Short Communication

Comparison of aglycon and glycosidic saponin extracts of *Cyclamen coum* tuber against *Candida* spp.

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Abstract

Background and Purpose: Candidiasis, an important fungal infection, is considered the fourth most common nosocomial blood stream infection. Nowadays, because of increased fungal resistance to antibiotics, the use of herbal medicine has gained particular attention. *Cyclamen* species are medicinal plants containing triterpenoid saponins, which are shown to have antimicrobial properties.

Materials and Methods: Three species of *Candida* including *C. albicans*, 10231 *C. tropicalis* 0750, and *C. krusei* and nine clinical samples were cultured on Sabouraud dextrose agar. Active substances of the tubers were extracted by fractionation method. Susceptibility of *Candida* to *Cyclamen coum* tuber extracts was evaluated via minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

Results: Our results demonstrated that ethyl acetate extract had no inhibitory effect on *Candida* strains, whereas the aqueous and *n*-butanolic extracts showed considerable activity. MIC and MFC of these extracts varied within the range of 2-32 µg/mL of saponin for different *Candida* samples. Aglyconic aqueous phase of the extract had the most effective anticandida activity. Glycosidic and aglyconic aqueous extracts were less active on *C. albicans* strains and *C. tropicalis*, respectively.

Conclusion: Tuber extract of *Cyclamen* was rich in triterpenoid saponins and had antifungal effect. Sugar chain structure, as well as type and concentration of the aglycones were effective in this activity.

Keywords: *Candida*, *Cyclamen coum*, Minimum inhibitory concentration, Saponin

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Introduction

Currently, the incidence of opportunistic fungal infections has caused serious concern due to the increasing morbidity and mortality rates, especially in immunocompromised patients [1]. Candidiasis, as an important fungal infection, is considered the fourth leading cause of nosocomial blood stream infections. Some of the most important causative agents of these severe infections are *Candida albicans* and non-*albicans Candida* spp., including *C. tropicalis* and *C. krusei* [2, 3].

Candida can alter defense mechanisms and gradually achieve resistance to common antifungal agents [4]. However, the mechanisms contributing to antifungal resistance have not been fully perceived yet. The variability in the susceptibility of clinical isolates to antifungals has been reported among the *Candida* spp., highlighting the importance of performing species identification and antifungal susceptibility experiments [3]. In recent years, the use of herbal medicines has received particular attention in order to overcome the increase in fungal resistance to antibiotics. Chemical structure of many pharmaceutical compounds administered to improve human health is originated from herbal chemicals; about 25% of globally prescribed drugs are developed from plants [5-6]. Lots of diverse natural products are involved in plant defense. Saponin and phenolic compounds with noticeable antimicrobial and antifungal activity are secondary metabolites that are widely distributed in plant species [5, 7-9].

Cyclamen belongs to the Primulaceae family and is a medicinal plant containing triterpenoid saponins [10]. Previously, antimicrobial properties of *Cyclamen* tuber extracts were revealed [11]. In this study, susceptibility of *Candida* to *Cyclamen coum* tuber extract iswas evaluated using minimum

inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

Materials and Methods

Fresh tubers of *Cyclamen coum* were collected from Naharkhoran forests in Golestan Province, Iran. The samples were washed, sliced , and dried at 70°C for 48 h. Three *Candida* species, including *C. albicans* (ATCC 10231 and 3 isolates), *C. tropicalis* (ATCC 0750 and 6 isolates), and one isolate of *C. krusei*, were obtained from microbiology lab of Alzahra University, Tehran, Iran. Clinical isolates were previously collected from urinary catheters of patients admitted to intensive care unit (ICU) of the 501 Army Hospital of Tehran, Iran.

Preparation of extracts from Cyclamen tuber

Active substances of the tubers were extracted by Soxhlet extractor according to Ma et al. [12]. After defatting of 100 g powdered sample by petroleum ether (150 ml) and diethyl ether (150 ml), extraction was followed by ethanol 100% (150 ml×3) and 70% (150 ml×3). The solvents were mixed and evaporated (hydroalcoholic extract). The extract was separated into aqueous and *n*-butanolic phases and after evaporation of the solvents sediments resolved in water and dimethyl sulfoxide (DMSO), respectively.

Hydroalcoholic extract was hydrolyzed by 1N HCl (1:10 V/V) at 80ºC [13]. Afterwards, two aglycone extracts, including aqueous and ethyl acetate phases, were obtained by fractionation process using ethyl acetate. The extracts were evaporated and sediments were solved in water and DMSO, respectively. All the extracts were stored at 4ºC.

