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Evaluation of the Antioxidant and Antimicrobial Activities of Ethyl Acetate Extract of *Saccharomyces cerevisiae*

Running head: Antioxidant and Antimicrobial Activities of *S. cerevisiae*

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SUMMARY

Research background. Antioxidants are described as important compounds that are present at low concentrations to inhibit oxidation processes. Due to the side effects of synthetic antioxidants, research interest has increased considerably towards finding natural sources of antioxidants that can replace synthetic antioxidants. The emergence and spread of antibiotic resistance require the development of new drugs or some potential sources of novel medicine. This work aims to extract the secondary metabolites of *S. cerevisiae* using ethyl acetate as a solvent and to determine the antioxidant and antimicrobial activities of these extracted metabolites.

Experimental approach. The antioxidant activities of the secondary metabolites of *S. cerevisiae* were determined using DPPH, ABTS, and FRAP assays. Furthermore, the antimicrobial potential of the ethyl acetate extract of *S. cerevisiae* in treating *Cutibacterium acnes*, *Staphylococcus aureus,* and *Staphylococcus epidermidis* was assessed.

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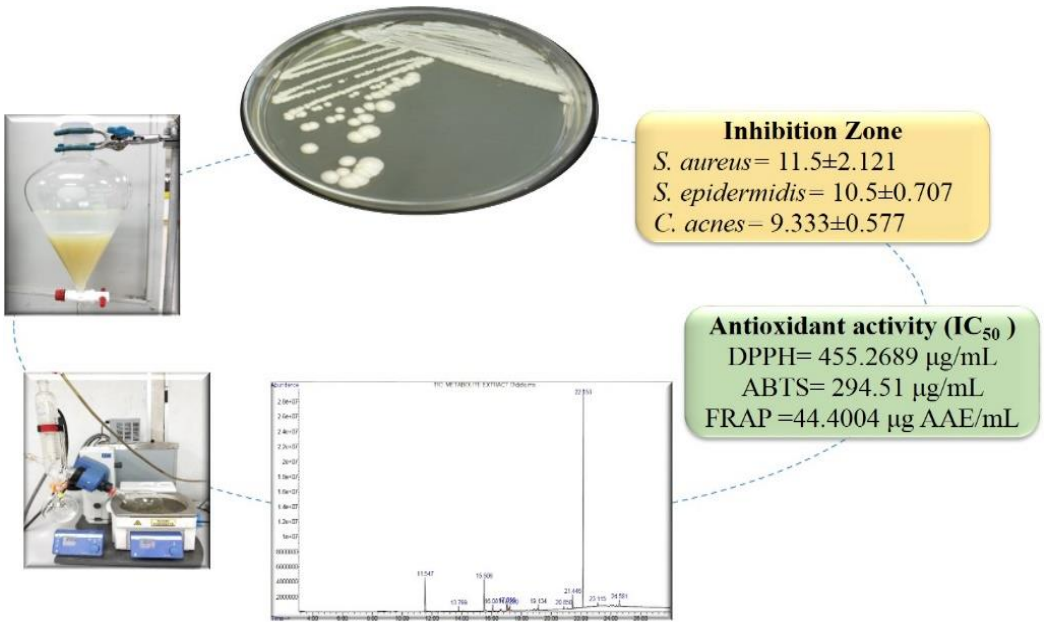
Results and conclusion. Five out of 13 of the extracted secondary metabolites were identified as antioxidants. The antioxidant activity of the *S. cerevisiae* extract exhibited relatively high IC_{50} of 455.2689 µg/mL and 294.51 µg/mL for DPPH and ABTS respectively while the FRAP value was obtained as 44.4004 µg AAE/mL. Moreover, the extracts presented a significant antibacterial activity ($p<0.05$) against *Staphylococcus aureus* and *Staphylococcus epidermidis* at the concentrations of 100 mg/mL and 200 mg/mL, respectively. However, no inhibitory effect was observed against *Cutibacterium acnes* as the extract was only effective against *Cutibacterium acnes* at the concentrations of 300 mg/mL and 400 mg/mL (inhibition zones ranging from 9.0±0 to 9.333±0.577) respectively ($p<0.05$). *Staphylococcus aureus* was highly sensitive to the extract, with a MIC value of 18.75 mg/mL.

Novelty and scientific contribution. This report confirmed the efficacy of the secondary metabolites of *S. cerevisiae* as a natural source of antioxidants and antimicrobials and suggest the possibility of employing them in drugs for the treatment of infectious diseases caused by the tested microorganisms.

Graphical Abstract



Key words: *Saccharomyces cerevisiae,* secondary metabolites, free radicals, bacterial pathogen

INTRODUCTION

S. cerevisiae is a eukaryotic microbe and belongs to the Saccharomycotina family. Yeasts, including *S. cerevisiae,* possess the ability to produce antimicrobial and antifungal compounds that inhibit the growth of pathogenic bacteria and fungi (*1*). *S. cerevisiae* produces toxic proteins or glycoproteins to combat other strains of yeast or bacteria (*2*). According to Hassan (*3*), glutathione (GSH), sulfur-containing amino acids, and Maillard reaction products are the components that contribute to the antioxidative properties of *S. cerevisiae*. GSH is the most abundant thiol in yeast cells that plays a considerable role in antiradical activity (*4*). Besides that, Meng *et al.* (*5*) reported that GSH and ascorbic acid can act as radical scavengers in *S. cerevisiae*. Ascorbic acid is a small water-soluble molecule that works with GSH to form a redox couple (*6*). GSH is described as a cofactor of oxidative stress enzymes that can modulate enzyme activity to maintain redox balance (*4*).

In recent years, natural antioxidants are becoming more likely to serve as alternatives to synthetic antioxidants probably due to the associated side effects of the synthetic antioxidants, such as their carcinogenicity and toxicity. Thus, there has been much interest in finding safer and more effective natural antioxidant sources (*7*). Secondary metabolites of *S. cerevisiae* have attracted more attention as a potential source of natural antioxidants owing to their strong bioactive properties in the human body (*8*). The usage of *S. cerevisiae* as a safe source of ingredients and additives in food processing has been widely accepted by the consumers (*9*). Perhaps these secondary metabolites of *S. cerevisiae* can act as natural antioxidants; therefore, it is crucial to evaluate their antioxidant activity for potential application in the food and pharmaceutical industries.

