Saudi Journal of Biological Sciences 29 (2022) 1842–1852

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Comparative study of the antioxidant activity of the essential oils of five plants against the H_2O_2 induced stress in Saccharomyces cerevisiae

Khadija Ridaoui [a,*], Ismail Guenaou [a], Ikram Taouam [a], Mounia Cherki [a], Noureddine Bourhim [a], Abdelaziz Elamrani [b], Mostafa Kabine [a]

[a] Department of Biology, Laboratory of Health and Environment, Faculty of Science Ain Chock, Hassan II University of Casablanca, Morocco
[b] Laboratory of Organic Synthesis, Extraction, and Valorization, Faculty of Sciences Aïn Chock, Hassan II University of Casablanca, B.P 2693 Maarif, Casablanca, Morocco

ARTICLE INFO

Article history:
Received 16 June 2021
Revised 10 October 2021
Accepted 15 October 2021
Available online 22 October 2021

Keywords:
Essential oils
Yeast
S.cerevisiae
Oxidative stress
Hydrogen peroxide
Antioxidants

ABSTRACT

The purpose of this work was to investigate the protective effect of five essential oils (EOs); *Rosmarinus officinalis, Thymus vulgaris, Origanum compactum* Benth., *Eucalyptus globulus* Labill. *and Ocimum basilicum* L.; against oxidative stress induced by hydrogen peroxide in *Saccharomyces cerevisiae*. The chemical composition of the EOs was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The *in vitro* antioxidant activity was evaluated and the protective effect of EOs was investigated. Yeast cells were pretreated with different concentrations of EOs (6.25–25 μg/ml) for an hour then incubated with H_2O_2 (2 mM) for an additional hour. Cell viability, antioxidants (Catalase, Superoxide dismutase and Glutathione reductase) and metabolic (Succinate dehydrogenase) enzymes, as well as the level of lipid peroxidation (LPO) and protein carbonyl content (PCO) were evaluated. The chemical composition of EOs has shown the difference qualitatively and quantitatively. Indeed, *O. compactum* mainly contained Carvacrol, *O. basilicum* was mainly composed of Linalool, *T. vulgaris* was rich in thymol, *R. officinalis* had high α-Pinene amount and for *E. globulus*, eucalyptol was the major compound. The EOs of basil, oregano and thyme were found to possess the highest amount of total phenolic compounds. Moreover, they have shown the best protective effect on yeast cells against oxidative stress induced by H_2O_2. In addition, in a dose dependent manner of EOs in yeast medium, treated cells had lower levels of LPO, lower antioxidant and metabolic enzymes activity than cells exposed to H_2O_2 only. The cell viability was also improved. It seems that the studied EOs are efficient natural antioxidants, which can be exploited to protect against damages and serious diseases related to oxidative stress.

Abbreviations: ANOVA, Analysis of variance; ABTS, 2,2-azino-bis(3-etilbenzotiazolin)-6-sulfonic acid; BHA, Butylated hydroxyanisole; BHT, Butylated hydroxytoluene; BSA, bovine serum albumin; CAT, catalase; DCIP, 6-Dichlorophenolindophenol; DNPH, 2,4- dinitro-phenylhydrazine reagent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, Ethylene diamine tetra acetic acid; EOs, essential oils; FID, flame ionization detector; GC, gas chromatography; GC/MS, gas chromatography-mass spectrometry; GR, Glutathione reductase; GSH, Reduced glutathione; GSSG, Oxidized glutathione; H_2O_2, Hydrogen peroxide; LPO, Lipid peroxidation; MDA, Malondialdehyde; $MgCl_2$, Magnesium chloride; NaAc, Sodium acetate; NaCl, Sodium chloride; NADH, Nicotinamide adenine dinucleotide; NADHP, Nicotinamide adenine dinucleotide phosphate; OH, Hydroxyl radical; PBS, Phosphate buffer saline; PCO, Protein carbonylation; PMSF, Phenylmethylsulfonylfluoride; ROS, Reactive oxygen species; RP, reducing power; *S.c, S. cerevisiae*; SD, standard deviation; SDH, Succinate dehydrogenase; SDS, Sodium dodecyl sulphate; SEM, Standard error of the means; SOD, Superoxide dismutase; TBA, Thiobarbituric acid; TBARS, Thiobarbituric acid reactive substances; TCA, Trichloroacetic acid; YPG, yeast-extract-peptone-glucose.

