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Article

Effects of *Cymbopogon winterianus* Jowitt ex Bor essential oil on the growth and morphogenesis of *Trichophyton mentagrophytes*

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Trichophyton mentagrophytes is a fungus causative agent of dermatophytosis, affecting humans worldwide. This has driven the search for products for the treatment of these infections. Accordingly, the aim of this study was to investigate the antifungal activity of the *Cymbopogon winterianus* essential oil against *T. mentagrophytes*. The antifungal tests consisted of antifungal screening, determination of MIC and MFC, analysis of the essential oil's effects on mycelial growth, germination of fungal spores, fungal viability, morphogenesis, cell wall (test with sorbitol) and cell membrane (cell leakage test) of *T. mentagrophytes*. Upon screening, the oil inhibited all strains, with zones of growth inhibition of 24-28 mm in diameter. The MIC was 312 µg/mL and CFM was 2500 µg/mL for almost all the strains tested. There were morphological changes in the conidia group, form and pigmentation of hyphae. The antifungal action of the product does not involve the cell wall and its action may involve the fungal plasma membrane. It is concluded that *C. winterianus* essential oil constitutes a potential antifungal product, especially for the treatment of dermatophytosis.

Uniterms: *Trichophyton mentagrophytes*/mycology. Dermatophytosis *Cymbopogon winterianus*/essential oil/ antifungal activity. Dermatophytes.

Trichophyton mentagrophytes é um fungo causador de dermatofitoses, afetando humanos em todo o mundo. Isto direciona a busca de produtos para o tratamento destas infecções. Assim, este estudo teve por objetivo investigar a atividade antifúngica do óleo essencial de *Cymbopogon winterianus* contra *T. mentagrophytes*. Os ensaios antifúngicos foram constituídos do *screening* antifúngico, da determinação CIM e CFM, da análise dos efeitos do óleo essencial no crescimento micelial, na germinação dos esporos, na viabilidade fúngica, na morfogênese, na parede celular (ensaio com sorbitol) e na membrana celular (ensaio de lise celular) de *T. mentagrophytes*. No *screening,* o óleo inibiu todas as cepas, com zonas de inibição de crescimento de 24-28 mm de diâmetro. A CIM foi de 312 µg/mL e a CFM foi de 2500 µg/mL para quase todas as cepas testadas. O óleo essencial inibiu o desenvolvimento micelial, a germinação dos esporos e a viabilidade fúngica. Houve alterações morfológicas no agrupamento dos conídios, na forma e pigmentação das hifas. A ação antifúngica do produto não envolve a parede celular e parece estar envolvida com a membrana celular fúngica. Pode-se concluir que o óleo essencial de *C. winterianus se* apresenta como um potencial produto antifúngico, especialmente para o tratamento das dermatofitoses.

Unitermos: *Trichophyton mentagrophytes*/micologia. *Cymbopogon winterianus*/óleo essencial/atividade antifúngica. Dermatofitoses/tratamento. Dermatófitos.

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INTRODUCTION

Dermatophytes are a group of related pathogenic fungi specialized in keratinous substrates such as the skin, hair and nails of humans and other animals producing dermatophytosis (Weitzman, Summerbell, 1995). Dermatophytosis affects approximately 40% of the world's population and represents 30% of all mycotic skin infections. *T. mentagrophytes,* among other dermatophytes, afflict man, other mammals and birds. It is distributed worldwide, affecting mainly the human scalp, feet and hands, nails and interdigital areas. Currently, it is one of the most commonly found dermatophytes in humans (Oyeka, 2000).

Unfortunately, the number of antifungal drugs available for combating dermatophytosis is limited. Furthermore, this infection may become resistant to treatment, which is consequently rendered ineffective (Favre *et al.,* 2003). The occurrence of fungal infections has increased significantly in recent years, and the use of antifungal agents has broadened while the consequent emergence of resistant strains has grown. Hence, investigations aiming to design novel antifungal products have become essential.

In this context, interest in plants with antifungal properties has increased because they represent a promising source of potential new products. Essential oils from medicinal plants have been the most used in the treatment of infectious pathologies in many parts of the body including skin (Rios; Recio, 2004). It has long been recognized that some essential oils are widely used for antimicrobial activities, especially in pharmaceutical, sanitary, cosmetic, agricultural, and food industries (Bakkali *et al.,* 2008).