To date, there are only a few yeast strains that can be referred to as producers of secondary metabolites with antioxidant properties. *Cutibacterium acnes*, a gram-positive anaerobic bacterium, is believed to be the main causative agent of acne. Furthermore, *Staphylococcus epidermidis* and *Staphylococcus aureus* have been reported as the causative agents of acne vulgaris and have been isolated in 53 % and 41 % aerobic cultures of pustular and nodulocystic skin lesions, respectively (*10*). Different acne treatments include lifestyle remedies, topical medication, oral medication, and medical procedures. However, patients may suffer from side effects from these treatments; hence, people nowadays prefer natural products as treatment options due to their body tolerance. This research focused on the extraction and identification of the secondary metabolites of *S. cerevisiae*. Different antioxidant assays, including DPPH, ABTS, and FRAP, were employed to determine the antioxidant activity of the

extracted metabolites. Finally, the antibacterial activity of the secondary metabolites of *S. cerevisiae* was investigated against *Cutibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

MATERIALS AND METHODS

Materials

Potato dextrose agar (PDA), ethyl acetate, methanol, ascorbic acid, potassium persulfate hydrochloric acid iron(III) chloride hexahydrate ($FeCl_3.6H_20$), dimethylsulfoxide (DMSO), Mueller-Hinton agar (MHA), tryptic soy agar (TSA), sterile MH broth (MHB), and sterile TS broth (TSB) were purchased from Merck (Darmstadt, Germany). Potato dextrose broth (PDB) was obtained from CONDA (Madrid, Spain). DPPH solution, Tris(2-pyridyl)-s-triazine (TPTZ), and iodonitrophenyltetrazolium violet (INT) were purchased from Sigma-Aldrich (St. Louis, United States).

Yeast cultivation

Potato dextrose agar (PDA) (Merck, Darmstadt, Germany) was prepared by dissolving 39 g of the dehydrated media in 1 L of distilled water. The medium was mixed, heated, and sterilized by autoclaving at 121 °C for 15 min. The medium was then poured into agar plates and allowed to solidify. The yeast, *S. cerevisiae,* was obtained from the laboratory unit of the Faculty of Industrial Sciences & Technology (FIST), Universiti Malaysia Pahang (UMP). The yeast was streaked on PDA with a sterile inoculating loop and incubated at 30ºC for 3 days. Potato dextrose broth (PDB) (CONDA, Madrid, Spain) was prepared by dissolving 26.5 g of the dehydrated media in 1 L distilled water. The medium was mixed, heated, and autoclaved at 121 °C for 15 min. A single yeast colony was picked from the PDA and cultivated in the PDB, followed by incubation in an Orbital Shaking incubator (BD115, Binder, Tuttlingen, Germany) for 3 days at 25-30 ºC with mild agitation at 130 rpm.

Extraction of secondary metabolites from yeast

The extraction was carried out according to the method described by Swathi (*11*). Ethyl acetate (Merck, Darmstadt, Germany) as solvent was added to the yeast liquid culture in 1:1 proportion. The mixture was shaken for 10 min in a separatory funnel for complete extraction of the secondary metabolites. Then, it was allowed to settle for a few minutes. Two layers of liquid were formed; the upper layer containing the metabolites was collected into falcon tubes while the bottom layer contains the yeast cells and PDB was washed thoroughly to ensure complete extraction of the metabolites before being

discarded. The separated upper layer was centrifuged at 2900 x *g* for 5 min (centrifuge Rotofix 32; Hettich, Schwerin, Germany) to remove any suspended yeast cells and media contaminants. The resulting supernatant was collected and evaporated to dryness at 35-40 ºC using an RV 10 digital Rotary Evaporator (N-1200A, Evela, Shanghai, China). A green-colored extract was obtained after the drying process and stored for further use.

Analysis of secondary metabolites of yeast using GC-MS

The crude extract was diluted in GC grade ethyl acetate before GC-MS analysis. The analysis was conducted using a GCMS 6890A system (Agilent, Santa Clara, United Stated). One (1.0) µL of the sample was introduced into the heated injector tube using a microliter volume syringe. The vaporized sample was carried through the SGE BPX5 GC column by helium gas at the rate of 1.0 mL/min. The components in the sample were separated and a gas chromatogram was obtained. Then, the effluent of the GC column was introduced directly into a mass spectrometer via a transfer line at 320 ºC. The gas molecules were converted into ions at an ion source temperature of 230 °C using electron energy of 70 eV. The scan range was set at 45-500 Da. The ions were detected by a detector and the information was sent to the computer. The components were identified based on their retention indices and by comparison of their mass spectra with the available data in the existing GC-MS library (NIST/EPA/NIH Mass, http://www.nist.gov/srd/nist1a.htm) (*12*).

Determination of antioxidant activity

DPPH assay

DPPH assay was conducted according to the suggested method by Hassan (*3*) with some modifications. DPPH solution (0.1 mM) (Sigma-Aldrich, St. Louis, United States) was first prepared in absolute methanol (Merck, Darmstadt, Germany) and added into different tubes at 1 mL volumes; Then, 1.0 mL of the yeast extract was added into each tube at different concentrations (125-2000 µg/mL) and mixed well. A set of blanks was prepared by adding 1.0 mL of methanol with 1.0 mL of yeast extract at different concentrations. All the mixtures were incubated in the dark at room temperature for 30 min. Negative control was prepared by the same procedure without yeast extract. Ascorbic acid (Merck, Darmstadt, Germany) solution was used as a positive control. The absorbance of the resulting mixtures was measured at 517 nm using a 10S UV-Vis Spectrophotometer (Genesys, Daly City, United States). The measurements were taken in triplicate and the mean values were calculated.

ABTS assay

The ABTS assay was carried out based on the method proposed by Hameed (*13*) with some modifications. 7 mM ABTS (Roche, Basel, Switzerland) was prepared by dissolving the ABTS powder in distilled water. The stock solution was prepared by reacting the prepared ABTS solution with 2.45 mM potassium persulfate (Merck, Darmstadt, Germany) at the ratio of 1:1 (*V*/*V*); the stock solution was kept in the dark at room temperature for 12-16 h before use (this is to generate $ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ solution was diluted with absolute methanol and adjusted to an absorbance range of 0.7 and 0.75 at 734 nm. 200 µL of the yeast extract at different concentrations (15.63-2000 µg/mL) and 1.8 mL of $ABTS^{\cdot+}$ solution were mixed well in the tubes and incubated in the dark at room temperature for 30 min. Negative control was prepared by the same procedure without yeast extract. Ascorbic acid solution was used as a positive control. The absorbance was measured at 734 nm using Infinite 200 PRO Microplate Reader (Tecan, Männedorf, Switzerland). The measurements were taken in triplicate and the mean values were calculated.

FRAP assay

The FRAP reagent was prepared by mixing 300 mM acetate buffer at pH 3.6, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, St. Louis, United States) in 40 mM hydrochloric acid (Merck, Darmstadt, Germany), and 20 mM iron(III) chloride hexahydrate ($FeCl_3.6H_20$) (Merck, Darmstadt, Germany) at the ratio of 10:1:1. The mixture was incubated for 15 min at 37 °C before use. Ascorbic acid was used in distilled water at different concentrations (200-1000 µg/mL) as the positive control to generate a standard curve. 150 µL of the yeast extract (in ethyl acetate) or standard was mixed thoroughly with 2.85 mL of the prepared FRAP reagent in falcon tubes. The formation of an intense blue color complex suggests the reduction of Fe^{3+} TPTZ complex to Fe^{2+}. The mixture was incubated in the dark for 30 min. The absorbance (*A*) of the solution was measured at 593 nm using the Infinite 200 PRO Microplate Reader. The measurements were taken in triplicate and the mean values were calculated.

