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"IN VITRO" ANTIFUNGAL ACTIVITY OF OZONIZED SUNFLOWER OIL ON YEASTS FROM ONYCHOMYCOSIS

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ABSTRACT

The "*in vitro*" antifungal activity of ozonized sunflower oil (Bioperoxoil®) was tested on 101 samples of yeasts originating from onychomycosis using the disk diffusion method. The oil was efficacious against several clinical fungal strains: *Candida parapsilosis*, *Candida albicans*, *Trichosporon asahii*, *Candida tropicalis* and *Candida guilliermondii*.

Key words: ozonized sunflower oil, yeasts, antifungal activity

Onychomycosis is a fungal infection of nails involved in an estimated 50% of nail disorders (24). It is caused by several species of pathogens but in particular anthropophilic dermatophytes and yeasts (5, 8, 22).

The treatment of such infections may be topical, systemic or both depending on the type, location and the number of sites (17, 19). Several drugs, including Azole compounds, Terbinafine and Ciclopirox olamine are particularly useful in treating onychomycosis (19, 23). Despite the availability of new systemic antifungal therapies, nail infections are difficult to eradicate and recurrence is reported in from 25 to 40% of cases (25).

Recent advances in treatment options have shown ozonized oil to be an alternative therapy against bacterial and fungal diseases (1, 2, 4, 6, 7, 9, 10, 13, 18, 20).

The aim of this study was to evaluate the potential "*in vitro*" antifungal activity of ozonized oil (Bioperoxoil ®) compared to Amphotericin B, Fluconazole, Ketoconazole and Itraconazole (12).

After the project of this study had been evaluated and approved by the Research Ethics Committee (CEP/FAMERP-2907/2004) all patients signed informed consent forms. From January 2004 to December 2006, all patients with clinical suspicion of onychomycosis attended in the Dermatology Service were included in this investigation. Daily observations were made over a 2-week period with yeast samples from nails being cultivated on Mycosel Agar and Sabouraud Dextrose Agar (DIFCO) incubated at 25°C and 37°C, respectively. Several tests were performed to identify the yeasts including digestion and fermentation of sugar, ID32® and CHROMagar®.

"*In vitro*" antifungal activity of ozonized sunflower oil (Bioperoxoil®) and Azole compounds was evaluated using the disk diffusion method, according to the CLSI M44-A

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document guidelines (16) (Filter paper discs with Amphotericin B, Ketoconazole, Fluconazole and Itraconazole - Cecon®). Mueller-Hinton agar supplemented with 2% dextrose and 0.5 mg/L methylene blue (10 cm plates with 25 mL medium) was used, and sterile filter paper discs (Whatman nº 1 - 5 mm in diameter) were impregnated with 35 mg of ozonized oil (Bioperoxoil®) composed of 30% ozonized sunflower seed oil and 0.5% alpha lipoic acid with a peroxide value equivalent to 356 meq O_2/kg. After the plates had been incubated at 35°C for 24 hours, the inhibition zones were measured in millimeters and their means calculated.

The results were then evaluated using one-way analysis of variance (ANOVA) and Mood's median test (15).

In total, 101 yeast samples were isolated from patients with onychomycosis with several species and phenotypic profiles being identified (Figure 1).

