

BRIEF REPORT

# Amorolfine 5% Nail Lacquer Exhibits Potent Antifungal Activity Compared to Three Acid-Based Devices Indicated for the Treatment of Onychomycosis: An In Vitro Nail Penetration Assay

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## ABSTRACT

**Introduction:** Onychomycosis is the most common infectious disease involving nails. The aim of this study was to evaluate the antifungal activity of amorolfine 5% nail lacquer and three different acid-based medical devices indicated in the treatment of onychomycosis using an in vitro nail penetration assay.

**Methods:** Four products were tested in vitro: (a) amorolfine 5% nail lacquer; (b) ethyl lactate and acetic acid; (c) citric acid and urea; (d) ethyl lactate, glycerin, lactic acid, and citric acid. Test products were applied to healthy human cadaver nails and allowed to dry. Disks were

cut from each piece of nail and placed on seeded agar plates of *Trichophyton rubrum*. Following incubation at 30 °C, zones of inhibition were measured.

**Results:** Amorolfine-treated nails exhibited inhibitory activity against *T. rubrum* with a mean zone of inhibition of 59.2 mm in diameter. In contrast, all three acid-based medical devices and the untreated controls showed no zones of inhibition (mean effective zones of 0 mm).

**Conclusion:** In this in vitro nail penetration model, head-on, comparative study, we showed that amorolfine 5% nail lacquer possesses potent antifungal activity, whereas no antifungal activity was detected for three commercially available acid-based medical devices under identical assay conditions.

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**Keywords:** Amorolfine; Fungicidal; Low pH; Medical devices; Onychomycosis

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## INTRODUCTION

Onychomycosis is a common infectious disease, which if inappropriately treated can lead to

spread of the infection, complications, diminished patient quality of life and may result in stigmatization [1].

A fungal nail psychosocial perception study carried out in Hong Kong revealed wide-ranging misconceptions about onychomycosis treatments since 26% of respondents thought disinfectant solutions, such as vinegar or alcohol, were effective treatments, 42% incorrectly believed that antibiotics were an effective treatment, and 14% thought the only cure was complete removal of the nail [2]. Another survey indicated that many people with mild, uncomplicated onychomycosis may never consult a physician [3], and hence are likely to rely on topical self-medication.

To be effective, a topical treatment for onychomycosis must have potent fungicidal activity and be able to penetrate the nail plate, consisting of dense cross-linked keratin fibers held together by cysteine-rich proteins and disulphide bonds [4].

Amorolfine 5% nail lacquer (Loceryl<sup>®</sup>, Galderma SA, Lausanne, Switzerland) is a topical antifungal with proven clinical efficacy in the treatment of onychomycosis caused by dermatophytes, yeasts and moulds [5–7]. The active substance, amorolfine hydrochloride (5.574 g in 100 mL ethanol-based nail lacquer), has a fungicidal effect and broad antimycotic spectrum.

A wide range of acid-based medical devices are commercialized to treat onychomycosis and common ingredients include acids to inhibit fungal growth, glycerin to hydrate and moisturize the nail, and urea to hydrate and gently dissolve the intercellular matrix of the nail plate, in addition to penetration enhancers and film-forming agents. However, there is a notable lack of published reports on their exact composition, mechanism of action and whether

they can penetrate through the nail down to the nail bed.

The objective of this study was to compare the antifungal activity of amorolfine 5% nail lacquer with three different commercially available acid-based medical devices using an in vitro nail penetration assay.

## METHODS

Four products were tested: (a) amorolfine 5% nail lacquer; (b) ethyl lactate and acetic acid (Excilor<sup>®</sup>, Vemedia, Diemen, The Netherlands); (c) citric acid and urea (Scholl Fungal Nail Treatment, Bayer Healthcare AG, Leverkusen, Germany); and (d) ethyl lactate, glycerin, lactic acid, and citric acid (Nailner<sup>®</sup>, YouMedical, Amsterdam, The Netherlands).

