

STUDIES ON THE ANTIFUNGAL AND ANTIBACTERIAL PROPERTIES OF MYCOPIROX CREAM

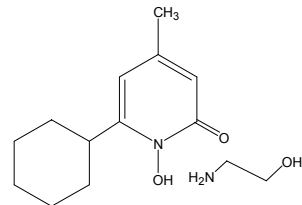
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Abstract

Mycopirox is a 1% aqueous cream of the N-hydroxypyrrone ciclopirox olamine, topical antifungal agent. The active ingredient has been shown to be superior to the azoles such as miconazole, clotrimazole and ketoconazole in that, unlike the azoles, it is fungicidal, bactericidal and has broad spectrum activity against both fungi and bacteria. In this study, we compared the antimicrobial properties of Mycopirox, as formulated, with two leading brands of miconazole cream, available in West Africa. Our studies show that Mycopirox is superior to the 'leading azoles', as it is rapidly fungicidal and bactericidal at applied doses and is potent against both gram positive and gram negative bacteria.

Introduction

Mycopirox® is the LaGray brand of 1% ciclopirox olamine, a member of the N-hydroxypyridone class of topical antifungal agents. The primary utility of Mycopirox® is in the treatment of uncomplicated dermatomycosis and in mixed fungal/opportunistic bacterial infections. Ciclopirox olamine has been shown to have broad spectrum antifungal and antibacterial activity. It has also been shown to have anti-inflammatory properties, making it an ideal agent for an antifungal cream formulation.



The purpose of this study is to demonstrate that Mycopirox®, as formulated, has:

1. Potent fungal growth inhibitory activity and, unlike azole antifungal creams, is rapidly fungicidal
2. Broad spectrum antifungal activity and is rapidly bactericidal

Experimental

a. Bacterial and fungus growth inhibition

Candida albicans (CA. KBTH.1) was inoculated into nutrient broth and incubated overnight. The optical density (OD) of the culture was determined at 600 nm. A loop full of the culture was streaked onto nutrient agar plates. Various concentrations of Mycopirox cream were prepared in 96%v/v alcohol. Sensitivity discs were soaked in the cream solutions for 20 minutes after which they were removed and allowed to dry at room temperature. Each of the dried discs was placed onto the agar plates. The plates were then incubated at 37±2°C for 21 hours and examined for zones of inhibition. The process was repeated using both Gram negative (*Escherichia coli* EC.37MH.1) and Gram positive (*Staphylococcus aureus* SA.KBTH.1) bacteria on the appropriate media

b. Comparative antifungal activity of Mycopirox with two brands of Azole Creams

Candida albicans (CA.KBTH.1) was inoculated into nutrient broth and incubated overnight at 25±2°C to provide an inoculum broth. The OD of the broth was determined at 600nm. Samples of Mycopirox® and the miconazole creams were added to nutrient broth and vortexed for 2 minutes. Each sample was heated in a water bath at 80°C for 30 minutes after which they were allowed to cool to room temperature. The inoculum broth (0.2mL) added to each sample and vortexed for 2 minutes. A sample of the resultant mixture (0.1mL) was quantitatively transferred immediately onto prepared agar plates and streaked evenly. Plates were incubated at 25±2°C and colonies counted after 48 hours from which viable cells were determined as colony forming units (CFUs). The CFUs were determined at 3hr intervals over 24 hours. Time kill-curves (plot of CFUs vs. time) were then generated for the creams.

c. Fungicidal Properties of Mycopirox Cream

A strain of *Candida albicans* (CA.KBTH.1) was inoculated into nutrient broth and incubated overnight at 25±2°C. The OD of the broth was determined at 600nm. Mycopirox cream (1g) was added to nutrient broth and the mixture vortexed for 2 minutes and heated in a water bath at 80°C for 30 minutes after which it was cooled to room temperature to provide an inoculum broth. A sample of the inoculum broth (0.1 mL) was inoculated into the cream mixture and vortexed for 2 minutes. 0.1mL of the resultant solution of cream was quantitatively transferred immediately onto prepared agar plates and streaked evenly. Plates were incubated at 25±2°C and colonies observed after 48 hours from which CFUs were determined. The procedure was repeated at 3 hr intervals over a period of 24 hours. Mean kill rates (ΔCFU/T) of *Candida albicans* was plotted against various concentrations of cream from which the minimum fungicidal concentration of Mycopirox cream was determined.