* Corresponding author at: Department of Biology, Laboratory of Health and Environment, Faculty of Science Ain Chock, University Hassan II Casablanca, Morocco.

E-mail address: khadija.rd07@gmail.com (K. Ridaoui).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.10.040
1319-562X/

1. Introduction

Oxygen is a highly reactive molecule. It can be somewhat reduced to reactive oxygen species (ROS) that are defined as small and highly chemically-reactive agents (Jamieson, 1998). They include free radicals such as; superoxide anion ($O_2^{\bullet -}$) and hydroxyl radicals (•OH) as well as non-radical oxygen species for instance; hydrogen peroxide (H_2O_2) and singlet oxygen($^1O2$) (Kurutas, 2015; Sato et al., 2013). The production of ROS by biological systems, mitochondria mainly, is very important as they take part in many physiological processes such as apoptosis, immunity, differentiation and activation of many transcriptional factors... (Rajendran et al., 2014). Nevertheless, the surplus of ROS, that can be caused by environmental factors (e.g. UV, ionizing radiations, pollutants, and heavy metals, smoking...), generates a phenomenon known as oxidative stress which is an imbalance in the generation of pro-oxidants and the ability of antioxidants to neutralize them (Aouacheri et al., 2015; Kurutas, 2015; Pizzino et al., 2017). This will eventually cause damages in the cellular components like membranes, proteins, lipids, nucleic acid (Dröge, 2002) leading to several diseases that have been proven to be associated with oxidative stress such as diabetes (Pizzino et al., 2017), kidney disease (Galle, 2001), cancer (Valko et al., 2004), cardiovascular diseases (Bahoran et al., 2007), neurological diseases (Allan Butterfield, 2002) and respiratory diseases (Caramori, 2004). Antioxidants compounds defined as a defense system, including enzymatic and non-enzymatic compounds, are developed by the organism in order to prevent oxidative stress after consumption (Halliwell, 2008; Pizzino et al., 2017). Hence, the interest in the search for natural antioxidants sources that would be efficient to scavenge the surplus of free radicals has increased over the past few years. Moreover, the studies on essential oils (EOs) and extracts of medicinal and aromatic plants have increased as well as their use as alternatives for treatment of diseases owing to the presence of compounds such as polyphenols, terpenes and flavonoids that were shown to have many beneficial effects including antioxidant activity and have been used to treat many diseases attributable to their ability of free radicals scavenging (Adams, 2007; Nait Irahal et al., 2020; Nait Irahal et al., 2021; Santos-Buelga and Scalbert, 2000). It has been reported that *Thymus vulgaris* (El-Nekeety et al., 2011), *Ocimum basilicum* extracts (Kaurinovic et al., 2011) and *Origanum compactum* (Bouyahya and Jamal, 2016) have shown antioxidant properties. Also, previous studies reported that *Rosmarinus officinalis* oil has shown many biological effects including antibacterial and antifungal activities (Satyal et al., 2017). In addition, the phytochemical studies, the antibacterial and antimicrobial activities of *Rosmarinus, Thymus, Origanum., Eucalyptus* and *Ocimum* have been extensively investigated and were focused mainly on the hexane, chloroform, ethylacetate, acetone, methanol, ethanol and aqueous extracts. However, very few reports are available on the use of the essential oils as a protective potential against the oxidative stress in yeast cells. Therefore, the objective of this study is to present a screening and a comparative study on the antioxidant activity of five different industrial essential oils *Rosmarinus officinalis, Ocimum basilicum* L., *Origanum compactum* Benth., *Eucalyptus globulus* Labill. and *Thymus vulgaris*. Moreover, *Saccharomyces cerevisiae* was chosen as a model organism since that the mechanisms of defense against oxidative stress in *S. cerevisiae* were shown to be similar to humans (Meng et al., 2017).

The different essential oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) and were investigated for their effect when it comes to the protection against oxidative stress induced by H_2O_2 in the yeast *Saccharomyces cerevisiae* by determining antioxidant and metabolic enzymes activity and level of lipid peroxidation and protein carbonylation.

