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Research Article

DOSE-DEPENDENT EFFECTS OF JUJUBE LEAF AND FRUIT EXTRACTS ON YEAST GROWTH

Mümin Sargın[1,a], Tülay Turgut Genç [2,b,*]

[1]*Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology, Çanakkale, Turkey*
[2]*ÇanakkaleOnsekiz Mart University, Faculty of Science, Department of Biology, Çanakkale, Turkey*

**Corresponding Author:*
E-mail: tturgutgenc@comu.edu.tr

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a: ORCID 0000-0003-4290-4457, b: ORCID 0000-0001-5074-3572

ABSTRACT. Structurally different secondary metabolites synthesized by plants are known as phytochemicals. It is known that many phytochemicals can extend the life span of organisms. The parts of the jujube plant (*Ziziphus jujuba* Mill.) have been used for many years in the treatment of different diseases among people. Jujube leaves and fruits have a very good antioxidant and anti-aging capacity as they have many important bioactive compounds. The feeding of *Drosophila melanogaster* with jujube extracts has been shown to increase lifespan. *Saccharomyces cerevisiae* is another model organism used to understand aging mechanisms which are similar to higher eukaryotes. In our study, the effects of jujube leaf and fruit extracts on yeast growth were analyzed. Hexane, acetone, methanol and water solvents were used for leaf and fruit extraction. Growth tests were done in different concentrations of each extract using a microplate reader. To determine the dose-dependent effect of the extracts on yeast growth, growth tests were performed using a microplate reader using different concentrations of each extract. The results showed that the hexane extracts of jujube leaf slowed down yeast growth, while acetone, methanol and water extracts positively affected yeast growth. On the other hand, yeast growth was slowed in all jujube fruit extracts except the low concentrations of hexane extract (0.2 mg/ml). As a result, polar extracts of jujube leaf were found to increase yeast growth more than fruit extracts. Therefore, it is necessary to analyze the leaf content and determine the bioactive compound that positively affects growth.

Keywords: *Growth, Plant extracts, Saccharomyces cerevisiae, Ziziphus jujuba*

INTRODUCTION

Aging is a gradual and continuous process that occurs in all living organisms, eventually leading to death [1]. Aging is associated with biological, physiological and environmental changes. With the increase in cell damage and accumulation of toxic substances in the cells, cellular systems begin to deteriorate over time [2]. The fruit fly (*Drosophila melanogaster*), worm (*Caenorhabditis elegans*) and mouse (*Mus musculus*) are multicellular organisms commonly used in aging studies. On the other hand, *Saccharomyces cerevisiae* is a single-celled model organism used to identify genes and signaling pathways that slow down cellular and organismal aging and age-related diseases. *S. cerevisiae* is also used to determine the effect of chemical compounds and different plant extracts on aging [3, 4, 5, 6, 7, 8]. The yeast has played an important role in determining two important pathways i.e. Sirtuin pathway and the TOR signaling pathway, related to aging and age-related diseases [9, 10, 11].

There are two types of aging mechanisms in *S. cerevisiae*: chronological and replicative. The replicative life span (RLS) refers to the average and the maximum number of daughter cells produced by a single parent cell before the cell division irreversibly ceases. The chronological lifespan (CLS) is defined as the time during which a non-dividing yeast cell can survive and re-enter the cell cycle to regenerate new cells with the addition of a fresh growth medium [12, 13, 14]. In other words, CLS refers to the average and maximum survival time of non-replicating yeast cells. Some plant extracts (PEs) have been shown to contribute to prolonging lifespan without affecting the basic biological functions of yeast cells. These natural phytochemicals found in plant extracts are called geroprotectors. In recent studies regarding phytochemicals that have the potential to increase life span, phenols and their subtypes of flavonoids have the greatest attention. some of these phytochemicals are resveratrol, quercetin, 4,4′-dimethoxychalcone, phloridzin, flavonoid glucoside, astaxanthin, chebulinic acid, boeravinone B, tangeretin, nobiletin, parishin, sesquiterpene glucosides, polysaccharides, vitamin C and esters (tschimganine, α-hibitakanine and β-hibitakanine) which affect the CLS of *S. cerevisiae* and fission yeast *Schizosaccharomyces pombe* [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28]. There are studies in the literature examining the effects of various plant extracts on yeast CLS. In one study, it was determined that 6 of 37 plant extracts caused a dramatic increase in yeast CLS [29]. The *Cimicifuga racemosa*, *Apium graveolens* and *Salix alba* extracts functioned through the mTOR pathway while the *Ginkgo biloba* and *Valeriana officinalis* extracts functioned through PKA pathway. However, the effect of *Passiflora incarnate* extract on longevity was not TOR or PKA dependent [30]. The root extract of *Ferula tschimganica* has been shown to extend the lifespan of *S. pombe* [27]. Extracts of *Gastrodia elata* and Shenzhou honey-peach fruit were determined to increase the RLS of *S. cerevisiae* in a TOR-dependent manner [25, 31]. In another study, the extracts of *Serenoa repens*, *Hypericum perforatum*, *Ilex paraguariensis*, *Ocimum tenuiflorum*, *Solidago virgaurea*, *Citrus sinensis*, *Humulus lupulus*, *Vitis vinifera*, *Andrographis paniculata*, *Hydrastis canadensis*, *Trigonella foenum-graecum*, *Berberis vulgaris*, *Crataegus monogyna* and *Taraxacum erythrospermum* plants were found to increase yeast lifespan [32]. In a study investigating the effect of 175 tropical plant extracts on yeast lifespan, it was shown that 7 plant extracts extend the yeast CLS, and leaf extracts of *Manihot esculenta* and *Wodyetia bifurcata* plants increased the yeast CLS in a dose-dependent manner [33].