Cymbopogon winterianus Jowitt ex Bor (Poaceae) popularly known as "citronella" or "java citronella", is a perennial herb which forms one-meter-high compact and strong clumps, with extensive use in popular medicine on the coast of Brazil. It is used as antimycotic, acaricide and repellent against a variety of insects (Pandey, Rai, 2003). This study was carried out to investigate the antifungal activity of *C. winterianus* essential oil, analyzing its effects on *T. mentagrophytes* strains.

MATERIAL AND METHODS

Plant material

Leaves of *C. winterianus* were collected from the Formation Center for Technicians at the Federal University of Paraiba (campus IV), Bananeiras city, Paraiba state-Brazil, in February 2007. Botanical identification of the plant was obtained and a voucher sample (JPB 41387) deposited with the Herbarium Professor Lauro Pires Xavier, at the Federal University of Paraiba.

Essential oil

Fresh leaves of *C. winterianus* were cut into pieces and subjected to water-distillation using a Clevenger apparatus. The essential oil obtained (density = 0.8790 g/mL) was kept in an amber bottle flask and maintained at temperature lower than 4 °C. The oil emulsion used in antifungal assays was obtained according to the following procedure: 34 µL of essential oil, 10 µL of tween 80 and q.s.f. 3 mL of sterile distilled water, were added to a sterile tube and shaken for 3 minutes using a vortex instrument, thus obtaining a stock emulsion with a final concentration of 10000 µg/mL. Seriate dilutions were performed in the proportion of two in order to obtain emulsions from 5000 – 5 µg/mL.

Microorganisms

Eight strains of *T. mentagrophytes* were used in antifungal assays. These dermatophytes were obtained from the collection of the Mycology Laboratory (LM), Pharmaceutical Science Department, Health Science Center, Federal University of Paraiba. Fungi were maintained on potato dextrose agar (PDA) – Difco® – at 28 °C and 4 °C until test procedures.

Inoculum preparation

Stock inoculums of *T. mentagrophytes* strains were prepared from 10-day cultures in PDA at 28 °C to induce sporulation. Fungal colonies were covered with 5 mL of sterile saline solution (NaCl 0.85 % w/v), the surface gently scraped with a sterile loop and the resultant mixture of fungal units was then transferred to a sterile tube. The turbidity of the final inoculum was standardized according to a McFarland scale 0.5 tube and adjusted to a fungal population of 10^6 colony former units (CFU). The confirmation of inoculum quantification was done by plating 0.01 mL of inoculum suspension in Sabouraud dextrose agar (SDA). The dishes were incubated at 28 °C and examined daily for the presence of fungal colonies which were counted as soon as growth became visible (Santos *et al*., 2006; Hadacek, Greger, 2000).

Antifungal activity screening

The solid medium diffusion method using filter paper discs was employed for antifungal activity scree-

ning (Hadacek, Greger, 2000; Adam *et al*., 1998). Sterile Sabouraud dextrose agar (SDA) – Difco® – was prepared and distributed uniformly into sterile Petri plates, where previously 1mL of the fungal suspension was inoculated. Subsequently, filter paper discs (diameter 6 mm) were soaked with essential oil and placed on the surface of the inoculated agar. Dishes were incubated at 37 °C for 8 days. At the end of the incubation period, the fungal growth inhibition zone diameter was measured and expressed in millimeters. Results were considered positive for antifungal activity where the mean values of the growth inhibition zone in two independent assays were greater than or equal to 10 mm in diameter.

