0			0			
	Outros	0	0,0			0			
Education	No schooling	1	6,7			1	20,0		
	Elementary School (1st degree) incomplete	1	6,7			1	20,0		
	Elementary School (1st degree) complete	2	13,3	1	20,0			1	20,0
	High school (2nd degree) incomplete	1	6,7	1	20,0				
	High school (2nd degree) Complete	7	46,7	3	60,0	2	40,0	2	40,0
	Graduated	3	20,0			1	20,0	2	40,0
Do you know what onychomycosis is?	Yes	10	66,7	4	80,0	3	60,0	3	60,0
	Not	5	33,3	1	20,0	2	40,0	2	40,0
Habit of walking barefoot	Yes	5	33,3	1	20,0	3	60,0	2	40,0
	Not	10	66,7	4	80,0	2	40,0	3	60,0
Time of nail injury	between 1 and 5 years	7	46,7	3	60,0	2	40,0	1	20,0
	between 5 and 10 years	6	20	2	40,0	1	20,0	3	60,0
	more than 10 years	2	13,3	0		2	40,0	1	20,0
Took the examlaboratory for diagnosis	Yes	8	53,3	3	60,0	2	40,0	3	60,0
	Not	7	46,7	2	40,0	3	60,0	2	40,0
Realização de tratamento tópico ou sistêmico	Yes	9	60,0	4	80,0	4	40,0	3	60,0
	Not	6	40,0	1	20,0	1	60,0	2	40,0
Realização de tratamento Podologia ou Estético	Yes	10	66,7	4	80,0	1,0	20	5	100,0
	Not	5	33,3	1	20,0	4,0	80	0	

Legend: Group (a): Treatment with maelaleuca oil, (b): manuka, (c) cyclopirox.

Table 1. Frequency of variables: age, gender, color, schooling, knowledge about onychomycosis, laboratory tests and forms of treatment.



Legend: A before the procedure and D after the procedure.

Figure 1: Before and after podiatry care to a female patient belonging to the intervention group (a) with the use of melaleuca oil.



Legend: A before the procedure and D after the procedure

Figure 2: Before and after patient care in the intervention group (b) in complementary use of manuka oil.



Legend: A before the procedure and D after the procedure.

Figure 3: Before and after patient care of the control oil group (c) with topical medication in solution.

also verified that *the OE of L. scoparium* has potential use as cosmetic insum in mouthwashes for infections in the oral mucosa and in tests was up to 20 times more potent against gram-positive bacteria than *melaleuca alternifolia oil*, known as Tea Tree (MADDOCKSJENNINGS et al., 2009). This same OE is also used as a strong antimicrobial and antifungal agent in creams, soaps, toothpastes and other cosmetic preparations (LIS-BALCHIN, 1997). This antimicrobial action of manuka OE is due to the presence of terpenes, terpenoides and aromatic groups obtained after distillation extraction process (MERONI et al., 2020).

CONCLUSION

The use of essential oils as organic insums of natural origin are a promising alternative for the treatment of patients with fungal infections and the safety of a product directly linked to their stability, guaranteed when there are no internal and/or external interferences that may compromise them. The melaleuca and manuka oils studied in the present study showed antifungal activity, opening a perspective of studies to characterize their antimicrobial principles for the treatment of onychomycosis and stimulate the development of new forms of treatment containing phytopharmaceuticals. Thus, the tests showed that such essential oils triggered improvement, although gradual of patients and with lower cost.

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