The jujube plant (*Ziziphus jujuba* Mill.), which belongs to the *Rhamnaceae* family, is a medium-sized thorny tree and its fruits are consumed as food and as medicine among the people. It is widely used, especially in China, as one of the most important herbal-based medicines. All parts of the jujube plant (leaf, flower, fruit, kernel, bark, wood and root) has been used medicinally for 3000 years [34]. The jujube plant is used especially in the treatment of sleep problems and in the regulation of digestion. Each component of the jujube plant is beneficial for health, and different parts of the plant are traditionally used in the treatment of many types of diseases such as diabetes, diarrhea, liver complaints, urinary disorders, obesity, skin infections, respiratory tract infections, anemia, insomnia, cancer [35, 36]. Jujube fruit is highly nutritious due to its high content of amino acids, carbohydrates, minerals and vitamins [35, 37]. Studies show that jujube fruit has many bioactive compounds such as triterpenic acids, flavonoids, cerebrosides, phenolic acids, α-tocopherol, β-carotene and polysaccharides [38]. Jujube fruits have many pharmacological properties such as neuroprotective, antioxidant, anti-cancer, anti-inflammatory, hepatoprotective, antimicrobial and immunomodulatory activities [34, 39,

40, 41, 42, 43, 44, 45]. It also has health-promoting effects such as anti-aging properties [46]. The jujube leaves contain saponins, triterpenic acids (cheanotic acid, epicheanotic acid, ceanotenic acid, alphytolic acid, maslinic acid, zizyberanalic acid, 2-hydroxyursolic acid, betulinic acid and oleanolic acid) and flavonoids (quercetin-3-O-rutinoside) [47]. Jujube leaves are used in the treatment of insomnia, nourishing the heart and calming the nerves, as well as the teas prepared with the leaves, are used in the treatment of bleeding and diarrhea. It has been shown that the oils obtained from the pulp, leaf and seed of the jujube plant are rich in sterols, fatty acids and triterpenes [48].

In previous studies, feeding fruit flies (*Drosophila melanogaster*) with jujube extracts resulted in increased lifespan and environmental stress tolerance [49]. In addition, jujube extract has been shown to have a very good antioxidant and anti-aging capacity in *D. melanogaster* [50]. Therefore, in this research, the effect of jujube fruit and leaf extracts on *S. cerevisiae* growth was investigated. The results showed that the polar extracts of jujube leaf were found to increase yeast growth more than fruit extracts.

MATERIALS AND METHODS

Plant Extract Preparation

The leaves and fruits of the jujube plant (*Ziziphus jujuba* Mill.) were collected from Güzelyalı village (40°04'39.37" N and 26°34'39.49" E) in central district of Çanakkale province (Turkey) in September 2020. Leaf and fruit samples were dried in a place out of direct sunlight and ground into powder in a mortar. A total of 30 gr jujube leaf and fruit powders were used for the extraction process which was completed in a Soxhlet device using 230 mL n-hexane, acetone, methanol and distilled water, respectively. The leaf and fruit extracts were filtered and concentrated with a rotary evaporator. The crude extracts were kept in sterile bottles at 4 ºC for further analysis. The working concentrations (0.2 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 5.0 mg/mL and 10.0 mg/mL) were prepared by diluting leaf and fruit extracts in appropriate volume of DMSO.

