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Evaluation of Inhibitory Effects of Citric and Tartaric Acids and Their Combination on the Growth of *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida albicans* and *Malassezia furfur*

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Abstract: The present study was carried out to evaluate the effects of citric and tartaric acids and their combination on the growth inhibition of some important pathogenic fungi *in vitro*. *Trichophyton mentagrophytes* var. *mentagrophytes*, *Candida albicans*, *Aspergillus fumigatus* and *Malassezia furfur* were cultured into specific media and subsequently, fungal conidia were harvested from the medium surface and counted by hemacytometer method. The antifungal susceptibility test of citric and tartaric acids and their combination against fungi were assayed by broth macrodilution technique. The results demonstrated that citric acid had more fungistatic and fungicidal activities than those of tartaric acid against all pathogenic fungi tested and its effect on filamentous fungi was higher than that on the yeasts. Antifungal activity of the acids combination was similar to citric acid, but higher than tartaric acid alone. Further research is needed to assess the efficacy of citric and tartaric acids as inhibitors of fungal growth in clinical trials, especially in treatment of patients with fungal infections.

Key words: Citric acid · Tartaric acid · Antifungal activity · Filamentous fungi · Yeasts

INTRODUCTION

Microbiological resistance to antifungals, particularly polyenes and azoles, has been increasingly reported. Despite the increase in frequency of resistance, reports of clinical antifungal failures or single stains becoming resistant to antifungal therapies remain distinctly uncommon [1,2]. On the other hand, the number of serious invasive fungal infections has continued to increase due to the fact that more immunosuppressed patients are at risk for these infections. Fortunately, the antifungal agents against these organisms have also increased with more effective and less toxic alternatives [3, 4].

Organic acids are widely used as preservatives in foods and have been used as buffer agents in medical solutions [5,6]. Several studies reported the inhibitory effect of these acids such as saturated fatty acids, formic and propionic acids, lactic acid and medium-chain fatty acids against different microorganisms [7-9]. In addition to their suppressing effect on the growth of food spoilage microorganisms, organic acids were shown to possess antibacterial activities against various infectious pathogens including *Staphylococcus aureus, Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli*, as well as *Clostridium botulinum* [10-13]. As far as we know, there is little information about the antifungal activity of organic acids, especially citric and tartaric acids against clinically important potential pathogens. The main objective of this study was to investigate the antifungal effects of two above mentioned acids against *T. mentagrophytes* var. *mentagrophytes*, *C. albicans*, *A. fumigatus* and *M. furfur*.

MATERIALS AND METHODS

Test Organisms: Clinical isolates of *T. mentagrophytes* var. *mentagrophytes* (isolated from a dog with dermatophytosis), *C. albicans* (isolated from a patient with cutaneous candidiasis), *A. fumigatus* (isolated from an ostrich with disseminated aspergillosis) and *M. furfur* (isolated from a patient with pityriasis versicolor) were obtained from fungal collection of Faculty of Veterinary Medicine, University of Tehran, Iran.

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Fungal Cultivation and Preparation of Inocula: Trichophyton mentagrophytes was cultured on Sabouraud glucose agar containing cycloheximide (*Merck Co., Darmstadt, Germany*) at 30°C for 3 weeks, *A. fumigatus* on Sabouraud glucose agar at 37°C for 3 days, *C. albicans* on Sabouraud glucose agar at 32°C for 3 days and *M. furfur* on Dixon agar (*Merck Co., Darmstadt, Germany*) at 32°C for 3 days. After incubation, *T. mentagrophytes* microconidia, *A. fumigatus* conidia, *C. albicans* and *M. furfur* yeasts were flooded with sterile saline containing 0.1% Tween 80 and scraped off with the aid of a loop. The fungal suspensions were then pipetted into a test tube and filtered through gauze to remove hyphal fragments and agar blocks. The concentration of fungal conidia was quantitated by counting with a hemacytometer and adjusted at a density of 3×10^6 cell/ml for *T. mentagrophytes*, 2.5×10^6 cell/ml for *A. fumigatus* and 1×10^7 cell/ml for both *C. albicans* and *M. furfur*.

Preparation of Citric and Tartaric Acids: Citric and tartaric acids (*Merck Co., Darmstadt, Germany*) were obtained as reagent-grade powders from their respective manufacturers.