MIC and MFC assays

The microdilution susceptibility test was carried out according to the Clinical and Laboratory Standards Institute protocol [14]. The strains were subcultured on Sabouraud dextrose agar (SDA). From a suspension of cells, a new dilution was prepared with final inoculum of $0.5\text{-}2.5\times10^3$ CFU/ml. Microtiter plates containing 100 µl of the determined dilutions of each extract were inoculated with 100 µl of inoculum and were incubated at 37°C for 48 h. Ketoconazole (512-0.25 µg/ml in DMSO) was tested as standard, and the medium without the test compounds was used for growth control. MIC was defined as the concentration that results in undetectable turbidity. MIC assay was carried out in triplicate independently. To determine the MFC, 5 µl of all the wells was subcultured on SDA at 37°C for 48 h. The MFC was recorded as the lowest concentration that impedes the growth of 99-99.5% of the inoculum.

Phytochemical analysis

The level of the two secondary metabolites, including saponins and phenolic compounds, were evaluated in the extracts according to Wu et al. [15] and Marrinova et al., respectively [16].

Statistical analysis

All the experiments were carried out at least three times. The statistical analyses were performed using SPSS version 19 [17]. The data were expressed as mean±standard deviation. Different treatment outcomes were compared by One-way analysis of variance. P-value less than 0.05 was considered statistically significant.

Results and Discussion

Our findings regarding anticandidal activity of *C. coum* tuber extracts are illustrated in Figure 1. The experiment demonstrated that ethyl acetate extract with low quantity of saponins had no inhibitory effect on *Candida* strains, whereas the other extracts showed considerable activity, but not on all the strains. For instance, aqueous phase of the aglycone extract could not inhibit growth of the isolates 2, 3, and 6 of *C. tropicalis*, while the MIC and MFC of this extract was evaluated to be within the range of 2-4 µg/ml for the other *Candida* samples. This extract did not have killing effect on standard strain of *C. tropicalis* 0750.

n-butanolic extract did not affect isolate 6 of *C. tropicalis*, but MIC and MFC were the same for the other isolates (5 µg/ml). The MIC for the aqueous phase of glycosidic extract was within the range of 8-32 µg/ml, the lowest effects were obtained on *C. tropicalis* isolates (16 and 32 µg/ml). In comparison with ketoconazole, the extracts were shown to have significant antifungal effects.

In recent years, have been considerable efforts have been made to find natural antimicrobials with inhibitory effect on fungal growth. Saponins and phenolic compounds are major groups, which are responsible for antimicrobial activity of plants [8, 18]. Saponins are antifungal agents against some fungi, including *Candida genera*, which have been the target of many studies to develop phytotherapeutic treatment for infections due to their low toxicity, high efficiency, and cost-effectiveness. It seems that the interaction between aglycone moieties of the saponins and fungal membrane sterols is the principal mechanism that causes the formation of transmembrane pores, destroys integrity, and leads to membrane lysis [14, 18].

The previous results have revealed that the saponins with short oligosaccaride chains are