d. Bactericidal activity of Mycopirox Cream

Escherichia coli (EC.37MH.1) was inoculated into nutrient broth and incubated overnight at 37±2°C. The inoculum (0.1mL) was transferred into 0.9 mL of normal saline and a 6-fold dilution subsequently prepared to provide an inoculum mixture. Mycopirox cream (1g) was added to nutrient broth and vortexed for 2 minutes from which serial dilutions were prepared using broth as diluent. The cream mixtures were heated in a water bath at 80°C for 30 minutes after which they were allowed to cool to room temperature. The inoculum mixture (0.1 mL) was added to each of the cream solution and vortexed for 2 minutes. A sample (0.1 mL) of each of the resultant mixture was quantitatively transferred immediately onto prepared agar plates and streaked evenly. Plates were incubated at 37±2°C and colonies counted after 24 hours from which CFUs were determined. CFUs were determined at 10 minutes intervals over 30 minutes.

Results

a. Growth inhibitory activity against fungi and bacteria

The results of the microbial growth inhibitory activities are shown in Figs. 1 to 3. In Fig. 1, large zones of inhibition were observed at the concentrations (0.2 – 0.35% cream in broth) studied.

Similarly, against both the Gram positive bacterium *S. aureus* (Fig. 2) and the Gram negative bacteria *E. coli* (Fig.3), Mycopirox showed clear zones of inhibition at all concentrations studied.

Subsequent studies will be done to determine the minimum inhibitory concentrations of the cream against the yeast *C. albicans*, the filamentous fungus *Aspergillus niger*, the Gram positive bacteria *S. aureus* and *S. epidermidis* and the Gram negative bacteria *E.coli*, *P. aeruginosa* and *K. pneumoniae*.

Fig 1. Zones of Inhibition of Various Concentrations of Mycopirox® against *Candida albicans* CA.KBTH.1)

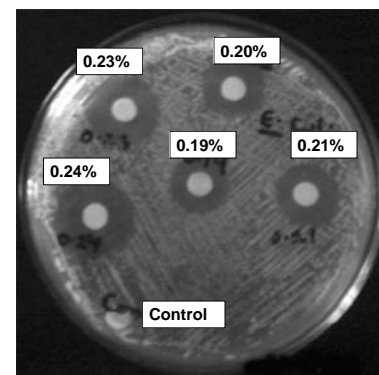
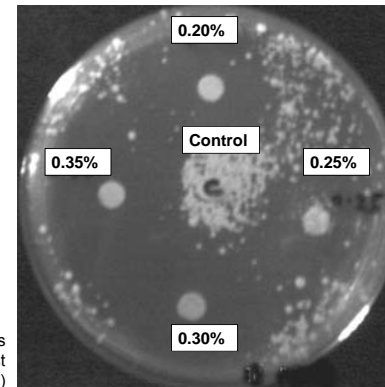


Fig. 2 Zones of Inhibition of Various Concentrations of Mycopirox® against *Staphylococcus aureus* SA.KBTH.1

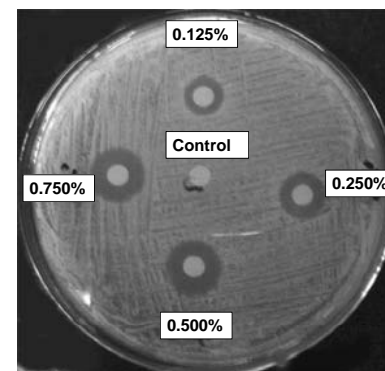


Fig. 3. Zones of Inhibition of Various Concentrations of Mycopirox® against *Escherichia coli* EC.37MH.1

b. Comparative antifungal activities of Mycopirox and two brands of Azole cream.

Results of the study are as shown in Table 1. The data in Table 1 was used in generating the Time-Kill curves in Fig 4.