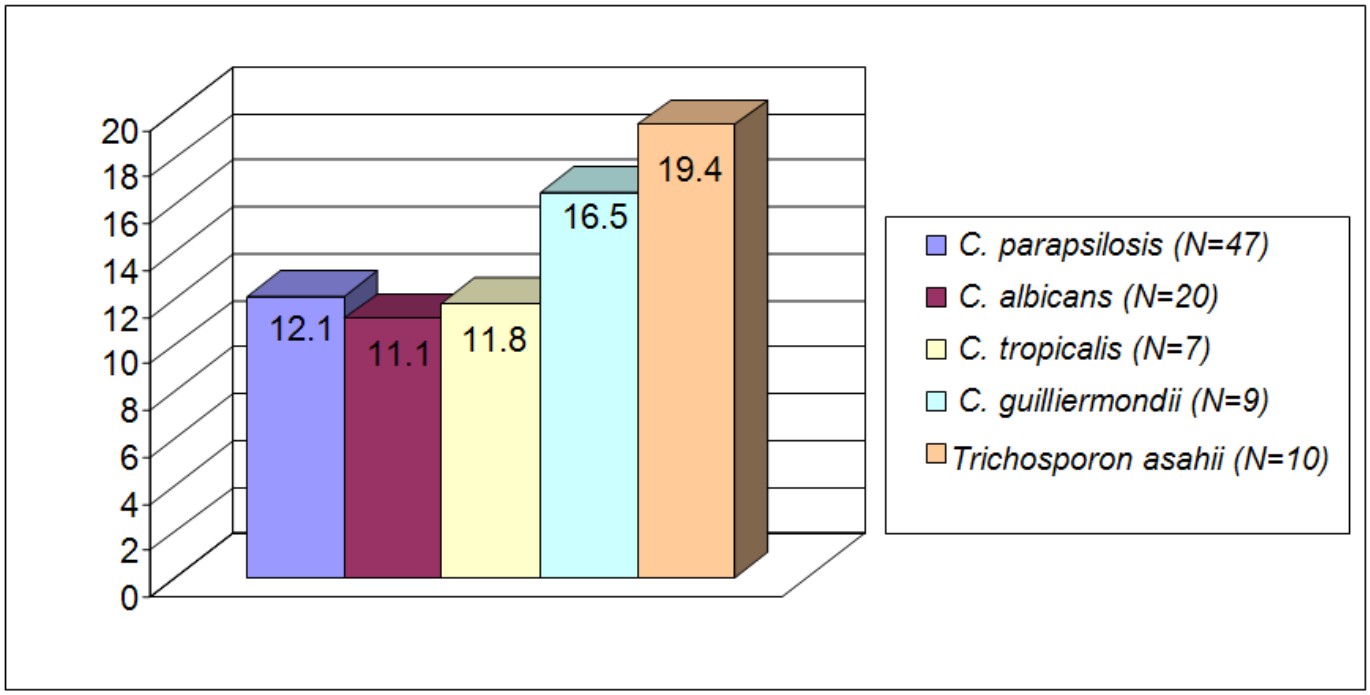

Figure 1. The inhibition zone (mm) of ozonized oil against the main species of yeast

Some samples were identified as rare species with high inhibition zones: *Phaeococcus* sp: 19 mm; *Rhodotorula rubra*: 14 mm; *Candida zeylanoides*: 55 mm; *Candida krusei*, 10 mm; *Hansenula anomala* 27 mm and *Saccharomyces cerevisiae* 21 mm. By ANOVA, there was no statistically significant difference between the halos of the *C. albicans*, *C. parapsilosis* and *C. tropicalis* species. However the inhibition zones of this group was significantly different to those of *C. guilliermondii* and *Trichosporon* asahii, which in turn were statistically similar.

By Mood's median test, a high correlation was seen for the halos of *C. albicans*, *C. parapsilosis* and *C. tropicalis*. However the halo of *C. guilliermondii* was statistically similar to those of *C. tropicalis* and *Trichosporon* sp. and significantly different to the other species.

The mean inhibition zone sizes with ozonized oil for the different yeast species were compared with those of Amphotericin B, Ketoconazole, Fluconazole and Itraconazole anti-fungicides (Table 1). All antifungal drugs inhibited growth; the inhibition caused by ozonized oil was less than those of conventional medications.

Table 1. Mean inhibition zone sizes (mm) of ozonized oil and conventional anti-fungal drugs against the main species of yeast

	Ozonized oil	Amphotericin B	Ketoconazole	Fluconazole	Itraconazole
C. parapsilosis	12	23	42	26	20
C. albicans	11	21	37	26	17
Trichosporon asahii	19	20	36	24	16
C. guilliermondii	16	22	42	24	16
C. tropicalis	12	22	38	29	21

In recent years there has been an increase in fungal resistance to conventional antibiotics used in the treatment of onychomycosis. There are several reasons for this, with a question of fundamental importance being the selection of resistant microorganisms that occurs when the therapeutic process fails. In general, the factors related to the ineffectiveness of treatment can be summarized as the prolonged treatment and its high cost for most of the population both of which culminate in the patient's failure to comply to therapy. In parallel, there has been a rise in the number of cases of several other species that were previously harmless to humans. Faced with this, more attention should be paid to the topical treatment of fungal infections, including onychomycosis (13, 19).