2. Materials and methods

2.1. Chemicals and reagents

All the following chemicals used in this study were of high quality. Yeast extract, Agar and peptone were received from BIOKAR Diagnostics. Tris (hydroxymethyl) aminomethane, Hydrogen peroxide (H_2O_2), Glycerol, Phenylmethylsulfonyluoride (PMSF), Guanidine hydrochloride and 2-β-mercaptoethanol were purchased from Fluka. Potassium persulfate ($K_2S_2O_8$) from SOCHID. Vitamin C and Trichloracetic acid (TCA) were bought from Scharlau (Spain). Bovine serum albumin (BSA) was purchased from Janssen Chimica. Potassium dihydrogen phosphate, Potassium cyanide (KCN), Ethylenediaminetetraacetic acid (EDTA), Sodium dodecyl sulphate (SDS) and Sodium carbonate were bought from Riedel-de Haën. Ethanol was received from Biosmart. Acetic acid, 2,6- Dichlorophenolindophenol (DCIP), Succinate, oxidized glutathione (GSSG), Thiobarbituric acid (TBA), Sodium chloride (NaCl), Nicotinamide adenine dinucleotide (NADH), Nicotinamide adenine dinucleotide phosphate (NADPH), 2,4- dinitrophenylhydrazine (DNPH) reagent and Magnesium chloride ($MgCl_2$) were purchased from Sigma-Aldrich.

2.2. Essential oils

In this study five different EOs were used: rosemary (*Rosmarinus officinalis),* basil *(Ocimum basilicum L.),* oregano *(Origanum compactum Benth.),* eucalyptus *(Eucalyptus globulus Labill.),* thyme *(Thymus vulgaris)* all received in pure concentration from Naturactive Laboratoires Pierre Fabre France. The geographical origin and batch number of the EOs are shown in Table 1.

2.3. Yeast strain and growth conditions

All the experiments were done using a wild type strain of *S. cerevisiae* YMES2, isolated from traditional Moroccan bread dough, kindly provided by Professor Faouzi Errachidi from Faculty of Sciences and Technologies of Fes (FST). Yeast strain was grown in liquid YPG medium (1% yeast extract, 1% peptone, 2% glucose) with an orbital shaker at 160 rpm, for 24 h at 30 °C with the ratio of flask volume/medium of 5/1.

2.4. Phytochemical analysis

2.4.1. Determination of total phenols

This assay was carried out using the Folin-Ciocalteau reagent and Gallic acid as standard by adding 0.5 mL of each essential oil and 2 mL of sodium carbonate (75 g. L^{-1}) to 2.5 mL of 10% (v/v) Folin-Ciocalteau as reported by (Slinkard and Singleton, 1977). The mixture was left at room temperature for 30 min, then proceeded to the absorbance measurement using a wavelength of 765 nm. Tests were carried out in triplicate.

2.4.2. Gas chromatographic (GC)

Gas chromatographic analyses were performed using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and a BP-5 capillary column (30 m x0.25 mm i.d., film thickness 0.25 μm SGE Ltd). The oven

Table 1
Geographic origin and batch numbers of essential oils.

Essential oil	Part used	Batch number	Collection region	Chemotype
O. basilicum L.	Aerial part	3,401,597,745,942	Asia / North Africa	Linalool
R.officinalis	Leaves	3,401,566,088,148	North Africa	1,8-cineole
O. compactum Benth.	Flowering top	3,401,597,747,083	North Africa	Carvacrol/ thymol
E. globulus Labill.	Leaves and twigs	3,401,597,746,543	Europe	1,8-cineole/ α-pinene
T. vulgaris	Aerial part	3,665,606,000,181	Europe	Thymol

temperature was programmed, 60–200 °C, at 3 °C.min-1, and then held isothermal for 5 min; injector and detector temperatures, 280 °C and 300 °C, respectively; carrier gas, nitrogen, adjusted to a linear velocity of 30 cm.s^{-1}. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.2 μL of a pentane-volatiles solution (1:1)