Determination of minimum inhibitory concentration (MIC)

The MIC values were determined for the fungi strains which were sensitive to the essential oil in solid medium diffusion assay. Broth microdilution bioassay was used to determine MIC of the *C. winterianus* essential oil (Moreira *et al*., 2010; Sahin *et al.,* 2004). For this, 96-well dishes (flat bottomed) and caps were used. The 96-well dishes were prepared by dispensing 100 µL of double concentrated Sabouraud dextrose broth (SDB) (Difco®) into each well. A 100 µL volume from the stock emulsion of essential oil was placed into the first wells. Subsequently, 100 µL from their serial dilutions was transferred into consecutives wells, excluding the last one. The last well contained 100 µL of broth inoculated with fungal inoculum to confirm cell viability (viability control). Positive control was carried out in a similar fashion with standard antifungal using Ketoconazole (Sigma-Aldrich®). In both cases, the highest concentration (5000 µg/mL) was placed into the first wells and the lowest concentration (5 µg/mL) into the penultimate wells. Sensitivity control of the assayed strains to the tween 80 without essential oil was carried out. A 100 µL volume of 5% tween 80 in broth was placed into wells and 10 µL of fungal suspensions were inoculated into each respective well. Also, a sterility control was performed to verify whether the broth used in antifungal assay was contaminated before test procedures. For this, 100 µL of broth was dispensed into a well, without essential oil or inoculum.

All the dishes were aseptically sealed then mixed on a plate shaker (300 rpm) for 30 seconds, incubated at 28 °C and read after 5 days of incubation. The MIC values were determined by visual inspection of the growth inhibition of each well compared with that of the control (without drugs) well. MIC was defined as the lowest essential oil concentration able to inhibit the fungal growth by 100%. The test was performed in duplicate and the geometric mean values were calculated (Santos *et al*., 2006).

Determination of the minimum fungicide concentration (MFC)

MFC was determined by the microdilution method to verify if the inhibition was reversible or permanent (Denning *et al.,* 1992; Rasooli; Abyaneh, 2004). Aliquots of 20 µL from the wells that did not show growth in the MIC procedure were transferred to 96-well dishes previously prepared with 100 µL of SDB. The dishes were aseptically sealed then mixed on a plate shaker (300 rpm) for 30 seconds, incubated at 28 °C and read 5 days after incubation. The test was performed in duplicate and the geometric mean values were calculated. MFC was defined as the lowest essential oil concentration for which no visible growth occurred when subcultured in the 96-well dishes containing broth without anti-fungal products.

Effects on mycelial growth

Analysis of the interference of the essential oil of *C. winterianus* on mycelial growth was performed by determining the dry mycelial weight of *T. mentagrophytes* LM02 (Rasooli, Abyaneh, 2004; Sharma, Tripathi, 2006). Flasks containing 2500, 625, 312 and 156 µg/mL of essential oil in SDB medium, were inoculated with suspension of the test *T. mentagrophytes* strain. In the correspondent control, the same amount of essential oil was replaced by distilled water. The system was incubated at 28 °C for 15 days. From the sixth day of incubation, mycelium dry weight was determined every 3 days. Flasks containing mycelia were filtered through a Whatman filter no. 1 (particle retention: 11 µm) and then washed with distilled water. The mycelia were dried at 60 °C for 6 h and then at 40 °C over night. The filter paper containing dry mycelia from two independent assays were weighed and the mean values obtained. Percentage growth inhibition based on the dry weight at each time of analysis, was calculated according to Sharma and Tripathi (2006).

Spore germination assay

To evaluate the power of essential oil of *C. winterianus* on the germination of fungal spores of *T. mentagrophytes* LM02, essential oil concentrations of 156, 312, 625 and 2500 µg/mL, a control with tween 80 (10% in distilled water), and another control with distilled water, were used. In sterile test tubes, 500 mL of double concentrated CSD plus the essential oil were evenly mixed with

500 mL of fungal conidia suspension and immediately incubated at 28 °C. Samples of this mixture were taken after 24 h of incubation for analysis. The whole experiment was performed in duplicate, where the number of conidia was determined in a Neubauer chamber and the inhibition percentage of spore germination at each time point was calculated by comparing the results obtained in the test experiments with the results of the control experiment. The analysis was conducted under an optical microscope (Zeiss® Primo Star) (Rana *et al*., 1997; Liu *et al.,* 2007).