Ozonized oils have been used in the topical treatment of superficial mycoses and bacterial infections (10, 13, 20) and recently Daud et al. (4) demonstrated the high efficacy of these oils to treat dermatophytosis caused by *Microsporum canis*. Although, scarce, controlled *in vitro* studies to assess the minimum effective inhibitory concentration and the "cutoff point for sensitivity", "dose-dependence" and "resistance", have been performed in Cuba and Russia where these therapies originated (3, 11, 21).

The atoxic and oxidant characteristics of these oils have gained interest. Approximately one in three diabetics suffer from onychomycosis; this leads to increased risk for other foot disorders and even limb amputations. The socioeconomic impact of diabetes is devastating both to the individual and to society and any treatment capable of stabilizing the oxygen metabolism and modulating oxidative stress, accompanied by germicidal actions, can improve the quality of life of these patients and reduce their use of medications (12).

This work demonstrated that the mean size of inhibition zones using ozonized oil was smaller than those obtained with other antifungal agents. However, it is impossible to say that this oil is less effective than the control agents as there are no data indicating the minimum inhibitory concentration required for sensitive or resistant strains to become established. A broad antifungal activity was observed as all species were inhibited; there was no evidence of resistance.

A controlled randomized phase III trial using ozonized oil in patients suffering of onychomycosis demonstrated that this treatment is effective and even better than that of Ketoconazole and no side effects were observed (14).

In conclusion ozonized oil (Bioperoxoil®), is a novel and promising alternative therapy against fungal agents which merits further studies using a quantitative methodology and clinical-laboratory correlations to better establish the limits of sensitivity and appropriate protocols for treatment.

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REFERENCES

1. Arias, L.; Jiménez, R.; Beauballet, P.; Gómez, M.; Menéndez, S. (1990). Tratamiento de la otitis externa crónica difusa con aceite ozonizado. Ensayo terapéutico. *Primer Congreso Iberolatinoamericano de Aplicaciones del Ozono*, CNIC-CIMEQ, 31 de Octubre - 3 de Noviembre.
2. Cardoso, C.C.; Macêdo, S.B.; Carvalho, J.C.T.; Dall'Aglio, R.; Ferreira, L.R.; Gomez, M.; Fraschini, F.; De Martini, G. (2002). Azione dell'olio

ozonizzato (Bioperoxoil®) nelle lesione chirurgiche dei modelli pre-clinici. *Farmaci & Terapia* XIX (1/2), 56-60.