Evaluation of antibacterial activity of secondary metabolites of yeast

Kirby-Bauer disc diffusion susceptibility test

In this test, the yeast extract sample was used against *Cutibacterium acnes* (ATCC 6919), *Staphylococcus aureus* (ATCC 6538), and *Staphylococcus epidermidis* (ATCC 12228). The concentrations of the yeast's secondary metabolites were 100 mg/mL, 200 mg/mL, 300 mg/mL and 400

mg/mL. The extract was dissolved in dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany). Gentamycin discs (Thermo Scientific™ Oxoid™) with 10 µg drug concentration acted as the positive control while the disc with DMSO solvent but without any extract acted as the negative control. 7 µL of the dissolved extract and solvent were impregnated into sterile discs and dried in the laminar flow hood (model AHC-4DI, ESCO, Selangor, Malaysia) to remove the solvent completely. The antimicrobial tests against *S. aureus* and *S. epidermidis* were carried out in Mueller-Hinton agar (MHA) (Merck, Darmstadt, Germany) while the disc diffusion test against *C. acnes* was carried out on Tryptic Soy agar (TSA) (Merck, Darmstadt, Germany) as *C. acnes* cannot grow on MHA. The three tested bacterial strains were cultured in broth and the concentration of the bacteria was adjusted to 0.5 McFarland. According to CLSI, 0.5 McFarland is equivalent to an optical density value range of 0.08 to 0.13 at 625 nm. The TSA plates with *C. acnes* were incubated in the anaerobic jar at 37 °C for 48-72 h while the MHA plates were incubated in the presence of oxygen at 37 °C for 8-16 hours. The formed inhibition zones were observed after every 6 h of incubation; the diameter of zones was measured in millimeters (mm). This test was performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC of the extracts was determined in a 96-round bottom well microplate. The initial concentration of the secondary metabolites of *S. cerevisiae* used for the MIC determination was the lowest concentration that formed an inhibition zone in the disc diffusion method for the three bacterial strains. Two-fold serial dilution was carried out to make a total of 8 different concentrations of the extract. Sterile MH broth (MHB) (Merck, Darmstadt, Germany) was added in every well for *S. aureus* and *S. epidermidis* while sterile TS broth (TSB) (Merck, Darmstadt, Germany) was added for *C. acnes*. The extract was prepared in 100 % DMSO at the concentration of 300 mg/µL; then, 100 µL of the prepared extract was transferred to the first column of the well, mixed, and 100 µL of the mixture also transferred to the next well for every row. Gentamicin was used as the positive control at the concentration of 1 mg/µL while the negative control was the broth and solvent without any extract. The suspension of the three tested bacterial strains was adjusted to the concentration equivalent to 0.5 McFarland using a UV-Vis spectrophotometer. 100 µL of the bacterial suspension was added into each well; then, the microplate was covered, sealed, and incubated at 37 °C. The microplates were then incubated for 6 h for *S. aureus* and *S. epidermidis* and 12 h for *C. acnes.* After the incubation period, 50 µL of 0.4 mg/mL of p-iodonitrophenyltetrazolium violet (INT) indicator (Sigma-Aldrich, Germany) was added into each well and

further incubated for 30 min. The color change of each well was observed. The color of the indicator turns pink when there is active bacterial growth in any well, while no color change indicates no active bacterial growth. The lowest concentration of the extract that inhibited bacterial growth was recorded as the MIC. This assay was performed in triplicate.

Statistical analysis

Statistical analysis of the data obtained was carried out using one way-ANOVA. The mean and standard deviation of the data was generated with the level of significance set at $p<0.05$. The data was displayed in the form of mean±standard deviation.

RESULTS AND DISCUSSION

Extraction of S. cerevisiae secondary metabolites and GC-MS analysis

The selection of a solvent for extraction processes depends on the nature of the desired bioactive compounds. Ethyl acetate, which was used as an extraction solvent in this study, has a medium polarity with a polarity index of 4.4. It has low toxicity on the tested strains and less effect on biological cells (*14*). When the liquid broth cultured with *S. cerevisiae* was mixed with 100 % ethyl acetate in the separatory funnel, there were two distinct layers formed as ethyl acetate is immiscible with water. Generally, organic compounds do dissolve in organic solvents. Ethyl acetate can even pull compounds from the aqueous layer. The GC-MS results were obtained by comparing the spectra and matching them against the library database (NIST/EPA/NIH Mass Spectral Library). The secondary metabolites recognized in *S. cerevisiae* extract were tabulated in Table 1.

Fig. S1 displayed the chromatogram of the extracted secondary metabolites from *S. cerevisiae*. Based on the results, the longest retention time of 24.58 min was recorded by l-Proline- N-allyloxycarbonyl-,undec-10-enyl ester. The retention time of any compound depends on the different strengths of the interaction of the compound with the stationary phase. Thus, this compound had the strongest interaction with the stationary phase and required more time to migrate through the column. 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester had the highest concentration in the extract of *S. cerevisiae* with 58.33 % of the total peak area. The GC-MS analysis of *Saccharomyces cerevisiae* has been reported to identify the presence of different compounds, such as Thieno [2,3-c] furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-, Oxime-, methoxy-phenyl-,Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl] hydrazide, 1-Aminononadecane, N-trifluoroacetyl, Androstane-11,17-dione,3-

[(trimethylsilyl)oxy]-,17-[O-(phenylme, Benzeneacetamide,α-ethyl-, 4-Benzyloxy-6-hydroxymethyl-tetrahydropyran-2,3,5-triol, 1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-,[S-(R*,R*)], Erythritol, 3,6,9,12,-Tetraoxatetradecan-1-ol,14- [4-(1,1,3,3- tetramethylbutyl, Urea,N,N'-bis(2-hydroxyethyl)-, Ergosta-5,22-dien-3-ol,acetate,(3β,22E)-, Ethyl iso-allocholate, (5β)Pregnane-3,20β-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azal,5,5'-Dimethoxy-3,3',7,7'-tetramethyl-2,2' binaphthalene-1,1',4,4',N-(4,6-Dimethyl2pyrimidinyl)-4-(4 nitrobenzylideneamino) benzene, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9 (2H,10H), 2-Methyl-9-β-d-ribofuranosylhypoxanthine, Dodecane,1-chloro-, 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazine, and 2-Bromotetradecanoic acid (*15*).