Yeast Strains, Media and Growth Conditions

S. cerevisiae yeast strain BY4741 (MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) was used in this study. Yeast cells were streaked in YPD medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose) to obtain a single colony. Since the experiments were performed in triplicate, 3 colonies were selected for 3 biological replicates. Selected yeast colonies were cultured in minimal growth medium YNBD-HLMU (0.67% Yeast nitrogen base, 2% (w/v) glucose) supplemented with histidine (40 mg/L), leucine (60 mg/L), methionine (40 mg/L) uracil (40 mg/L), at 30°C incubator shaker up to the exponential stage.

Dose-Dependent Effects of Jujube Extracts on Yeast growth

Once the yeast cultures reach to log stage, they were used for a 96-well plate liquid culture assay to determine the dose-dependent effects of leaf and fruit extracts on yeast growth. The growth test was performed as described in previous studies [51, 52]. The log phase yeast culture was diluted and transferred to fresh YNBD-HLMU culture supplemented with leaf and fruit extracts (0.2 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 5.0 mg/mL and 10.0 mg/mL), and without extracts (control). The cell suspensions including plant extract (200 µL) were transferred into three wells of a sterile 96-well microplate. 200 µL of jujube extract (leaf or fruit) without yeast cells was distributed into the fourth well of

the assay plate as a blank. As a control, yeast cells suspended in YNBD-HLMU culture were also distributed into the well. Cells were incubated at 27 °C without shaking. The outgrowths of yeast cultures in the 96-well microplate were monitored by measuring the OD_{600} every 30 min until 24 h using a MultiskanTM FC Microplate reader. A standard growth curve was prepared in YNBD-HLMU medium by plotting the log of cell numbers against time (30 min/3 days). All culture assays for each extract were repeated three times. Results were given as mean values of three independent experiments that were measured in triplicate.

RESULTS AND DISCUSSION

To determine the dose-dependent effects of jujube leaf and fruit extracts on yeast growth, the 96-well plate screening assay was used according to the previously reported protocols with some modifications [51, 52]. The exponentially growing yeast cells were used instead of stationary phase yeast cells. The leaf and fruit extracts were prepared with different solvents: hexane, acetone, methanol and distilled water. The cell suspensions with different final concentrations of extracts (0.2 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 5.0 mg/mL and 10.0 mg/mL) were used to determine the dose-dependent effect on yeast growth. The growth of yeast cells was recorded by measuring the OD_{600} every 30 min until 24 h. The cell number at each reading point was determined with the standard growth curve. The relative cell numbers were calculated by dividing the extract-treated cultures' average cell number to that of the control culture. The relative number of cells in the control culture (i.e. 1.0) was assumed to be 100% and the relative yeast growth in the extract-treated cultures was calculated as a percentage. Values below 100% were considered as slowing yeast growth, while values above 100% were evaluated as increased yeast growth.

The effect of leaf-hexane extracts on yeast cell growth was given in Fig. 1. Generally, a decreased yeast growth was observed at all concentrations of the hexane extract. It was determined that 0.2 mg/mL, 0.5 mg/mL and 1.0 mg/mL of hexane concentrations reduced the yeast growth 79%, 43% and 27%, respectively. In addition, the yeast growth was completely diminished at 5.0 mg/mL and above concentrations. The effect of acetone extracts on the growth of yeast cells was given in Fig. 2. The 5.0 mg/mL and 10.0 mg/mL acetone concentrations increased the yeast growth by 50% and above, while 0.2 mg/mL acetone concentration increased by 31%. It was observed that 0.5 mg/mL and 1.0 mg/mL acetone concentrations did not affect yeast growth, and the growth level determined at these concentrations was the same as the control. Interestingly, all used concentrations of the methanol extract were found to increase yeast growth by at least 25% (Fig. 3). The concentration that increased yeast growth the most was 5.0 mg/mL methanol concentration, which caused an increase of 81%. The other methanol extract concentrations, 0.2 mg/mL, 0.5 mg/mL, 1.0 mg/mL and 10.0 mg/mL, increased the yeast growth by 26%, 41%, 43% and 35%, respectively. Similar to the result observed with the methanol extract, all concentrations of the water extract were found to increase yeast growth by at least 25% (Fig. 4). The percent stimulation of yeast growth with different concentrations of water extract was as follows: 25% at 0.2 mg/mL, 40% at 0.5 mg/mL, 33% at 1.0 mg/mL, 68% at 5.0 mg/mL and 61% at 10.0 mg/mL. It was observed that the highest leaf-water extract concentration that increased yeast growth was 5.0 mg/mL, while growth started to decrease at a concentration of 10.0 mg/mL. These results indicated

that acetone, methanol and water extracts of jujube leaf generally increased yeast growth, while hexane extracts slowed yeast growth.