A total of 6 non-diseased big toe nails were taken from six fresh human cadavers and washed before use. For each test product, 3 pieces of nail plate from different donors were treated with 25  $\mu$ L test product/cm<sup>2</sup>. The compound was applied to the center of uncut nails and allowed to spread evenly. After air-drying for 24 h, the nail was inverted and two disks of 4 mm diameter (biopsy punch) were cut from the center of each piece of nail (a total of 6 assays per test product). Each disk was placed, with the treated side facing upwards, at the center of a seeded agar plate of *Trichophyton rubrum* (ATCC<sup>®</sup> MYA-4438<sup>™</sup>, Manassas, VA, USA) ( $2\text{--}5 \times 10^5$  conidia/mL). Disks from untreated nails were used as controls. Following 4-day incubation at 30 °C, the zone of inhibition was measured with an electronic caliper. Each test was performed in duplicate.

The mean zone of inhibition  $\pm$  the standard error of the mean (SEM) was calculated and compared between groups. A mean zone of

inhibition >10 mm was chosen to indicate potent antifungal activity.

No formal statistical testing was considered necessary to compare the amorolfine 5% nail lacquer group with the other treated groups (which gave values of zero without any variability).

This article does not contain any new studies with human or animal subjects performed by any of the authors.

## RESULTS AND DISCUSSION

The mean nail thickness ranging from 0.86 to 1.09 mm was similar for assays for all test products (Table 1). Nail disks treated with amorolfine 5% nail lacquer showed a mean zone of inhibition  $\pm$  SEM of  $59.2 \pm 3.4$  mm in diameter (Table 1 and Fig. 1a). In contrast, the three acid-based medical devices (Fig. 1b–d) and the untreated control (Fig. 1e) all showed no zones of inhibition (mean effective zones of  $0 \pm 0$  mm) (Table 1). Amorolfine 5% nail lacquer demonstrated potent antifungal activity when compared to the three medical devices tested (Fig. 2).

The disk-diffusion method has been widely used to demonstrate antifungal activity of various drugs [8, 9]. In this study, amorolfine 5% nail lacquer demonstrated effective antifungal activity (inhibition zone 59.2 mm in diameter), corroborating previous findings showing that amorolfine 5% nail lacquer penetrates the nail to the site of infection in the subungual compartment [5, 10, 11]. Due to its high potency, even small amounts of amorolfine reaching the nail bed inhibit or kill the pathogens [12] and amorolfine concentrations deep in the nail bed still exceeded the minimum inhibitory concentrations of *T. rubrum* (<0.001–0.13  $\mu$ g/mL) [6]. Conversely, the three acid-based medical products tested did not show

any antifungal activity in the human nail penetration model used.

This easy-to-use in vitro nail penetration model was developed taking care to ensure that the antifungal effects witnessed were from the test compound penetrating the nail plate and the test substance could not spread over the edge of the nail biopsy to influence the growing fungi beneath the nail plate. The compound was applied to the center of uncut nails, allowed to spread evenly and dry before biopsy punches were taken from the center of the nail specimen to ensure there was no spillage of the test compound. By inverting the nail before taking the biopsy, the disks were cut in the direction of unexposed nail surface to exposed nail surface to ensure that the test compound was not artificially dragged from the biopsy punch. It is noteworthy that it would also be possible to increase the biopsy size or seal the margins of the biopsied nail plate. However, presumably there was no spreading over the edges since no zones of inhibition were observed with the acid-based medical devices under identical assay conditions in this head-on comparative study.