Table 1

Antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity of the *S. cerevisiae* extract was evaluated based on its ability to reduce DPPH free radicals. DPPH solution is a purple solution that turns yellow as the odd electron of the DPPH radical pairs with hydrogen from the antioxidant to form the reduced DPPH-H (*16*). Fig.1a depicts the radical scavenging capability of the *S. cerevisiae* extract compared to ascorbic acid. The highest DPPH radical scavenging activity for ascorbic acid and the yeast extract was 95.91 % and 90.96 % at the concentration of 250 μg/mL and 2000 μg/mL, respectively. Ascorbic acid exhibited a significantly higher DPPH radical scavenging activity compared to the *S. cerevisiae* extract. Ascorbic acid is one of the active reducing agents and scavengers of free radicals in biological systems, acting as a scavenger of free oxidizing radicals and harmful oxygen species (*17*). Hassan (*3*) documented that 25 mg/mL of baker's yeast autolysate showed (69.06±1.22) % DPPH radical scavenging activity due to the reduction of the molarity of the DPPH solution from 0.2 to 0.1 mM. Moreover, the extraction method might affect the antioxidant capability of an extract (*18*). The study by Sugiyama *et al*. (*19*) evaluated the antioxidant activity of indole derivatives from a marine sponge-derived yeast. All compounds showed weak activities in the DPPH assay. The extract of *S. cerevisiae* has been reported to exhibit the highest DPPH radical scavenging activity due to its high tryptophol content from alcoholic fermentation (*20*). Ethanolic extracts of *Hibiscus sabdariffa* and *Croton caudatus* leaves have been evaluated for free radical scavenging activity in the model system of *S. cerevisiae*. *H. Sabdariffa* and *C. caudatus* demonstrated an immense free radical scavenging capacity of DPPH with an IC_{50} value of 184.88 and 305.39 μg/mL at

a concentration of 500 µg/mL, respectively (*21*). Furthermore, it has been demonstrated that soybean with *Tricholoma matsutake* and *S. cerevisiae* exhibited considerable DPPH radical scavenging activity (*22*).

ABTS radical scavenging activity

The ABTS radical scavenging activity of the *S. cerevisiae* extract was assessed based on its ability to reduce ABTS radical cation ($ABTS^{\cdot+}$). $ABTS^{\cdot+}$ was produced from the oxidation of ABTS with potassium persulfate which involves loss of an electron by the nitrogen atom of ABTS (*23*). The $ABTS^{\cdot+}$ solution is bluish-green in methanol; the absorbance of the solution was determined at 734 nm to minimize interference from other absorbing components and sample turbidity. Fig. 1b showed the relative ability of the *S. cerevisiae* extract to scavenge $ABTS^{\cdot+}$ compared to that of ascorbic acid. The standard ascorbic acid and yeast extract recorded the highest inhibition percentage values of 99.17 % and 92.20 % respectively, at the concentration of 2000 µg/mL. This result indicated that both ascorbic acid and yeast extract effectively inhibited the formation of $ABTS^{\cdot+}$. It has been reported that endophytic yeasts isolated from the lower stem and roots of *Phragmites australis* Cav showed 88 % radical scavenging activity with the ABTS assay (*24*). Furthermore, *Aspergillus awamori* DT11 exhibited $ABTS^{\cdot+}$ scavenging activity of 34.07 % compared to ascorbic acid (44.5 %) that was used as a positive control (*25*).

Fig. 1

FRAP assay

The FRAP assay of *S. cerevisiae* extract was evaluated based on the ability to reduce ferric iron (Fe^{3+}) to the ferrous ion (Fe^{2+}) state via electron donation to the sample. This reduction was monitored by measuring the change in absorption at 593 nm. The assay was conducted at low pH conditions (pH 3.6) to maintain iron solubility and electron transfer (*26*). The FRAP values for each yeast extract at different concentrations were tabulated in Table 2. In general, the absorbance readings increased when the concentration of ascorbic acid and yeast extract increased from 200 µg/mL to 1000 µg/mL. This was due to the reduction of the ferric TPTZ complex to the intensely blue-colored ferrous form (*26*). The standard ascorbic acid and yeast extract recorded the highest absorbance of 3.6291 and 0.1611 respectively, at the concentration of 1000 µg/mL. The yeast extract exhibited the highest FRAP value of 44.4004 µg AAE/mL, indicating that *S. cerevisiae* can act as a potent antioxidant to reduce ferric iron.

Trolox, a water-soluble vitamin E analog, was used as a reference standard to determine the antioxidant capacity of yeast microcarriers. The Trolox equivalent values for yeast cell wall particles (YCWPs) and native yeast were reported as 0.20 μM and 7.48 μM, respectively (*27*). Chen *et al.* (*28*) reported that *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* significantly improved FRAP in comparison to their non-fermented counterpart, and both activities were attributed to the released phenolics during the solid-state fermentation (SSF).

Table 2

Benzeneethanol, 4-hydroxy, known as tyrosol, showed antioxidant activity in *vitro* and was able to inhibit or slow down the oxidation reactions brought by dioxygen or peroxides in animal tissues (*29*). However, tyrosol with its derivatives appeared to be less active than hydroxytyrosol and its analogs due to the catechol structure influence of the latter (*29*). Antenucci (*30*) reported that oligotyrosol exhibited higher antioxidant activity than tyrosol using DPPH, FRAP, and hydroxyl radical scavenging assays. 1H-Indole-3-ethanol is indolyl alcohol substituted with a 1H-indol-3-yl group. 1H-Indole-3-ethanol plays a role as a metabolite of both plants and *S. cerevisiae*. Hexadecanoic acid is a fatty acid ester of plant and animal origins that that play acts as an insect repellent. Hexadecanoic acid extracted from wild-growing mushrooms exhibited antioxidant activity at the concentration of 0.10 mg/mL (*31*). Hexadecanoic acid is one of the twenty bioactive compounds isolated from *Thesium humile* Vahl with reported antioxidant activity (*32*). Gondwal (*33*) reported that the water extract from the seeds and fruit pulp of *Skimmia anquetilia* can be used as a natural antioxidant due to its hexadecanoic acid content. Octadecanoic acid is a fatty acid ester and an algal metabolite that acts as a defoaming agent in processing beet sugar and yeast. According to a previous study, hexane extract of *Sinapis alba* exhibited slightly higher antioxidant activity than the standard ascorbic acid in phosphomolybdenum assay due to the existence of octadecanoic acid and other photo components (*34*). Pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro isolated from a marine bacteria *Bacillus tequilensis* MSI45 exhibited high antioxidant activity (*35*). The antioxidant activity of different pyrrolizidines, including pyrrolo[1,2-a]pyrazine-1,4-dione, and hexahydro-3-(phenylmethyl) in the ethyl acetate extract of *Streptomyces omiyaensis* has been reported by Tangjit (*36*). Also, *Streptomyces* strain MUSC 149(T) showed a strong antioxidant activity due to its pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- content (*37*).

Antimicrobial activity

Disc diffusion method is a popular antimicrobial test in microbiology laboratories due to its simplicity and ability to test multiple antimicrobial agents (*38*). *S. epidermidis* and *S. aureus* can grow in a range of pH 4.0-9.8, with an optimum of 6-7 pH (*39*). *C. acnes* showed optimal growth in the range pH 6.0 to 7.0 than in a more acidic and alkaline environment; however, *C. acnes* can grow in the pH range of 5.0 to 8.0 (*39*). The pH value of the dissolved extract ranged from 6-7 which is considered suitable for bacterial growth. Four concentrations of the dissolved extract (100 mg/mL, 200 mg/mL, 300 mg/mL and 400 mg/mL) were used in this assay. The dry mass of the extract loaded onto sterile discs was 0.7 mg, 1.4 mg, 2.1 mg, and 2.9 mg. The observed antibacterial activity of the *S. cerevisiae* extract was presented in Table 3 and Fig. 3. The results revealed that *S. cerevisiae* extract effectively suppressed the growth of the tested bacteria at variable potency levels.