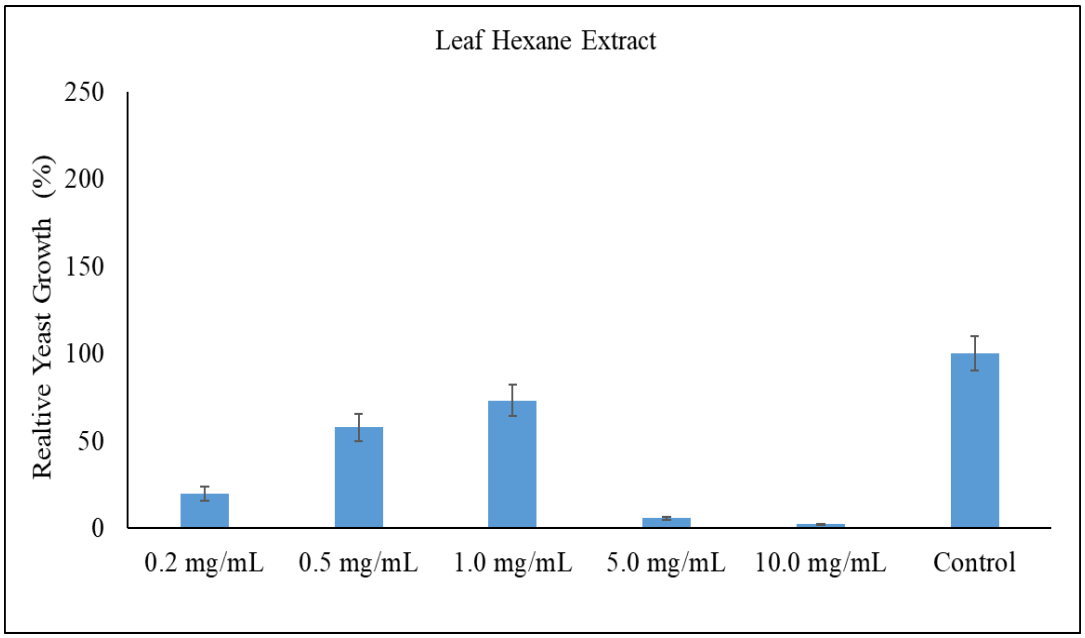

Fig. 1. *The effect of jujube leaf hexane extracts on yeast cell growth*

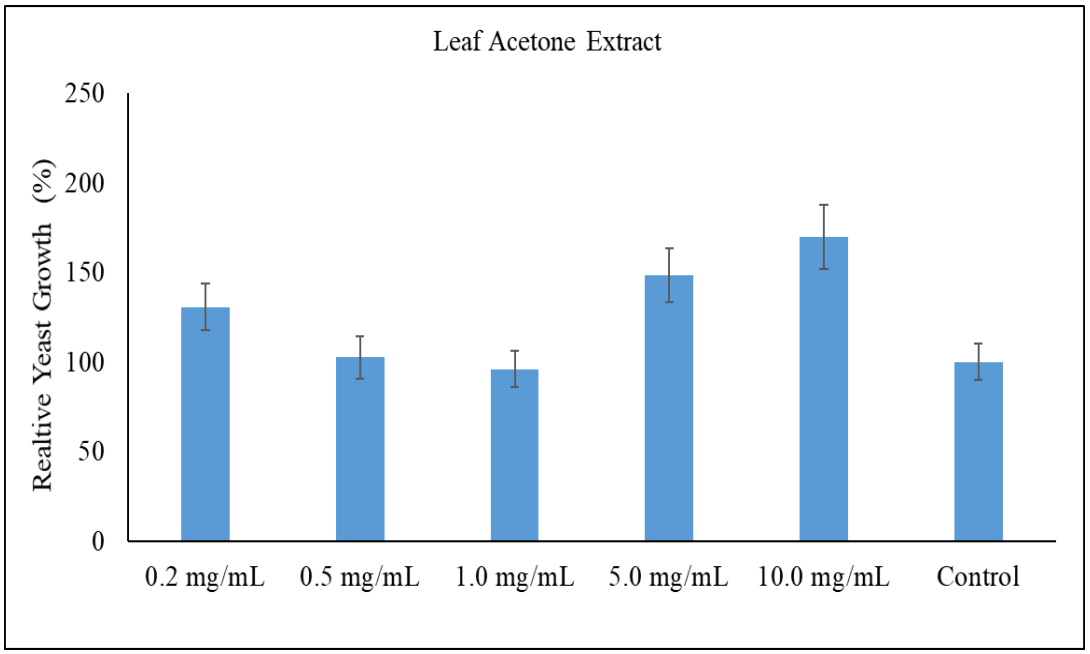

Fig. 2. *The effect of jujube leaf acetone extracts on yeast cell growth*

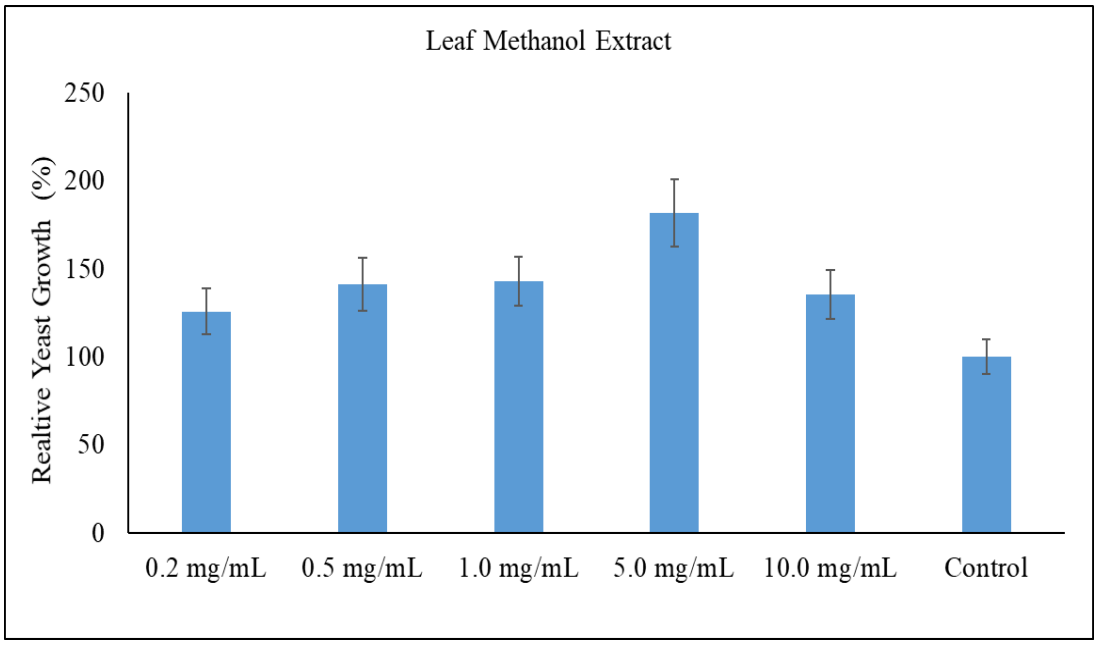

Fig. 3. *The effect of jujube leaf methanol extracts on yeast cell growth*

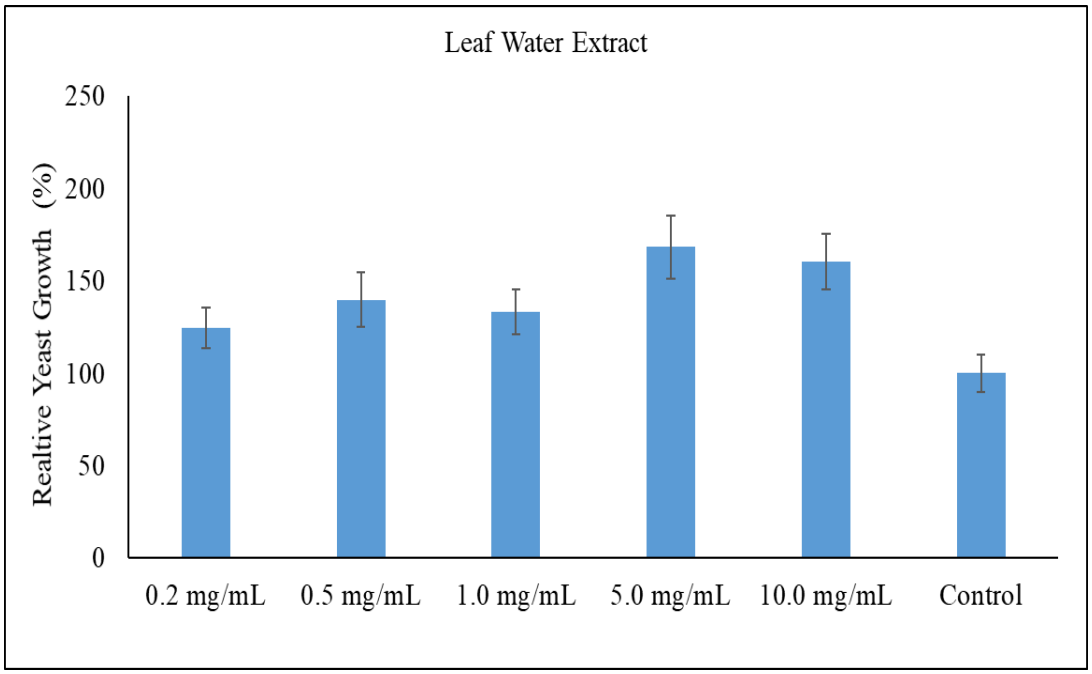

Fig. 4. *The effect of jujube leaf water extracts on yeast cell growth*

The effect of fruit-hexane extracts on the growth of yeast cells was given in Fig. 5. Although 0.2 mg/mL concentration of hexane extract increased yeast growth only slightly (10%), it was determined that growth decreased 