The role of acidity (low pH) in the pathogenesis of dermatophytes is complex [13]. Transmission electron microscopy has shown that many fungal cells were necrotic when *T. rubrum* or *Candida albicans* was treated for 60 min in direct contact with 50% K101 nail solution (Moberg Pharma, Bromma, Sweden), a topical formulation of pH 4 containing 50 g propylene glycol, 15 g lactic acid and 10 g urea [14]. The most prominent changes were observed with *T. rubrum*; the cell wall was clearly damaged, the membrane was disrupted and the content in the cytoplasm was degraded. The authors suggested that the presence of a diol (propylene glycol) disturbed cell wall integrity by an unspecific mode of action and the low pH may have contributed to the efficacy

**Table 1** Measurement of zones of inhibition

Test compound	Donor	Nail disk	Nail thickness (mm)	Inhibition zone diameter (mm)
(a) Amorolfine 5% nail lacquer	Donor 1	1	0.83	70
	Donor 1	2	1.09	51
	Donor 2	3	1.08	53
	Donor 2	4	1.09	68
	Donor 3	5	1.25	52
	Donor 3	6	1.25	61
Mean ( $\pm$ SEM)			1.09	59.2 $\pm$ 3.4
(b) Ethyl lactate and acetic acid	Donor 4	1	0.5	0
	Donor 4	2	0.54	0
	Donor 2	3	1.02	0
	Donor 2	4	0.98	0
	Donor 5	5	0.98	0
	Donor 5	6	1.11	0
Mean ( $\pm$ SEM)			0.86	0 $\pm$ 0
(c) Citric acid and urea	Donor 1	1	0.69	0
	Donor 1	2	0.83	0
	Donor 6	3	1.43	0
	Donor 6	4	1.46	0
	Donor 3	5	1.16	0
	Donor 3	6	0.96	0
Mean ( $\pm$ SEM)			1.09	0 $\pm$ 0
(d) Ethyl lactate, glycerin, lactic acid, and citric acid	Donor 4	1	0.5	0
	Donor 4	2	0.5	0
	Donor 6	3	1.01	0
	Donor 6	4	1.01	0
	Donor 5	5	1.15	0
	Donor 5	6	1.09	0
Mean ( $\pm$ SEM)			0.88	0 $\pm$ 0

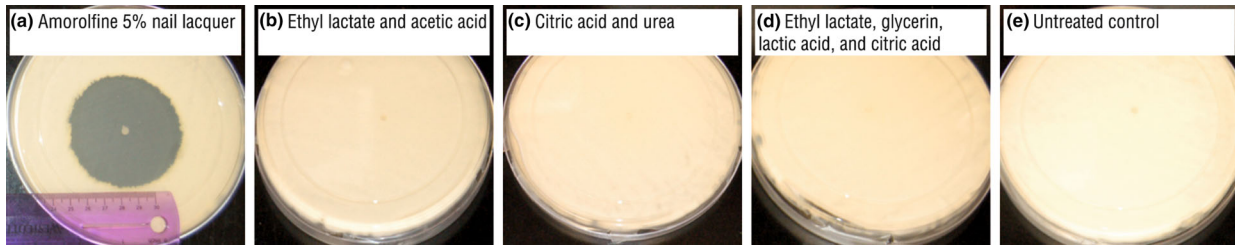
**Table 1** continued

Test compound	Donor	Nail disk	Nail thickness (mm)	Inhibition zone diameter (mm)
(e) Untreated control	Donor 1	1	1.26	0
	Donor 1	2	1.09	0
	Donor 2	3	1.33	0
	Donor 2	4	0.87	0
	Donor 3	5	0.75	0
	Donor 3	6	1.02	0
Mean ( $\pm$ SEM)			1.05	0 $\pm$ 0