Table 3

Among the tested bacteria, both *S. aureus* and *S. epidermidis* were susceptible to 400 mg/mL of the *S. cerevisiae* extract compared to the other tested bacteria. As depicted in Table 3, the most susceptible bacterium to the extract at the extract concentration of 400 mg/mL was *S. aureus* (11.5 mm), followed by *S. epidermidis* and *C. acnes* with mean inhibition zones of 10.5 mm and 9.333, respectively. At the concentrations of 100 mg/mL and 200 mg/mL of the *S. cerevisiae* extract, *C. acnes* had no inhibition zone, whereas the other bacteria show mean zones of inhibition in the range of 8.5 mm to 10 mm. The inhibition zones of the extract at 100 mg/mL and 200 mg/mL were significantly different when compared to the positive control (gentamycin 10µg) ($p<0.05$) for all the tested bacteria except for *C. acnes*. Moreover, the inhibition zones of the extract at 100 mg/mL and 200 mg/mL were significantly different when compared to that of 300 mg/mL and 400 mg/mL for *S. aureus* and *S. epidermidis* ($p<0.05$). Based on the mean value of the zones of inhibition, the antibacterial activity of the *S. cerevisiae* extract was concentration-dependent. Al-Jassani *et al*. (*15*) found the inhibition zones of *S. cerevisiae* extract at 90 µl concentration to be 5.33 mm and 5.10 mm for *S. epidermidis* and *S. aureus,* respectively. *S. cerevisiae* is employed as a human probiotic and influences the host's health by inactivating bacterial toxins, antimicrobial effect, nutritional effect, trophic effects, immunomodulatory effects, quorum sensing, anti-inflammatory effects, maintenance of epithelial barrier integrity, and cell restitution (*40*). It has been documented that *s. cerevisiae* showed moderate anti-microbial activity against bacteria and fungi; furthermore, cell lysate has been shown to show better antimicrobial activity than whole-cell and culture

supernatant. Again, the isolate showed better antibacterial activity against gram negative bacteria than gram-positive (*41*). Additionally, *S. cerevisiae* appeared to have bacterial activity against *Pseudomonas* sp. *Salmonella* sp., *E. coli*, *Vibrio cholera,* and *Staphylococcus aureus* as reported by (*42*). Yu-Jie *et al*. (*43*) evaluated the antimicrobial activity of *S. cerevisiae* through the inhibition of the growth of pathogenic *E. coli* O8 (MIC=0.025 g/mL), as well as its influence on the characteristics of its cell surface. High antimicrobial activities were exhibited by *C. intermedia*, *C. kefyr,* and *C. lusitaniae* against *E coli* while *C. tropicalis*, *C. lusitaniae*, and *S. cerevisiae* showed moderate antimicrobial activities against *E. coli*. However, all the tested yeasts demonstrated a very low activity against *P. aeruginosa* (*44*). Benzeneethanol,4-hydroxy- is one of the identified metabolites of *S. cerevisiae* that possess antibacterial activity against human pathogenic bacteria. 1-Methyl-3,3-diphenylurea and 1H-Indole-3- have also been reported to possess antimicrobial, cardioprotective, and anticarcinogenic properties. Diethyldithiophosphinic acid, known as O, O-diethyl dithiophosphate, has been shown to inhibit the growth of *E. coli, S. aureus, and Aspergillus fumigatus* (*45*). 9-hexadecenoic acid, present in *J. curcas* leaf extracts, has been shown to possess antimicrobial properties (*46*). Many fatty acids, such as hexadecanoic and octadecanoic acid have been documented to show antibacterial and antifungal activities (*47*,*48*). Pyrrolo[1,2-*a*]pyrazine-1,4-dione,hexahydro isolated from a marine bacteria *Bacillus tequilensis* exhibited a potent inhibitory effect against multidrug-resistant *S. aureus* (MIC=15± 0.172) mg/L and (MBC=20±0.072) mg/L (*37*). Furthermore, pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- (PPDHP) extracted from *Streptomyces* sp. has reported antifungal activity (*49*).

Determination of minimum inhibitory concentration (MIC)

The MIC of the extract was determined using a colorimetric method in which the clear-cut end-points were determined via a color change. Visual detection of bacterial growth via turbidity or formation of pellet in the wells may be difficult and could lead to inaccurate results (*50*). To detect the presence of bacterial growth, *p*-iodonitrophenyltetrazolium violet (INT) was used as an indicator. When there is active bacterial growth, INT changes from light yellow to pink or violet (Fig. S2). The MIC of the extracted metabolites was found as 18.75 mg/mL, 31.25 mg/mL, and 75 mg/mL for *S. aureus*, *S. epidermidis*, and *C. acnes,* respectively. This showed the potential of developing these metabolites into compounds with promising bioactivities against pathogenic microorganisms (Fig. 2). The obtained MIC of the extract against *S. aureus* showed consistency at the value of 18.75 mg/mL in the triplicate tests. The mean MICs for *S. aureus* was the lowest among the three bacterial isolates; this indicates that the secondary

metabolites of *S. cerevisiae* extract showed better antibacterial activities against *S. aureus* compared to the other two bacteria. *C. acnes* showed the highest level of resistance against both gentamycin and the tested secondary metabolites of *S. cerevisiae*.

Fig. 2

CONCLUSIONS

This study provides new scientific information about *S. cerevisiae*, based on its secondary metabolites, antioxidant and antibacterial potential. These secondary metabolites that produced by *S. cerevisiae* can act as natural antioxidants and can be used in industrial and pharmaceutical applications. Additionally, the secondary metabolites extracted from *S. cerevisiae* displayed good antimicrobial activities against the tested pathogens. The antibacterial activity of *S. cerevisiae* may be attributed to the various phytochemical constituents present in the extract. The individual compounds which responsible to this property can be in the future used in cosmetic industry.

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AUTHORS' CONTRIBUTION

M. AlMatar participated in designing and performing experiments, processing and interpreting data, as well as in preparation, writing and revising the manuscript. E.A. Makky assisted in designing experiments, processing and interpreting data, and revising the manuscript. M.H. Mahmood also participated in designing and performing experiments, and processing and interpreting data. O.W. Ting was involved in designing and performing experiments, and processing and interpreting data. W.Z. Qi designed and performed experiments, and processed and interpreted data.

SUPPLEMENTARY MATERIALS

All supplementary materials are available at: www.ftb. com.hr.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Piškur J, Zhou N, Dashko S, Compagno C. Why, when, and how did yeast evolve alcoholic fermentation. FEMS Yeast Res. 2014;14(6):826-32. https://doi.org/10.1111/1567-1364.12161

2. Melvydas V, Bružauskaitė I, Gedminienė G, Šiekštelė R. A novel *Saccharomyces cerevisiae* killer strain secreting the x factor related to killer activity and inhibition of *S. cerevisiae* K1, K2 and K28 killer toxins. Indian J Microbiol. 2016;56:335-43. https://doi.org/10.1007/s12088-016-0589-1

3. Hassan H. Antioxidant and immunostimulating activities of yeast (*Saccharomyces cerevisiae*) autolysates. World Appl Sci J. 2011; 15: 1110-9.