Viability assays

A fungal viability study was carried out with the essential oil of *C. winterianus* at 156, 312, 625 and 2500 µg/mL, using the viable fungal structures count method. A 4 mL volume of CSD was inoculated with 1mL of the fungal suspension. The essential oil was then added to the amount necessary to achieve the concentrations previously reported. A 1 mL volume of inoculum and then the essential oil was added to the amount necessary to achieve the concentrations reported previously. The whole system was incubated at 28 °C. At different time intervals (3, 6, 9 and 12 days) post incubation, an aliquot of 0.5 mL from the tubes was diluted in sterile water, spread on SDA Petri dishes, and incubated at 28 °C for 5 days. A control with tween 80 (10% in distilled water) and another control without essential oil, were used. The counting of the number of viable colonies was performed and expressed as logCFU/mL (Rasooli *et al.,* 2004).

Fungal morphogenesis study

The evaluation of the micromorphological alterations caused by the essential oil of *C. winterianus* in *T. mentagrophytes* LM02 was performed in duplicate by the slide culture technique. First, an SDA block containing the essential oil (78, 156, 312 µg/mL) was transferred to the center of a glass slide in a Petri dish with a piece of filter paper. Subsequently, a mycelium sample was taken from the periphery of a 10-day-old fungal colony grown on PDA, and inoculated onto the center of the sides of the agar medium block. About 1.5 mL of sterile water was placed in the Petri dish bottom and incubated at 28 ºC for 5 days. After incubation, slides with reproductive structures were fixed in lacto-phenol–cotton blue stain and observed under an optical microscope (Olympus® model CH-30) at 400x to examine morphological abnormalities. Structural changes observed on optical microscopy in test assays were recorded and compared with the normal growth found in the control experiment. The control assay without essential oil was tested in the same way (Gunji *et al.,* 1983; Frost *et al.,* 1995).

Sorbitol protection assay

MIC values of the *C. winterianus* essential oil were determined using *T. mentagrophytes* strains by the broth microdilution procedure described previously. Duplicate dishes were prepared: one of these contained 2-fold dilutions of essential oil while the other contained the essential oil plus 0.8 M sorbitol as osmotic support, in each of the wells. MICs were read at 5 days (Escalante *et al*., 2008).

Cellular leakage effects

The essential oil of *C. winterianus* at 78, 156, 312 µg/mL was added to 10 mL of fungal suspension of *T. mentagrophytes* LM02 using sterile tubes. Alcoholic potassium hydroxide solution at 25% (diluted with ethanol at 70 %) was used as a reference compound, which produces 100% cellular leakage. MIC of amphotericin B (0.60 µg/mL) was used as a positive control. Cells were incubated at 28 °C, and samples were taken at time intervals (2, 4 and 24 h) and spun at 3000 rpm for 5 min in centrifuge tubes. The supernatants were collected for absorbance analysis at 260 nm in a Varian Cary 50 spectrophotometer. Results are expressed as the means of percentage cellular leakage values from two independent assays (Escalante *et al*., 2008; Lunde, Kubo, 2000)

Statistical analysis

Statistical analysis for the study of the effects of essential oil on mycelial weight was performed to determine statistically significant differences ($P < 0.05$) employing analysis of variance (one-way ANOVA) using the Kruskal-Wallis test, followed by the Dunn post-test. For this, the implementation of statistical analysis was performed using GraphPad Prism version 4.03 for Windows, San Diego, California, USA.

RESULTS AND DISCUSSION

Results of antifungal activity of *C. winterianus* essential oil on *T. mentagrophytes* strains are shown in Table I. The essential oil *in natura* was able to inhibit all the tested strains with inhibition zones diameters of 24-28 mm. MIC values are also summarized in Table I. As can be seen, MIC of the essential oil was 312 µg/mL for all the essayed strains, except one. The lowest MIC value was 156 µg/mL for *T. mentagrophytes* LM07. Ketoconazole

showed a lower MIC than essential oil, where the MIC values were 78, 156 and 312 µg/mL for ketoconazole. Control results showed absence of fungal growth inhibition by tween 80, whereas fungal growth in broth without addition of drugs was detectable (sterility control). Fungicide effect was stronger for *T. mentagrophytes* LM07, evidenced by lowest MFC value of 1250 µg/mL. MFC values were around eight times higher than MIC for all the tested strains. However, MFC for ketoconazole were about sixteen times higher than MIC values (1250-5000 µg/mL).

