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Antiproliferative effects of *Matricaria chamomilla* on *Saccharomyces cerevisiae*

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ABSTRACT

Introduction: The *Matricaria chamomilla* plant is one of the most important plants used for the therapeutic purposes. More than 120 chemical constituents have been identified in Matricaria chamomile plant including 28 terpenoids and 36 flavonoids. This plant has a variety of therapeutic applications including the treatment of diabetes, eczema, wounds and gastrointestinal diseases. The *Saccharomyces cerevisiae* yeast is a non-pathogenic organism that is used as a model for pathogenic yeasts in order to identify compounds with antifungal properties and also to identify functional mechanism of these compounds. The aim of this study is to investigate the antifungal effect of *Matricaria chamomilla* hydroalcoholic extract on *S. cerevisiae* yeast.

Methods: In this study *Matricaria chamomilla* extract was prepared by maceration method. In order to study the extract effect on growth and survival rate of the yeast cell, the spectrophotometry and methylene blue staining methods were used. Excel and SPSS 11 softwares were used to determine amounts and to infer the difference between control and treatment samples.

Results: Results obtained from spectrophotometry and analyses of methylene blue staining showed that the *Matricaria chamomilla* extract at the concentration of 3000 µg/ml caused a significant decrease in the yeast growth and reduced the cells survival rate up to 48% ($p< 0.05$).

Conclusion: Results of this research confirm that the hydroalcoholic extract of *Matricaria chamomilla* has antiproliferative effect on *Saccharomyces cerevisiae.*

Implication for health policy/practice/research/medical education:
Chamomilla hydroalcholic extract has antiproliferative effect on *Saccharomyces cerevisiae* and might be beneficial in the treatment of cancer and fungal infection diseases.

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Introduction

The use of plants for the treatment of human diseases dates back to antiquities. One of the most important plants used for treatment purposes is *Matricaria chamomilla* that is of the Asteraceae family (1,2). This plant has more than 120 compounds including 28 terpenoids and 36 flavonoids (3). Apigenin is the most important flavonoid compounds existed in the *Matricaria chamomilla* that much of this compound is as glucoside and a small amount of it is free. The main constituents of chamomile oil include terpenoid, α-bisabolol, and its oxides (≤78%) and azulenes including chamazulene, chamazulene carboxylate and proazulenes (1,4). *Matricaria chamomilla* has a variety of therapeutic applications including the treatment of diabetes, eczema, wounds, and gastrointestinal diseases and also it has anti-inflammatory and anti-stress properties. This plant has some antioxidant and antimicrobial activities and significant antiplatelet property (5). Compounds isolated from essential oil of the *Matricaria chamomilla* extract like flavonoids and α bisabolol have strong antifungal effect on the *Candida albicans* yeast (6). *Saccharomyces cerevisiae* is one of species of yeast in the *Saccharomycetaceae* family (7). This yeast possibly is the best intensively studied eukaryote organism that is referred to as an ideal model organism since its gens are significantly conserved during the evolution. *Saccharomyces cerevisiae* yeast is a non-pathogenic organism that is used as a model for pathogenic

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yeasts. This organism is used to identify some compounds with antiproliferative characteristic and also to study the performance mechanism of these compounds. Also since this cell is less complex than cancer cells and there is a high degree of similarity between yeast cell and cancer cells, this organism is an excellent model system for cancer cells (8). Compounds bearing antiproliferative properties are excellent candidates as compounds with anti-cancer antifungal property that their performance details can be achieved using yeast (9,10). This research has proceeded to determine the antiproliferative effect of the hydroalcoholic extract of *Matricaria chamomilla* on *Saccharomyces cerevisiae* yeast.

Materials and Methods

Preparation of Chamomilla Hydroalcoholic extract

Chamomilla Hydroalcoholic extract was prepared by the maceration method. To prepare the extract, first dried Chamomilla flower powder was weighted and 2% W/V suspension was prepared in 70% alcohol then was Soaked into the lid closed container for 48 hours. Next, it was passed through the filter paper and the extract was separated from the alcohol by the distiller device. The extract was poured into open lid glass palate and was put into incubator at 30 ˚C for 72 hours in order to dry the extract completely (11) and then was maintained at the 4 ˚C (5).

Yeast cultures and growth conditions

Yeast cultures was prepared from University of Shahrekord, Iran. In order to reproduce yeast, the special medium for yeast growth, YPD, containing 1% yeast extract, 2% glucose and 2% peptone was used (12). Yeast cells are cultured in mediums containing different concentration of Chamomilla extract at 35 ˚C. To dissolve the extract the 100% DMSO solution was used (10).

Studying cells growth level using the spectrophotometer device

To obtain optical density after treatment with different concentrations, the absorption spectrum in wavelength of 600 nm was measured using the spectrophotometer device.

Studying viability of yeast cell by methylene blue staining method

An equal volume of yeast sample and methylene blue solution were mixed on a microscopic slide, then, the dead and living cells were counted by the optical microscope. Dead cells are blue and living cells are colorless. To obtain viability the following formula was used (13). All measured tests were repeated 3 times.

$$\frac{(Total\ cells) - (Dead\ Cells)}{Total\ Cells} \times 100 = Viability$$

Statistical Analysis

In order to analyze data statistically in this study the Excel program and the SPSS software version 11 were used. Amounts on diagram were stated based on means ± the standard error. Difference between the control sample and the treatment sample was determined by Student's *t*-test and P values less than 0.05 were considered significant.