4. Đorđević NO, Todorović N, Novaković IT, Pezo LL, Pejin B, Maraš V, Tešević VV, Pajović SB. Antioxidant activity of selected polyphenolics in yeast cells: The case study of montenegrin merlot wine. Molecules. 2018;23(8):1971. https://doi.org/10.3390/molecules23081971

5. Meng D, Zhang P, Li S, Ho CT, Zhao H. Antioxidant activity evaluation of dietary phytochemicals using *Saccharomyces cerevisiae* as a model. J Funct Foods. 2017; 38: 36-44. https://doi.org/10.1016/j.jff.2017.08.041

6. Winkler BS, Orselli SM, Rex TS. The redox couple between glutathione and ascorbic acid: A chemical and physiological perspective. Free Radic Biol Med. 1994;17(4):333-49. https://doi.org/10.1016/0891-5849(94)90019-1

7. Mathew S, Abraham TE. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. Food Chem. 2006;94(4):520-8. https://doi.org/10.1016/j.foodchem.2004.11.043

8. Rahmat E, K Youngmin. Yeast metabolic engineering for the production of pharmaceutically important secondary metabolites. Appl Microbiol Biotechnol. 2020; 104:4659–4674. https://doi.org/10.1007/s00253-020-10587-y

9. Kallscheuer N. Engineered microorganisms for the production of food additives approved by the european union—A systematic analysis. Front Microbiol. 2018; 9: 1746. https://doi.org/10.3389/fmicb.2018.01746

10. Hassanzadeh P, Bahmani M, Mehrabani D. Bacterial resistance to antibiotics in acne vulgaris: An in vitro study. Indian J Dermatol. 2008;53(3):122. https://doi.org/10.4103/0019-5154.43213

11. Swathi J, Sowjanya km, Narendra K, Satya A. Bioactivity assay of an isolated marine *Fusarium* sps. Int J Bio Sci Biotechno. 2013;5:179-86. http://dx.doi.org/10.14257/ijbsbt.2013.5.5.19

12. Oberacher H, Whitley G, Berger B. Evaluation of the sensitivity of the 'Wiley registry of tandem mass spectral data, MSforID' with MS/MS data of the 'NIST/NIH/EPA mass spectral library'. J Mass Spectrom. 2013;48(4):487-496. https://doi.org/10.1002/jms.3184

13. Hameed A, Hussain SA, Yang J, Ijaz MU, Liu Q, Suleria HAR, Song Y. Antioxidants potential of the filamentous fungi (*Mucor circinelloides*). Nutrients. 2017;9(10):1101. https://doi.org/10.3390/nu9101101

14. Abarca-Vargas R, Peña Malacara C, Petricevich V. Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea x buttiana holttum and standl,(Var. rose) extracts. Antioxidants. 2016;5(4):45. https://doi.org/10.3390/antiox5040045

15. Al-Jassani M, Mohammad G, Hameed I. Secondary metabolites analysis of *Saccharomyces cerievisiae* and evaluation of antibacterial activity. Int J Pharm Clin. 2016;8:304-15.

16. Ser HL, Palanisamy UD, Yin WF, Abd Malek SN, Chan KG, Goh BH, Lee LH. Presence of antioxidative agent, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- in newly isolated *Streptomyces mangrovisoli* sp. nov. Front Microbiol. 2015;6(854).
https://doi.org/10.3389/fmicb.2015.00854

17. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4(8): 118–126.
https://doi.org/10.4103/0973-7847.70902

18. Delfanian M, Kenari RE, Sahari MA. Influence of extraction techniques on antioxidant properties and bioactive compounds of loquat fruit (*Eriobotrya japonica* Lindl.) skin and pulp extracts. Food Sci Nutr. 2015; 3(3): 179–187.
https://doi.org/10.1002/fsn3.201

19. Sugiyama Y, Ito Y, Suzuki M, Hirota A. Indole derivatives from a marine sponge-derived yeast as DPPH radical scavengers. J Nat Prod. 2009;72(11):2069-71.
https://doi.org/10.1021/np900483g

20. Guerrini S, Mangani S, Romboli Y, Luti S, Pazzagli L, Granchi L. Impact of *Saccharomyces cerevisiae* strains on health-promoting compounds in wine. Fermentation. 2018;4(2):26.
https://doi.org/10.3390/fermentation4020026

21. Subhaswaraj P, Sowmya M, Bhavana,V, Dyavaiah M, Siddhardha B. Determination of antioxidant activity of *Hibiscus sabdariffa* and *Croton caudatus* in *Saccharomyces cerevisiae* model system. J Food Sci Technol. 2017; 54(9):2728–2736.
https://doi.org/10.1007/s13197-017-2709-2

22. Lee JH, Hwang CE, Son KS, Cho KM. Comparisons of nutritional constituents in soybeans during solid state fermentation times and screening for their glucosidase enzymes and antioxidant properties. Food Chem. 2019; 272, 362–371.
https://doi.org/10.1016/j.foodchem.2018.08.052

23. Tai A, Ohno A, Ito H. Isolation and characterization of the 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation-scavenging reaction products of arbutin. J Agric Food Chem. 2016;64(38):7285-90.
https://doi.org/10.1021/acs.jafc.6b02847

24. Hipol RM, Magtoto LM, Tamang SMA, Damatac AM. Antioxidant activities of fungal endophytes isolated from strawberry fragaria x ananassa Fruit. Electron j biol. 2014; 10:107-112

25. Hipol RM. Antioxidant potentials of culturable endophytic yeasts from *Phragmites australis* Cav. (Trin) ex Steud. from copper-contaminated mining site in Mankayan, Benguet. Philipp Sci Lett. 2014;7:337-346

26. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods. 2015;18:757-81. https://doi.org/10.1016/j.jff.2015.01.047

27. Young S, Nitina N. Thermal and oxidative stability of curcumin encapsulated in yeast Microcarriers. Food Chem.2019; 275:1–7 https://doi.org/10.1016/j.foodchem.2018.08.121

28. Chen Y, Wang Y, Chen J, Tang H, Wang C, Liab Z, Xia Y. Bioprocessing of soybeans (*Glycine max* L.) by solid-state fermentation with *Eurotium cristatum* YL-1 improves total phenolic content, isoflavone aglycones, and antioxidant activity. RSC Adv. 2020; 10:16928-16941 https://doi.org/10.1039/C9RA10344A

29. Vlachogianni IC, Fragopoulou E, Kostakis IK, Antonopoulou S. In vitro assessment of antioxidant activity of tyrosol, resveratrol and their acetylated derivatives. Food Chem. 2015;177:165-73. https://doi.org/0.1016/j.foodchem.2014.12.092

30. Antenucci S, Panzella L, Farina H, Ortenzi MA, Caneva E, Martinotti S, Ranzato E, Burlando B, d'Ischia M, Napolitano A, Verotta L. Powering tyrosol antioxidant capacity and osteogenic activity by biocatalytic polymerization. RSC Adv. 2016;6(4):2993-3002. https://doi.org/10.1039/C5RA23004G

31. Luo Y, Huang Y, Yuan X, Zhang L, Zhang X, Gao P. Evaluation of fatty acid composition and antioxidant activity of wild-growing mushrooms from Southwest China. International journal of medicinal mushrooms. 2017 19(10):937-947 https://doi.org/0.1615/IntJMedMushrooms.2017024388

32. Belakhdar G, Benjouad A, Abdennebi E. Determination of some bioactive chemical constituents from *Thesium humile* Vahl. J Mater Environ Sci. 2015;6(10):2778-83.