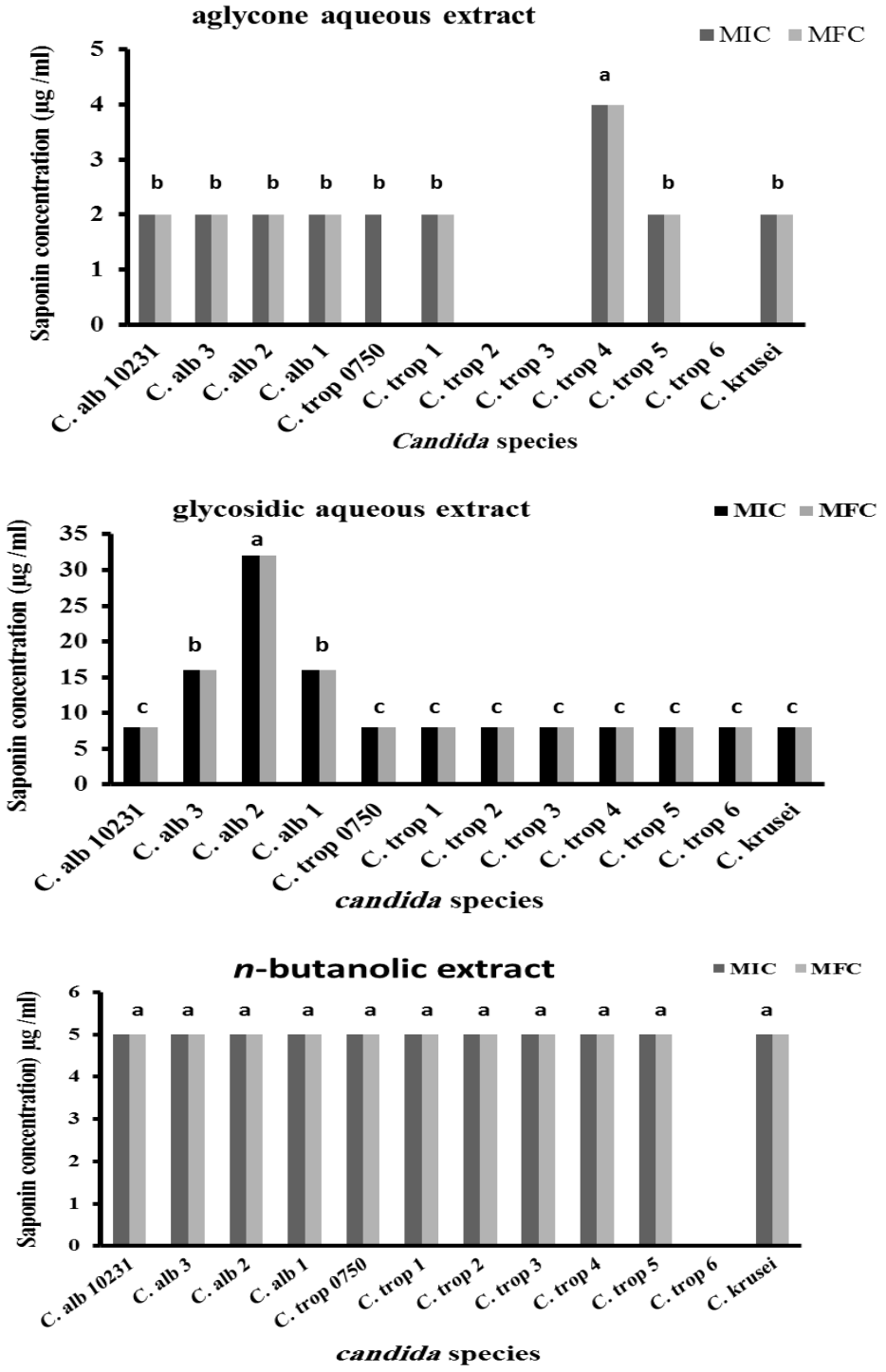

Figure 1. Minimum inhibitory concentration and minimum fungicidal concentration of the extracted saponins from *Cyclamen coum* tuber against *Candida* species

isolated from crude extract by *n*-butanol [5, 19]. Glycosidic aqueous and *n*-butanolic phases displayed the antifungal activity against all the studied fungal strains, suggesting the important role of sugar chains in promoting saponin activity.

Aglycone aqueous extract could not inhibit growth of a number clinical isolates of *C. tropicalis*, but it showed activity against other isolates at concentration of 1 µg/ml. This finding provides evidence for the role of the sugar part of saponins. Aqueous phase of aglycone extract with low saponin and high phenolic content had the highest activity. Type and concentration of the compounds, as well as the interaction between them can influence their anticandidal activity.

Our results revealed that high dose of the extracts had moderate potential of anticandidal activity and that growth of *C. albicans* and *C. tropicalis* was increased at concentrations greater than 20 µg/ml of *n*-butanolic extract and *C. tropicalis* and *C. krusei* at concentrations greater than 10 µg/ml of aglycone aqueous extract. This effect probably can be explained by the increasing sugar content, which is consumed by yeast cells and results in increased growth.

Difference in antimicrobial properties of the saponins may reflect variation in number, type, and sequence of the sugar residues and aglycone part [18-20]. Cyclaminorin, deglucocyclamin, cyclacumin, cyclamine, isocyclamin, and mirabiline isolated from *C. mirabile* tubers and mirabiline lactone isolated from *C. coum* showed significant antifungal properties [10, 21]. Mirabiline, a pentaglycoside oleanolic acid, had showed moderate activity against *C. albicans*, *C. parapsilosi*, and *C. tropicalis*.

Consequently, our findings were consistent with those of previous reports regarding the fact that saponins have antimicrobial activity depending on their chemical structures. Glycoside saponins from *C. coum* tuber were more active than the aglycone form. Further investigations should be performed to evaluate cytotoxicity of the extract.

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Author's contribution

All the authors contributed to designing and performing the experiments, analyzing the data, and writing the manuscript.

Conflicts of interest

None declared.

Financial disclosure

There were no financial interests related to the materials of the manuscript.

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