Results

Effect of Chamomilla plant extract on wild-type growth (OD 660 nm) is shown in the Figure 1. According to obtained results there was no significant difference in growth of yeast cells than the control sample in concentrations of 500, 1000 and 2000 µg/ml of Chamomilla extract but this difference is significant in concentration of 3000 µg/ml ($p< 0.05$).

Results obtained from staining with methylene blue showed that 500, 1000 and 3000 µg/ml concentrations of Chamomilla extract decreased viability of *Saccharomyces cerevisiae* cells up to 4, 13 and 48%, respectively (Figure 2).

Discussion

Saccharomyces cerevisiae yeast is an excellent model system to identify compounds with antiproliferative property and

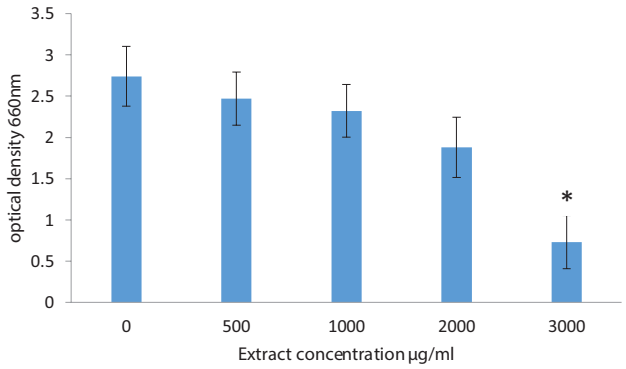
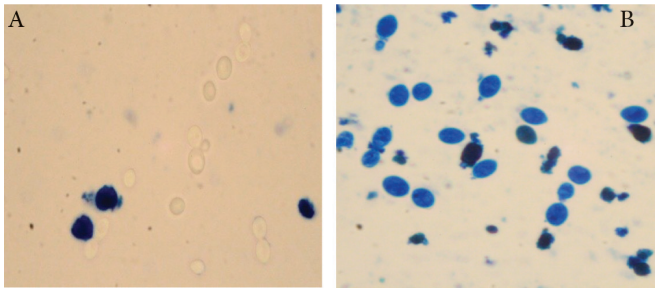


Figure 1. Growth of wild-type (OD 660 nm) via different extract concentrations. The growth rate of yeast cells was measured at the presence of plant extract in 660 nm. Extract was diluted in 100% DMSO, the "no compound" control, contained 1% DMSO. (*p <0.05)



Extract concentration (µg/ml)	0	500	1000	3000
Viability of wild-type	64	60	51	16

Figure 2. Methylene blue staining analysis. (A) Representative images of methylene blue staining wild-type cells in the absence (left) or presence of 3000 µg/ml Chamomile extract (right) for 24 h. Cells. Dead cells were stained blue and living cells are unstained. (B) The viability rate was measured at the presence of various concentrations OF plant extract by staining with methylene blue and plate counts. Cells were counted in three field of microscope, and the average of them is showed. Extract was diluted in 100% DMSO, the "no compound" control, contained 1% DMSO.

also to study performance mechanism of these compounds. Compounds with antiproliferative property are excellent candidates as compounds with anti-cancer and antifungal property (10). Plants extract with antiproliferative property can be replaced by synthetic drugs since they have fewer side effects. One of the most important plants used for therapeutic purposes is *Matricaria chamomilla* (1). Based on results of this study the hydroalcholic extract of this plant in concentrations of 3000 µg/ml inhibits growth significantly and decreases survival of *Saccharomyces cerevisiae* cells up to 48% that these results confirm antiproliferative effect of *Matricaria chamomilla* hydroalcholic extract. Performed study in the year 1991 by Kadzia on essential oil of the *Matricaria chamomilla* plant showed that compounds separated from essential oil like flavonoids and α-bisabolol have antifungal effect on the *Candida albicans* yeast (6). *Matricaria chamomilla* hydroalcholic extract has antifungal property on *Candida albicans* and the lowest concentration of this extract that has this property is 62.5 mg/ml. This difference in effective concentration amount on *Saccharomyces cerevisiae* and *Candida albicans* may indicate that the antiproliferative property of extract is more for the *Saccharomyces cerevisiae* yeast than the *Candida albicans* yeast. The antiproliferative effect of this plant has been studied on cancer cells. Results of this study showed that *Matricaria chamomilla* hydroalcholic extract in concentrations of 100-400 µg/ml decreased cell survival from 14.7 to 61.9% in different classes of cancer cells (5). Other study conducted on the *Candida albicons* yeast did not confirm the antiproliferative property of Chamomilla extract (11). This difference in results can be caused by difference in extraction methods or difference in studied parts of the plant.

Conclusion

Regarding the results of this study it can be concluded that *Matricaria chamomilla* hydroalcholic extract has the antiproliferative effect on the *Saccharomyces cerevisiae*. In order to validate the antiproliferative property of *Matricaria chamomilla* extract, it is recommended to study the effective substance and possible molecular mechanism of this extract performance for functional usages of this plant in the treatment of cancer and also fungal infection diseases.

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Authors' contributions

All authors wrote the paper equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

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