33. Gondwal M, Prakash O, Vivekanand, Pant AK, Padalia RC, Mathela CS. Essential oil composition and antioxidant activity of leaves and flowers of *Skimmia anquetilia* NP Taylor & Airy Shaw. J Essent Oil Res. 2012;24(1):83-90. https://doi.org/10.1080/10412905.2012.646034

34. Sujatha M, Karthika K, Sivakamasungari S, Mariajancyrani J, Chandramohan G. GC-MS analysis of phytocomponents and total antioxidant activity of hexane extract of *Sinapis alba*. Int J Pharm Chem Biol Sci. 2014;4(1):112-7.

35. Kiran GS, Priyadharsini S, Sajayan A, Ravindran A, Selvin J. An antibiotic agent pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro isolated from a marine bacteria *Bacillus tequilensis* MSI45 effectively controls multi-drug resistant *Staphylococcus aureus*. RSC Adv. 2018;8(32):17837-46.
https://doi.org/10.1039/C8RA00820E

36. Tangjitjaroenkun J. Evaluation of antioxidant, antibacterial, and gas chromatography-mass spectrometry analysis of ethyl acetate extract of *Streptomyces omiyaensis* SCH2. Asian J Pharm Clin Res. 2018;11:271-276
https://doi.org/10.22159/ajpcr.2018.v11i7.25692

37. Ser HL, Tan LTH, Palanisamy UD, Abd Malek SN, Yin WF, Chan KG, GOh BH, Lee LH. *Streptomyces* antioxidans sp. nov., a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. Front Microbiol. 2016;7:899.
https://doi.org/10.3389/fmicb.2016.00899

38. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. 2009.

39. Lund B, Baird-Parker AC, Baird-Parker TC, Gould GW, Gould GW. Microbiological safety and quality of food: Springer Science & Business Media; 2000.

40. Moslehi-Jenabian S, Lindegaard L, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients. 2010;2(4):449-73.
https://doi.org/10.3390/nu2040449

41. Fakruddin MD, Md. Nur Hossain MD, Ahmed MM. Antimicrobial and antioxidant activities of *Saccharomyces cerevisiae* IFST062013, a potential probiotic. BMC Complement Altern Med. 2017; 17:64.
https://doi.org/10.1186/s12906-017-1591-9

42. Rajkowska K, Kunicka-StyczynBska A, Rygala A. Probiotic Activity of *Saccharomyces cerevisiae* var. boulardii Against Human Pathogens. Food Technol Biotech.2012; 50: 230–236.

43. Yu-jie C, Chun-jie W, Wen-qian H, Xiao-shuo W, Bing-ga G, Si-mu-ji-de H, Si-qin Y, A-qi-ma W, Yu-fei Z, Ying-ga W, Ao-ri-ge-le C. Effects of antibacterial compounds produced by *Saccharomyces cerevisiae* in Koumiss on pathogenic *Escherichia coli* O8 and its cell surface characteristics J Integr Agric. 2017;16: 742–748

https://doi.org/10.1016/S2095-3119(16)61516-2

44. Younis G, Awad A, Dawod RE, Yousef NE. Antimicrobial activity of yeasts against some pathogenic bacteria. Vet World. 2017; 10: 979-983.

https://doi.org/10.14202/vetworld.2017.979-983

45. Abdel-Wahab MA, Bahkali A, El-Gorban AM, Hodhod MS. Natural products of *Nothophoma multilocularis* sp. nov. an endophyte of the medicinal plant *Rhazya stricta*. Mycosphere. 2017;8(8):1185-200.

https://doi.org/10.5943/mycosphere/8/8/15

46. Yin Y, Bi Y, Chen S, Li Y, Wang Y, Ge Y, Ding B, Li Y,Zhang Z. Chemical composition and antifungal activity of cuticular wax isolated from Asian pear fruit (cv. Pingguoli). Sci Hortic 2011;129(4):577-82.

https://doi.org/10.1016/j.scienta.2011.04.028

47. McGaw L, Jäger A, Van Staden J. Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia. 2002;73(5):431-3.

https://doi.org/10.1016/S0367-326X(02)00120-X

48. Altieri C, Bevilacqua A, Cardillo D, Sinigaglia M. Antifungal activity of fatty acids and their monoglycerides against *Fusarium* spp. in a laboratory medium. Int J Food Sci Technol. 2009;44(2):242-5.

https://doi.org/10.1111/j.1365-2621.2007.01639.x

49. Sanjenbam P, Krishnan K. Bioactivity of Pyrrolo[1,2-A]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- Extracted from *Streptomyces* sp. VITPK9 Isolated from the Salt Spring Habitat of Manipur, India. Asian J Pharm. 2016;10: 265.

http://dx.doi.org/10.22377/ajp.v10i04.865

50. Weseler A, Geiss HK, Saller R, Reichling J. A novel colorimetric broth microdilution method to determine the minimum inhibitory concentration (MIC) of antibiotics and essential oils against *Helicobacter pylori*. Pharmazie. 2005;60(7):498-502.

Table 1. Secondary metabolites identified in the ethyl acetate extract of *S. cerevisiae*

No	Compound	Molecular formula	t_R/min	Peak area/%
1	Benzeneethanol,4 hydroxy-	$C_8H_{10}O_2$	11.548	12.14
2	1-Methyl-3,3-diphenylurea	$C_{14}H_{14}N_2O$	13.796	1.26
3	1H-Indole-3-ethanol	$C_{10}H_{11}NO$	15.505	10.68
4	Diethyldithiophosphinic acid	$C_4H_{11}PS_2$	16.082	2.09
5	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	17.020	1.66
6	Diethyldithiophosphinic acid	$C_4H_{11}PS_2$	17.057	1.90
7	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	$C_9H_{14}O_2$	17.230	1.80
8	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	19.133	1.15
9	Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$	20.852	0.69
10	Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	21.444	3.74
11	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	22.152	58.33
12	2,3-Diphenyl-5,8-dimethoxy-6-acetamidoquinoxaline	$C_{24}H_{21}N_3O_3$	23.117	1.21
13	l-Proline,N-allyloxycarbonyl-,undec-10-enyl ester	$C_{20}H_{33}NO_4$	24.579	3.35