3. Cardoso, C.C.; Rodrigues, K.L.; Pichara, N.L.; Aglio, R.D.; Fiorini, J.E.; Frasch, F.; Diana, G.M.; Drago, L.; Vecchi, E.; Carvalho, J.C.T. (2004). Ozonized sunflower oil associated with alpha lipoic acid and lactobacilli: pre-clinic study on antiulcer, anti-inflammatory and antimicrobial activities. *Farmaci.* 28 (4), 97-110.
4. Daud, F.V.; Ueda, S.M.Y.; Navarini, A.; Mímica, L.M.J. (2011). The use of ozonized oil in the treatment of dermatophitosis caused by *Microsporum canis* in rabbits. *Braz. J.Microbiol*. 42, 274-281.
5. Faergemann, J.; Baran, R. (2003). Epidemiology, clinical presentation and diagnosis of onychomycosis. *The Brit. J..3 Dermatol.* 149, 1-4.
6. Fernández, I.; Quinsan, S.; Menéndez, S.; Gómez, M. (1988). Evaluación mutagénica del aceite ozonizado administrado intragástricamente. *I Conferencia Nacional de Aplicaciones del Ozono*, CNIC, Diciembre.
7. Fiorini, C.M.; Fiorini, J.E.; Macedo, S.B.; Ferreira, L.R.; Cardoso, C.C. (2002). Síndrome de Fournier. Relato de um caso tratado com Ozonoterapia Tópica. *Rev. Clín. Terap.* 28 (5), 185-187.
8. Gupta, A.K.; Ryder, J.E.; Summerbell, R.C. (2004). Onychomycosis: classification and diagnosis. *J. Drug. Dermatol.* 3, 51-55.
9. Hernández, F.; Hernández, D.; Zamora, Z.; Díaz, M.; Ancheta, O.; Rodriguez, S.; Torres, D. (2009). Giardia duodenalis: effects of an ozonized sunflower oil product (Oleozon) on in vitro trophozoites. *Exp. Parasitol.* 121 (3), 208-212.
10. Lezcano, I.; Nunez, N.; Gutierrez, M.; Molerio, J.; Regueiferos, M.G.; Diaz, W. (2006). Actividad in vitro del aceite de girasol ozonizado (Oleozon) frente a diferentes espécies bacterianas. *Rev. CENIC Ciênc. Biol.* 27, 46-49.
11. Lincheta, L.F.; Simón, R.D.; Cepero, S.M.; Díaz, N.L.; Duqye, S.M. (2000). Solución para La epidermofitosis de los pies de lãs fuerzas armadas revolucionarias. *Rev. Cubana Med. Milit.* 29 (2), 98-102.
12. Martinez-Sanches, G.; Al-Dalian, S.M.; Menendez, S. Re, L.; Giuliani, A.; Candelario-Jalil, E.; Álvarez, H.; Fernadéz-Montequín, J.I.; Léon, O.S. (2005). Therapeutic efficacy of ozone in patients with diabetic foot. *Eur. J. Pharmacol.* 523, 151-161.
13. Menendez, S.; Falcon, L.; Simon, D.R.; Landa, N. (2002). Efficacy of ozonized sunflower oil in the treatment of tinea pedis. *Mycoses.* 20 (45), 329-332.
14. Menendez, S.; Falcon, L.; Maqueira, Y. (2010). Therapeutic efficady of topical Oleozon in patients suffering from onychomycosis. *Mycoses.* May 17.
15. Mood, A.M. (1941). On the joint distribuition of the medians in samples from a multivariate population. *The Annals of Mathematical Statistics.* 12 (3), 268-278.
16. National Committee for Clinical Laboratory Standards. (2004). Method for Antifungal disk Diffusion Susceptibility Testing of Yeasts: *Approved Guideline M44-A.* NCCLS, Wayne, PA, USA.
17. Prado, F.C. (1999). *Atualização Terapêutica*. Artes Médicas, 19ª edição.
18. Rodrigues, K.L.; Cardoso, C.C.; Caputo, L.R.; Carvalho, J.C.T.; Fiorini, J.E.; Schneedof, J.M. (2004). Cicatrizing and antimicrobial properties of ozonised oil from sunflower seeds. *Imflammopharmacol.* 12 (3), 261-270.
19. Sampaio, S.A.P.; Rivitti, E.A. (2000). *Dermatologia.* Artes Médicas, São Paulo.
20. Sechi, L.A.; Lezcano, I.; Nunez, N.; Espim, M.; Dupre, I.; Pinna, A.; Molicotti, P.; Fadda, G.; Zanetti, S. (2001). Antibacterial activity of ozonized sunflower oil (Oleozon). *J. Appl. Microbiol.* 90, 279-284.
21. Siqueira Junior, J.F.; Rôças, I.N.; Cardoso, C.C.; Macedo, S.B.; Lopes, H.P. (2000). Efeitos antibacterianos de um novo medicamento – óleo ozonizado- comparados às pastas de hidróxido de cálcio. *Rev. Bras. Odont.* 57 (4), 252-6.
22. Svejgaard, E.L.; Nilsson, J. (2004). Onychomycosis in Denmark: prevalence of fungal nail infection general practice. *Mycoses.* 47, 131-135.
23. Warshaw, E.M.; Fett, D.D.; Bloomfield, H.E. (2005). Pulse versus continuous terbinafine for onychomycosis: a randomized, double-blind, controlled trial. *J. Amer. Acad. Dermat.* 53 (4), 578-584.
24. Winston, J.A.; Miller, J.L. (2006). Treatment of onychomycosis in diabetic patients. *Clin. Diabet.* 24(4), 160-166.
25. Woodfolk, J.A. (2005). Allergy and Dermatophytes. *Clin. Microbiol. Rev*. Jan. 18, 30 - 43.