It has long been recognized that some essential oils have antimicrobial properties which are possibly related to the function of these compounds in plants (Burt, 2004). Earlier, this essential oil was shown to exhibit an antimicrobial effect against various pathogenic microorganisms to humans and plants, being useful for controlling mycotic diseases in large plantations, in addition to its activity as repellent and insecticide (Simic *et al.*, 2008; De-Blasi *et al.,* 1990). Previous studies reported that citronellal and geraniol showed antifungal activity against *Aspergillus niger, Fusarium oxysporum* and *Penicillium digitatum* (Moleyar, Narasimham, 2004). However, scant information exists on the effects of *C. winterianus* essential oil against classical important fungi involved in human infections, including species of dermatophytes.

The effects of *C. winterianus* essential oil on mycelial growth are expressed as percentage of inhibition in dry mycelial weight of *T. mentagrophytes* LM02 and depicted in Figure 1. This study revealed that all concentrations of essential oil inhibited the development of mycelium. At each separate time point, all the concentrations tested differed ($P<0.05$) from results of 156 µg/mL. Also, at 156 µg/mL, the impediment of mycelial growth increased with the time of interaction between drug and fungal cells. With the other concentrations of essential oil, there was 100 % inhibition at all times analyzed. Dermatophytes produce hyphae which can penetrate the innermost layer of the skin and aggravate the damage in the host (Zurita, Hay, 1987). Therefore, some researchers are investigating essential oils' potential for inhibiting mycelial growth of pathogenic fungi, due to their importance in mycosis development.

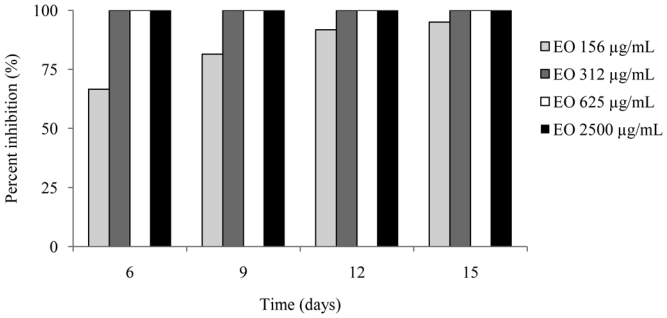

FIGURE 1 - Percentage inhibition of dry mycelial weight of *T. mentagrophytes* LM02 in the presence of several concentrations of *C. winterianus* essential oil (EO).

The inhibition percentage of conidia germination of *T. mentagrophytes* LM 02 caused by different essential oil concentrations of *C. winterianus* are displayed in Figure 2. In general, all concentrations of the essential oil exerted a strong inhibitory effect on conidia germination of the tested strain. As the essential oil concentration increased from 156 to 625 µg/mL, the inhibition percentage also increased, reaching values close to 100 %. Although these results are numerically relevant, the absence of germinated conidia in the culture, indicating total germination inhibition, was observed only at 2500 µg/mL. By comparing these results, it is notable that the percentage of inhibition for 156 and 625, 2500 µg/mL was significantly

TABLE I - Antifungal activity of *C. winterianus* essential oil against *T. mentagrophytes*

Strains	Inhibition zones	Essential oil		Ketoconazole	
		MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)
T. mentagrophytes LM02	24	312	2500	156	2500
T. mentagrophytes LM07	28	156	1250	78	1250
T. mentagrophytes LM11	24	312	2500	156	2500
T. mentagrophytes LM28	25	312	2500	78	1250
T. mentagrophytes LM79	24	312	2500	156	2500
T. mentagrophytes LM202	26	312	2500	156	2500
T. mentagrophytes LM308	26	312	2500	156	2500
T. mentagrophytes LM962	26	312	2500	312	5000

*range of screening results of the essential oil is expressed in fungal growth inhibition zone diameters (mm).

different (P <0.05), supporting the idea that the increased concentration somehow enhances the inhibitory effect on germination. Finally, it was found that tween 80 did not affect conidia germination. Due to the importance of proper spore germination in dermatophytosis pathogenesis, the interference in the process caused by the essential oil proves relevant because this product was shown to be a promising tool for intervention of this infectious process.