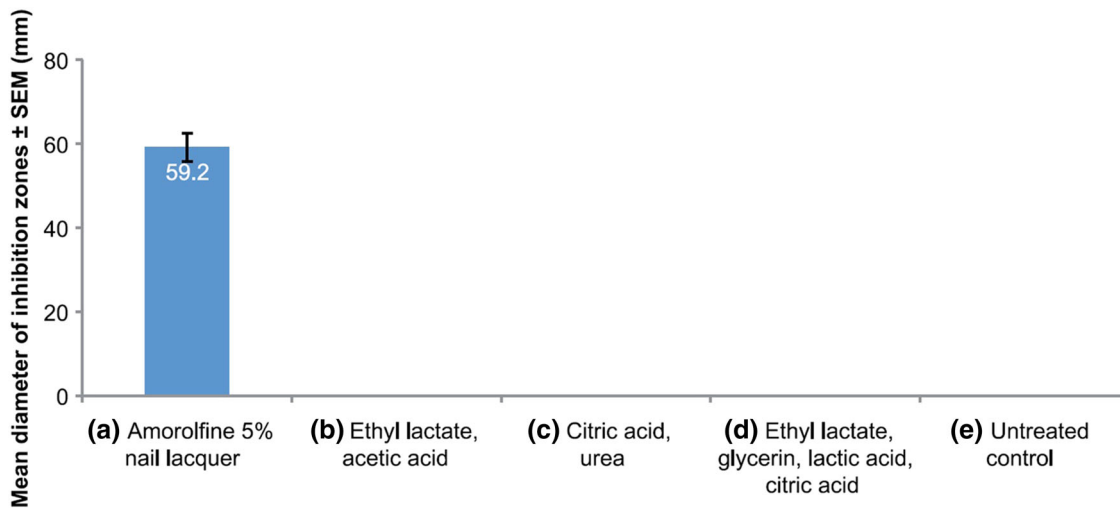
[14]. Although pH 4 is not low enough to inhibit growth, the production of *T. rubrum* arthroconidia has been shown to be dependent on pH with 85% less arthroconidia produced at pH 4.5 compared to pH 7.5 [15].

A pH of  $\leq 3$  was found to be fungicidal when Sabouraud dextrose broths of different pH values were inoculated with *T. rubrum* and incubated for 14 days at 25 °C [16]. However, a penetration test in an in vitro porcine nail model showed that even after 120 applications of acetic acid (Excilor), the pH measured in the nails was only 3.37, i.e., not low enough to be fungicidal [16]. Furthermore, the lowest pH of 3.37 was only measured in the superficial parts of the nail (0.5 mm depth) [16].

Although acid-based solutions may penetrate and acidify the nails, as stated by the manufacturers, the difficulty in obtaining a low enough pH throughout the nail and in the nail bed may explain why the three acid-based medical devices demonstrated no fungicidal activity in the nail penetration model.



**Fig. 1** Representative plates showing zones of inhibition of *Trichophyton rubrum* growth in the nail penetration assay with **a** amorolfine 5% nail lacquer; **b** ethyl lactate and acetic acid; **c** citric acid and urea; **d** ethyl lactate, glycerin, lactic acid, and citric acid; **e** untreated control



**Fig. 2** Effect of amorolfine 5% nail lacquer and three medical devices against *Trichophyton rubrum* in a nail penetration assay

Possible limitations of this in vitro study are that nails only received a single application of test product, and extrapolating from in vitro to in vivo may not be entirely accurate as in the nail environment in vivo, natural compounds may influence fungal growth. However, this in vitro nail penetration model has been shown to closely simulate in vivo testing and visually demonstrates both nail penetration and antifungal activity after a single application of amorolfine 5% nail lacquer [11].

Clinical evidence of efficacy of acid-based solutions against onychomycosis remains scarce [17, 18]. The K101 nail solution (a mixture of

propylene glycol, lactic acid and urea, pH 4) has been clinically demonstrated to be effective in the treatment of distal subungual onychomycosis [18–20]. In a randomized placebo-controlled study ( $n = 346$  K101,  $n = 147$  placebo), more patients with  $\leq 50\%$  nail involvement achieved mycological cure after 26 weeks in the K101 group (27.2%) than in the placebo group (10.4%;  $P = 0.0012$ ) [20].

## CONCLUSION

This study confirms penetration through the nail of amorolfine 5% nail lacquer with potent

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