Antifungal Assay: The *in vitro* fungistatic and fungicidal concentrations were determined by a serial dilution method using broth macrodilution technique as previously described with modifications [14], which Sabouraud glucose broth (for *T. mentagrophytes*, *A. fumigatus* and *C. albicans*) and Dixon broth (for *M. furfur*) were used instead of RPMI 1640 for test isolates. Briefly, dilutions of citric and tartaric acids were prepared from stock solutions of 50% (50 mg/ml) in deionized water. A series of concentrations of each acid and their combination, 50% (50 mg/ml), 45% (45 mg/ml), 40% (40 mg/ml), 35% (35 mg/ml), 30% (30 mg/ml), 25% (25 mg/ml), 20% (20 mg/ml), 15% (15 mg/ml), 10% (10 mg/ml), 5% (5 mg/ml), 2.5% (2.5 mg/ml), 1.25% (1.25 mg/ml) and 0.75% (0.75 mg/ml) were prepared in Dixon broth and Sabouraud glucose broth (1:1) into the tubes. Then, each tube was inoculated with 10 µl of the fungal suspensions. The tubes were incubated at 30°C for 7 days for *T. mentagrophytes*, at 37°C for 48 h for *A. fumigatus*, at 32°C for 48 h for both *C. albicans* and *M. furfur* and then scored for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC_{90}) was defined as the lowest concentration of the acid which produced no visible growth (90% inhibition). Each MIC_{90} determination was performed in triplicate. Drug-free and fungal control tubes were included for each isolate tested. The minimum fungicidal concentration (MFC) was determined in triplicate by subculturing 0.1 ml aliquots from all MIC_{90} tubes showing no visible growth on specific media.

Statistics: Student's t-test was used to assess statistical differences between the groups. Probabilities of 5% were taken to be statistically significant.

RESULTS AND DISCUSSION

The antifungal activity of citric and tartaric acids and their combination against all tested fungi, as measured by agar dilution test, was presented in Table 1. All of the fungal organisms tested were affected by citric and tartaric acids and their combination when applied at concentration more than 2.5 %. Citric acid was active against all pathogenic fungi tested. Conversely, tartaric acid showed modest activity against all fungi tested, but was less active than citric acid against fungi. The MIC_{90} and MFC values of the acids combination approximated the similar to those of citric acid but less than tartaric acid alone; thus, overall, neither significant synergism nor antagonism was seen from this screening assay with the combination of citric plus tartaric acids. Totally, the mean MIC_{90} and MFC values for citric acid and their

Table 1: Determination of MIC_{90} and MFC (%) of citric and tartaric acids and their combination against *T. mentagrophytes*, *A. fumigatus*, *C. albicans* and *M. furfur*

	Fungus							
	T. mentagrophytes		*A. fumigatus*		*C. albicans*		*M. furfur*	
Acid	*MIC_{90}	**MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC
Citric acid	5	2.5	5	1.25	5	2.5	10	5
Tartaric acid	5	2.5	15	10	20	15	15	10
Combination	5	2.5	5	1.25	5	2.5	10	5

*MIC_{90} : Minimum inhibitory concentration (90% inhibition)

** MFC : Minimum fungicidal concentration

combination on different fungal isolates were 3.3 and 2.2-fold higher than those for tartaric acid. The higher activity of citric acid may be attributed to have several inhibitory mechanisms such as depression of internal pH of microbial cell by ionization of undissociated acid molecules and disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force [15,16]. Conversely, tartaric acid, as an antimicrobial agent, is believed to act only by lowering the pH of the cell [17]. In addition to the inhibition of energy production, tartaric acid prevents the production of malic acid, which is a key intermediate in the production of glucose in the process of gluconeogenesis, the principal fuel for the cells [18]. Several studies showed that citric acid and its salts inhibit the growth of the most common bacterial pathogens such as *Arcobacter* spp., *Campylobacter* spp., lactobacilli, *E. coli* O157:H7 and *L. monocytogenes* [19, 20]. No previous study has been carried out on antifungal activities of these acids tested, except Lee *et al.* [11] on *C. albicans* isolate. This study showed that citrate salt was active against Gram-positive species and *C. albicans* but showed little activity against Gram-negative species; acetate salt showed the opposite results. Their combination did not show synergism or antagonism.

The present results showed that citric and tartaric acids are more activity against filamentous fungi (*T. mentagrophytes* and *A. fumigatus*) than yeasts (*C. albicans* and *M. furfur*). The reasons for this may be related to the different structures of the fungal cell walls. The main target of organic acids and its relatives are cell wall and membrane proteins. The hyphae wall of filamentous fungi generally contains less protein than the cell wall of yeast [21]. If proteins are considered to contribute to the transport through the membrane, the first-named organisms may be affected to a lower concentration.

In conclusion, citric and tartaric acids are active antifungal agents *in vitro*. However, further research is required to assess the correlation between antifungal activity *in vitro* and the actions *in vivo* and the successful results might in the future be applied in the treatment of patients with fungal infections.

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