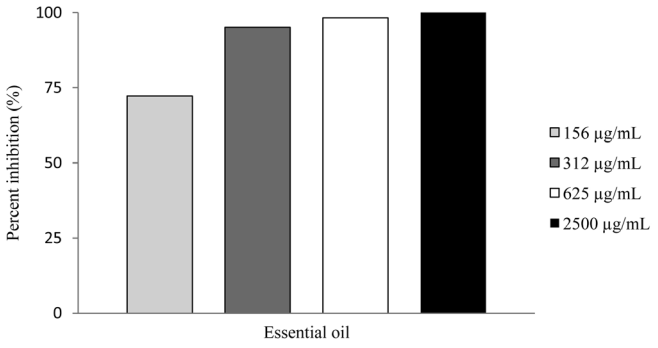

FIGURE 2 - Inhibition percentage of conidia germination of *T. mentagrophytes* LM 02 after 24 hours of interaction with the essential oil of *C. winterianus*.

Figure 3 shows the results regarding the effects of the essential oil *C. winterianus* on the viability of *T. mentagrophytes* LM 02. After statistical analysis, it was observed that values differed (P<0.05) at each time of interaction analyzed. These observations can be seen by the logCFU/mL values of essential oil always being lower than those observed in the control experiment. The essential oil at 312 µg/mL was able to reduce fungal viability throughout the experimental period, reaching 100 % fungi viability inhibition from nine days of interaction. At 156 µg/mL this effect was also evident, although a longer oil-fungi interaction period was needed (12 days of exposure). However, it is notable that at the highest concentrations, fungi failed to develop from the third day of interaction, making conditions completely impractical for growth. Tween 80 did not affect the fungal viability.

Fungal mortality curves are sufficiently sensitive as a test designed to dynamically measure the capacity of a compound to act on the viability of a microorganism. The estimate of mortality of fungal structures can also be inferred for a given concentration of an antimicrobial compound, showing the speed of a fungicidal effect or the duration of a fungistatic effect (Burt, 2003; Cantón; Pemán, 1999). Thus, considering the results obtained, a fungicidal effect was confirmed for all concentrations of essential oil of *C. winterianus*, differing only in the time needed to exhibit this effect.

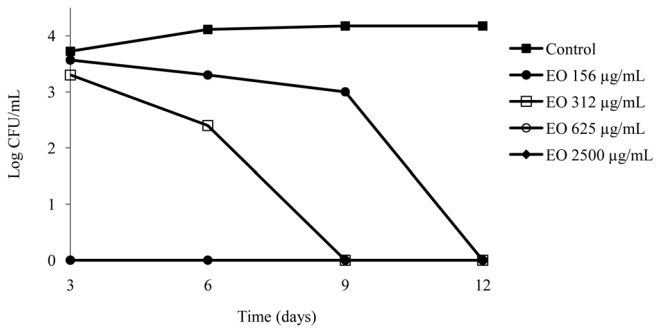

FIGURE 3 - Fungal viability of *T. mentagrophytes* LM 02 in LogCFU/mL in the presence of *C. winterianus* essential oil (EO).

Observations of *T. mentagrophytes* LM02 examined under a light microscope at 400 x magnification after exposure to *C. winterianus* essential oil showed some morphological abnormalities (Figure 4). Essential oil induced similar alterations at all concentrations but increased markedly when the concentration of oil was increased. Although the form of *T. mentagrophytes* conidia did not change, their typical cluster was heavily damaged and they were shown to be highly scattered at all concentrations of essential oil used. The vast majority of hyphae were often wider than normal hyphae, with great losses of pigmentation and presence of vacuoles widely distributed within them (Figure 4 C, D). Microscopic examination of the control mycelium (untreated cell) showed a regular cell structure with homogenous cytoplasm, abundant