40% at 0.5 mg/mL concentration. At other concentrations (1.0 mg/mL, 0.5 mg/mL and 1.0 mg/mL) growth was repressed by at least 85%. Yeast growth was slightly reduced (up to 33%) when yeast cells were grown

in different concentrations of acetone extract (Fig. 6). Acetone extract concentrations between 0.2-1.0 mg/mL slowed yeast growth by approximately 20% relative to control. When the extract concentration increased to 5.0 mg/mL, although yeast growth slightly increased, it started to decrease again at 10.0 mg/mL concentration. Similar results were obtained for methanol and water extracts of jujube fruit. The effects of methanol and water extracts of jujube fruit on the growth of yeast cells were given in Fig. 7 and Fig. 8, respectively. Yeast growth was observed to decrease between 8% and 25% at all concentrations of methanol and water extracts. At the concentration of 1.0 mg/mL methanol and water extracts, yeast growth was higher than the other concentrations (0.2 mg/mL, 0.5 mg/mL, 5.0 mg/mL and 10.0 mg/mL), but it was still less than the control (around 10%). As with the acetone extract, yeast growth began to decrease at a concentration of 10.0 mg/mL of methanol and water extracts. These results showed that hexane, acetone, methanol and water extracts of jujube fruit generally slowed down yeast growth except at 0.2 mg/mL hexane extract concentration.