Time/hrs	Total Colony Forming Units/mL (x 10 ⁸)			
	Mycopirox Cream	Miconazole Cream Brand 1	Miconazole Cream Brand 2	Control
0	12.700	13.10	15.60	14.50
3	11.700	13.50	16.10	20.40
6	9.190	21.60	22.10	30.20
9	2.420	21.80	31.20	45.80
12	1.110	25.90	37.80	53.00
24	0.202	28.60	42.70	90.60

Table 1. Viable counts vs. time for antifungal creams

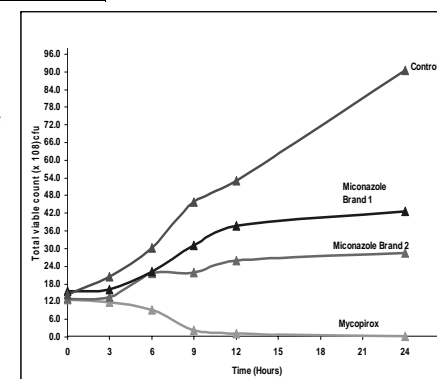


Fig 4. Time-Kill Curves of antifungal creams against *C. albicans*

The results show that while all the creams inhibited fungal growth, Mycopirox® was several orders of magnitude more inhibitory than the azole creams.

The azole creams did not produce any loss in cell viability, while Mycopirox® demonstrated rapid fungicidal activity, with virtually no viable counts at 24 hours.

d. Fungicidal Activity of Mycopirox Cream

The data of viable counts vs different concentrations of cream at different time intervals is shown in Table 2 and presented as Time-Kill curves in Fig. 5. From this data, the minimum fungicidal concentration of ciclopirox olamine in Mycopirox was calculated to be 97.3 mcg/mL.

Table 2. Viable counts of *C. albicans* at various concentrations against time

Time/hrs	Total Colony Forming Unit(CFU/mL)					
	A	B	C	D	E	F
0	54.0	64.0	78.0	73.0	66.0	78.0
3	18.0	39.0	69.0	68.0	74.0	84.0
6	8.0	31.0	52.0	63.0	71.0	110.0
9	7.5	28.0	51.0	62.0	63.0	190.0
12	5.0	27.0	50.0	64.0	66.0	220.0
24	1.0	9.5	39.0	57.0	60.0	910.0

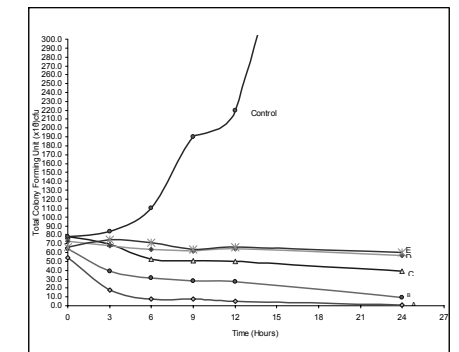


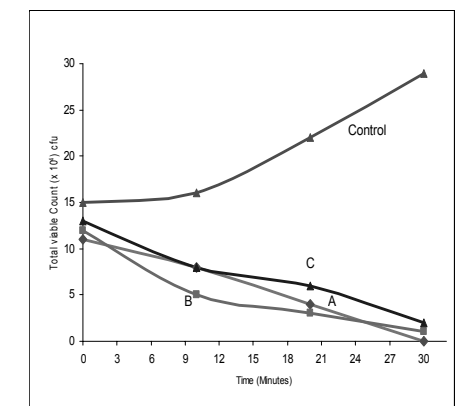
Fig. 5 Time-Kill curves of Mycopirox against *C. albicans* at various concentrations

c. Bactericidal Properties of Mycopirox Cream

The results of the study of viable counts against time is shown in Table 3 and as a Time-Kill curve in Fig. 6. As shown in the figure, Mycopirox is rapidly bactericidal at the three concentrations tested.

Table 3 Viable Counts of *E. coli* Vs. Time

Time/Minutes	Colony Forming Units (x 10 ⁴)			
	A	B	C	Control
0	11.0	12.0	13	15
10	8.0	5.0	8	16
20	4.0	3.0	6	22
30	0.0	1.0	2	29



A = 0.1%w/v, B = 0.05%w/v and C = 0.025%w/v Ciclopirox Olamine in Mycopirox Cream

Conclusion

- Mycopirox® cream inhibits fungal growth. It shows pronounced zones of inhibition even at very low concentrations.
- Mycopirox® cream is fungicidal compared to the two brands of Miconazole creams, which are fungistatic. Mycopirox is rapidly fungicidal even at low concentrations.
- Mycopirox® has broad spectrum antibacterial activity.
- Mycopirox® is bactericidal and even rapidly bactericidal at low concentrations.

References

K. Kokjohn et Al., (2003) Evaluation of in vitro activity of ciclopirox olamine, butenafine HCl and econazole nitrate against dermatophytes, yeasts and bacteria. *International Journal of Dermatology* 42: 11–17