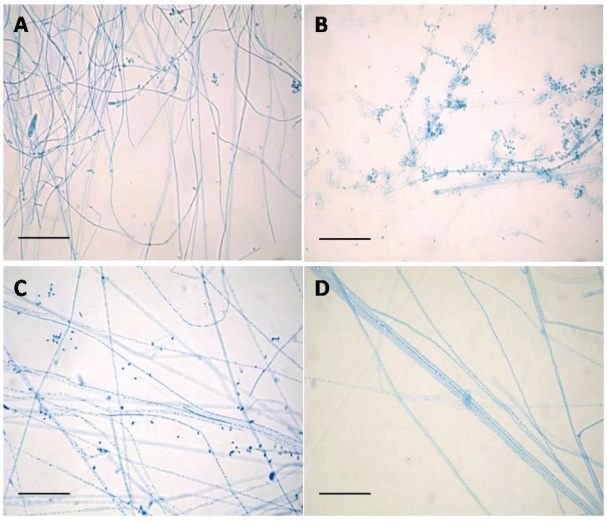

FIGURE 4 - Light microphotographs of *T. mentagrophytes* LM02 mycelium growing on ASD with or without *C. winterianus* essential oil after 5 days of incubation at 28ºC. Control experiment showing typical forms of the species (A, B). Changes in the development of hyphae induced by essential oil at 156 µg/mL (C, D). Bars: 100 µm.

conidiation, long clear septate hyphae, with very rounded microconidia clustered on branched conidiophores. A few macroconidia were present as cigar shaped, thin walled and narrow attachments to hyphae (Figure 4 A, B). The results in this report directly impact disease pathogenesis, since dermatophytosis depends on normal morphogenesis capacity of fungi and their growth in infection locus.

These modifications in fungal morphogenesis may be related to the interference of the essential oil with the enzymes responsible for synthesis or for maintenance of fungal cell wall, as previously cited by other researchers, impairing normal growth and cell morphogenesis (Debillerbeck *et al*. 2001; Gunji *et al.,* 1983).

Considering a possible interference of the essential oil on fungal cell wall, the oil was tested in the whole-cell sorbitol protection assay. In this test, sorbitol-protected cells can grow in the presence of inhibitor products of the fungal cell wall, while their growth is inhibited in the absence of sorbitol. This effect is detected by an increase in the MIC value obtained with sorbitol compared to MIC values without sorbitol (Svetaz *et al*., 2007). MIC values in both experiments were identical, thus suggesting that *C. winterianus* essential oil does not act through the inhibition of fungal cell wall synthesis or assembly.

An important characteristic of *C. winterianus* essential oil and its phytochemicals (e.g. mono-terpenes) is their hydrophobicity and consequently they can interact with fungal membrane, interfering in its integrity. This irreversible harm can be detected by measuring 260-nm-absorbing materials released to the medium, primarily representing nucleotides, of which uracil-containing compounds exhibited the strongest absorbance at different time intervals (2, 4 and 24 h). Amphotericin B was used as a positive control because it can complex with ergosterol in the fungal membranes, thereby compromising their barrier function to the point of causing leakage of cellular contents (Odds *et al.,* 2003). The results showed that the essential oil and amphotericin B produced 100 % cell leakage at 4h of interaction, as shown in Table II. Although the result obtained for MIC of the essential oil had been higher than the MIC of the positive control in 2 hours, both were statistically similar.

These results confirm that the antifungal activity of *C. winterianus* essential oil is related to the interference with the integrity and functionality of *T. mentagrophytes* cell membrane. Due to the lipophilic character of essential oils, they can partition fungal membranes into lipids rendering them more permeable, damaging their integrity and ultimately causing mycelial death (Sikkema *et al.,* 1995; Cox *et al.,* 2000; Burt *et al.,* 2004). Therefore, the results found in this study may be considered relevant and promising. In conclusion, these results support the rational use of *C. winterianus* essential oil for the inhibition of dermatophyte growth. Furthermore, *C. winterianus* essential oil could lead to future developments involving its possible rational use for treatment of dermatophytosis.

TABLE II - Percentage cellular leakage values of *C. winterianus* essential oil (EO) and amphotericin B for *T. mentagrophytes* LM02

Products	Time intervals		
	2 h (%)	4 h (%)	24 h (%)
EO 78 µg/mL	35	100	100
EO 156 µg/mL	55	100	100
EO 312 µg/mL	68	100	100
Amphotericin B 0.60 µg/mL	60	100	100

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