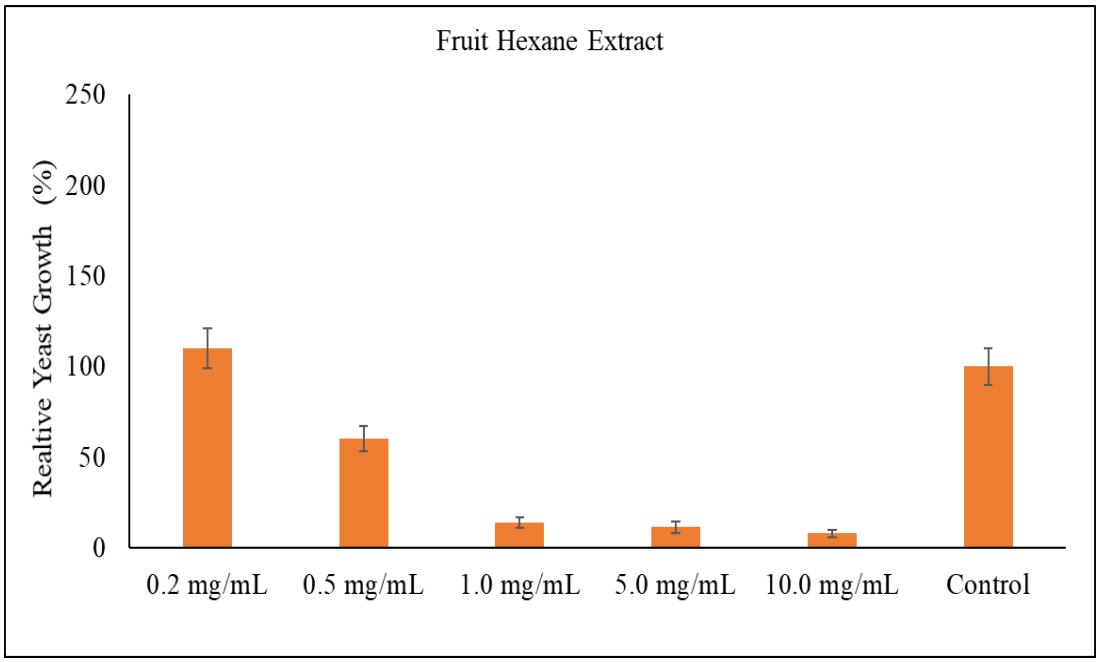

Fig. 5. *The effect of jujube fruit hexane extracts on yeast cell growth*

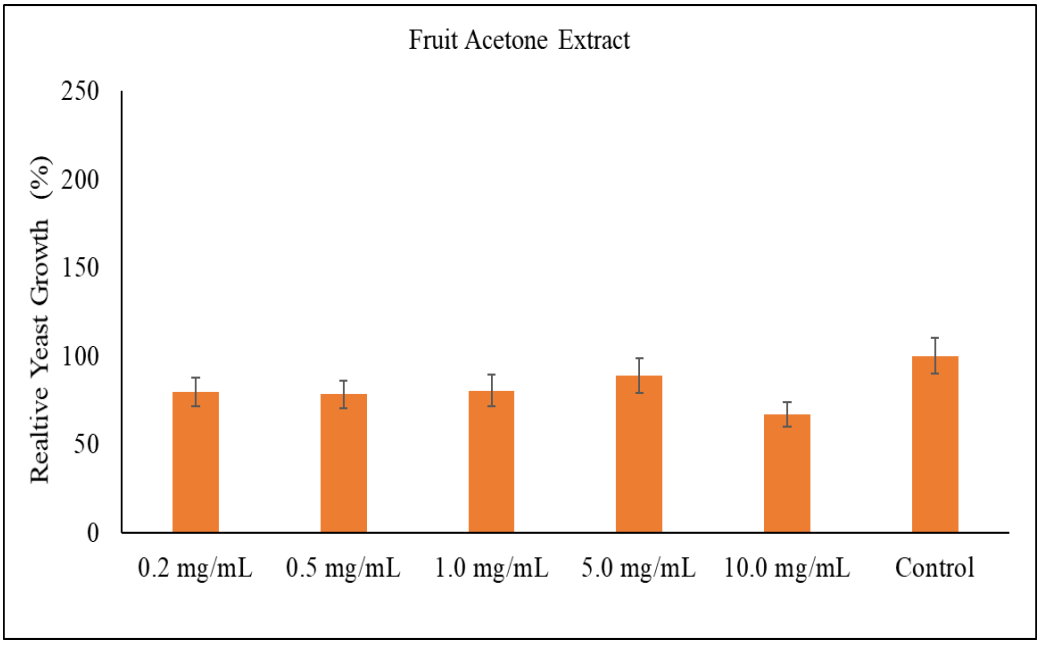

Fig. 6. *The effect of jujube fruit acetone extracts on yeast cell growth*

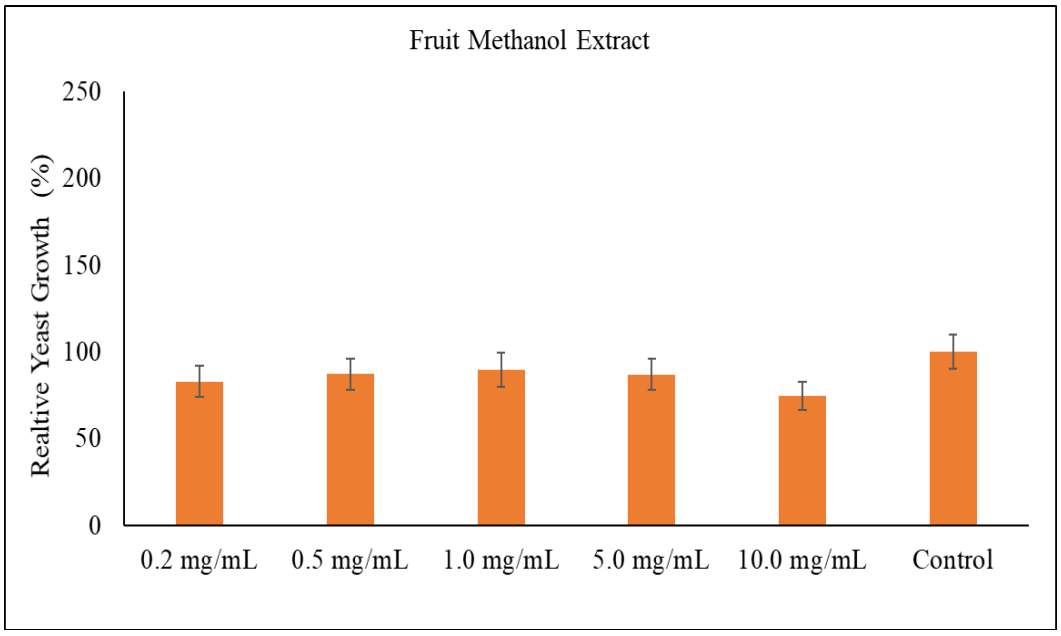

Fig. 7. *The effect of jujube fruit methanol extracts on yeast cell growth*

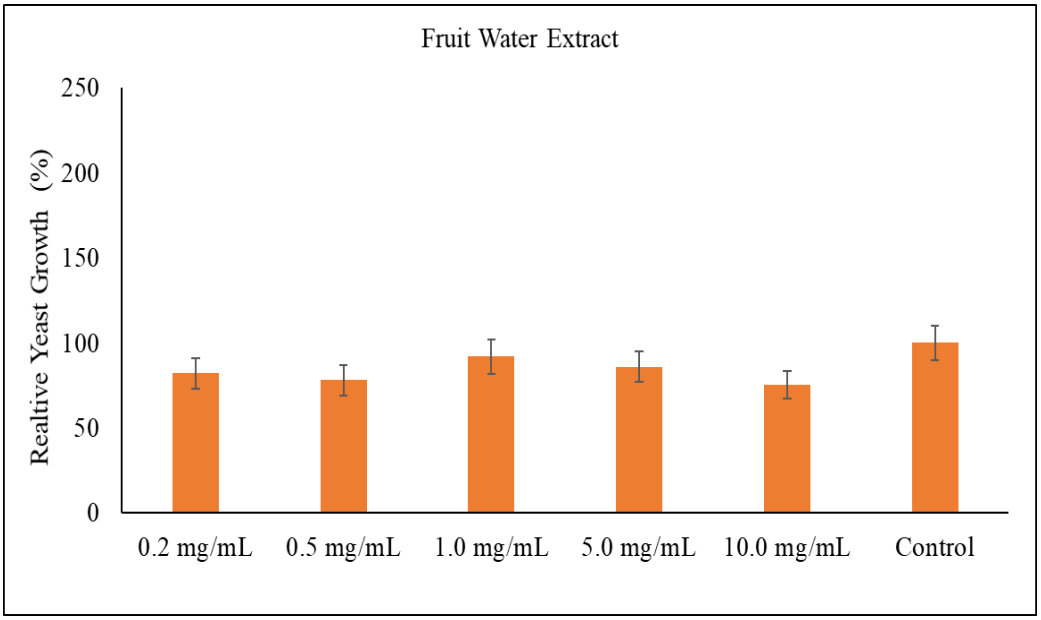

Fig. 8. *This The effect of jujube fruit water extracts on yeast cell growth*

CONCLUSION

In this study, the effects of jujube leaf and fruit extracts on yeast growth were analyzed. The nonpolar and polar solvents (hexane, acetone, methanol and distilled water) were used for the extraction process. Methanol and water are polar solvents while hexane is non-polar. Acetone, on the other hand, is grouped as a polar solvent due to the polarity in the carbonyl group, although it exhibits the properties of both polar and non-polar substances. It was determined that hexane extracts of jujube leaf slowed down yeast growth, while acetone, methanol and water extracts positively affected yeast growth. These results show that polar bioactive compounds of jujube leaf positively increase yeast growth. In contrast to jujube leaf, polar bioactive compounds of jujube fruit did not increase yeast growth, on the contrary, increasing extract concentrations slowed yeast growth. However, the fact that a low concentrations of hexane extract (0.2 mg/mL) had a slight positive effect on yeast growth indicated that nonpolar bioactive content can be more effective on yeast growth. In future studies, it would be appropriate to determine the bioactive content of leaf (10.0 mg/mL acetone, 5.0 mg/mL methanol and 5.0 mg/mL water) and fruit (0.2 mg/mL) extracts, which have positive effects on yeast growth.

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