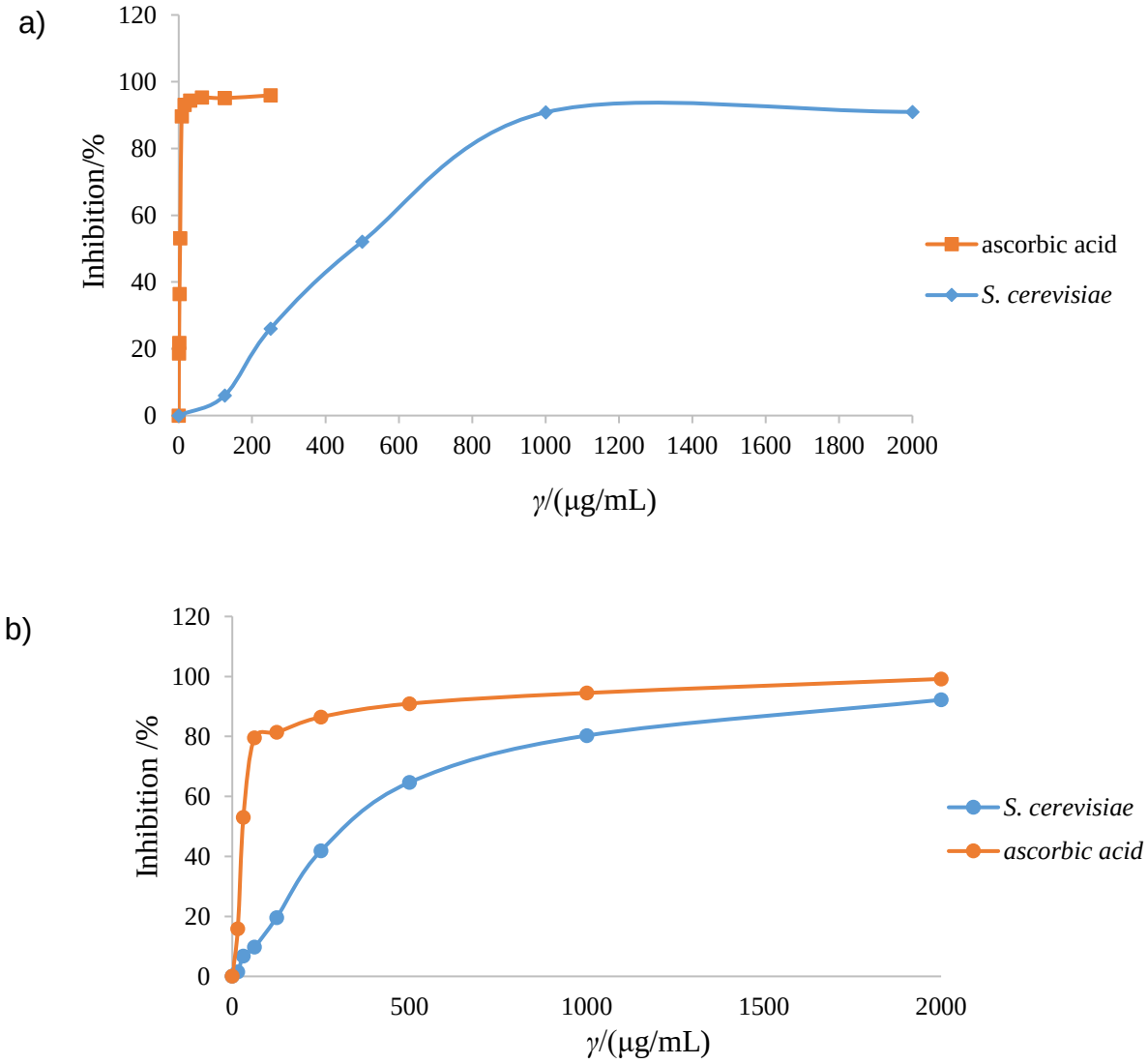

Fig. 1. (a) DPPH radical scavenging activity of standard ascorbic acid and *S. cerevisiae* extract. (b) ABTS radical scavenging activity of ascorbic acid and *S. cerevisiae* extract

Table 2. FRAP values obtained from different yeast extract concentrations

γ/(μg/mL)	*A*	FRAP/(μg/mL)
0	0.0000	0
200	0.0402±0.0007	36.7327
400	0.0742±0.0039	39.1041
600	0.1130±0.0075	43.1121
800	0.1364±0.0006	43.9787
1000	0.1611±0.0041	44.4004

Table 3. Antimicrobial screening test of *S. cerevisiae* extract against some bacterial strains.

Pathogenic bacteria	*d*(inhibition)/mm				
	γ(extract)/(mg/mL)				*c*(gentamycin)/μM (10 μg)
	100	200	300	400	
S. aureus	9.5±2.121	10.0±1.414	11.0±1.414	11.5±2.121	18.667±0.577
S. epidermidis	8.5±0.707	8.5±0.707	10.0±0	10.5±0.707	22.333±0.577
C. acnes	0.0	0.0	9.0±0	9.333±0.577	12±0

Values are expressed as mean±SEM. Analysis was performed with one-way ANOVA, where $p<0.05$

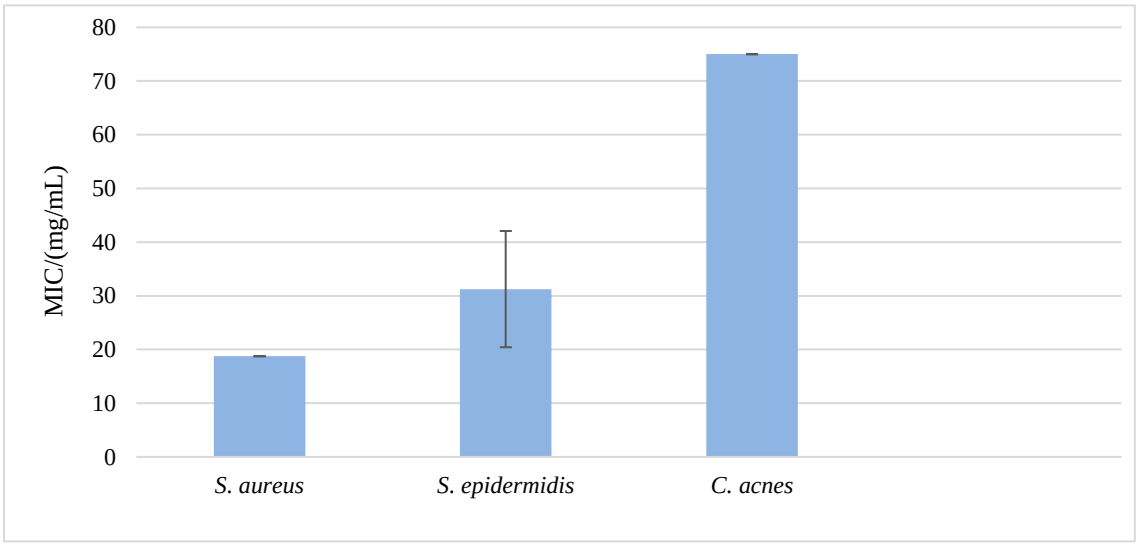

Fig. 2. Minimum inhibition concentration (MIC) of *S. cerevisiae* extract against the tested microbes