2.4.3. GC–MS assay

A gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) were used to analyze the essential oils. The GC–MS unit consisted on a Shimadzu GC-2010 gas chromatograph, equipped with BP-5 capillary column (30 m × 0.25 m m i.d., film thickness 0.25 μm; SGE, Ltd.), and interfaced with a Shimadzu QP2010 Plus mass spectrometer (software version 2.50 SU1). The oven temperature was programmed as described for GC analysis; transfer line temperature, 300 °C; ion source temperature, 200 °C; carrier gas, helium, adjusted to a linear velocity of 36.5 cm.s^{-1}; split ratio, 1:40; ionization energy, 70 eV; scan range, 40 400 u; scan time, 1 s. Component identification was carried out by comparison of their retention indices relative to C_9-C_{20} *n* alkanes on the BP-5 column (Adams, 2007), confirmed by comparison of recorded mass spectra with those of a computer library (Shimadzu corporation library and NIST05 database/ ChemStation data system) and from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercial available standards and other literature data.

2.5. Antioxidant activity

2.5.1. DPPH free radical scavenging assay

This assay was performed according to (Wu et al., 2019) and with some modifications. First of all, 50 μL of each EO at different concentrations (0,6–10 mg/mL) was added to 2 mL DPPH ethanol solution (60 μM). After that, the mixture was left in the dark at room temperature for 30 min then the absorbance was measured at 517 nm. The following equation was used to calculate the activity:

$$\mathrm{DPPHscavengingactivity}(\%) = ((A_0 - A_t)/A_0) \times 100$$

A_0 is the absorbance of the control after 30 min; A_t is the absorbance of each EO after 30 min. Ascorbic acid was used as positive control. Tests were carried out in triplicate.

2.5.2. $ABTS^+$ free radical scavenging assay

This test was performed in triplicate and as described by (Dorman et al., 2004). Thus, following to the reaction, of $K_2S_2O_8$ (2.45 mM) with ABTS (7 mM) aqueous solution, in the dark and at room temperature during 16 h, the radical $ABTS^{\bullet+}$ solution was generated. Ethanol was added to the prepared solution to adjust the absorbance to 0.7. Samples at different concentrations were added to 9 mL of $ABTS^{\bullet+}$. The absorbance was measured at 734 nm at time 0 (A_0) and after 6 min (A_1). Ascorbic acid was used as positive control.

2.5.3. Reducing power

According to the method reported by (Oyaizu, 1986), the reductive power of the EOs samples was defined. The mixture contained: 0.2 M phosphate buffer (pH 6.6), 1% (w/v) potassium ferricyanide, 10% TCA and 0.1% ferric chloride. The absorbance was measured at 700 nm and the ascorbic acid was used as positive control. The assay was carried out in triplicate.

2.6. Cytotoxicity of EOs

The sensitivity of *S. cerevisiae* to all five EOs was carried out according to Tran and Green (2019) with minor modifications. EOs were used at different concentrations (6.25, 12.5, 25, 50 and 100 μg/mL) was determined after exposing yeast cells to EOs for 2 h then plated in agar for an additional 72 h. Under the same conditions a control was performed but without adding any EOs.

2.7. Oxidative stress induction

In this study, oxidative stress in *S. cerevisiae* was induced using Hydrogen peroxide (H_2O_2). Yeast cells at the first exponential phase growing in liquid YPG medium with an initial OD_{600nm} = 0.26 were directly or pretreated with different concentrations of EOs (6,25–100 μg/ mL) for an hour, then incubated with H_2O_2 (2 mM) during 1 h at 30 °C/160 rpm (de Sá et al. 2013).

2.8. Tolerance determination

The determination of cell viability was determined in normal condition and after inducing oxidative stress, on cells treated or not with EOs. The analysis was performed in triplicate, by plating cells on solidified YPG medium (1% yeast extract, 1% peptone, 2% glucose and 2% agar) after a (10000 x) dilution (Castro et al., 2007; Dani et al., 2008). An incubation at 30 °C/72 h was done then colonies were counted. Survival was expressed as percentage.

2.9. Biochemical assays

2.9.1. Preparation of cell-free extract

After the treatment with H_2O_2 for 1 h at 30 °C/160 rpm, a centrifugation at 6000g for 5 min at 4 °C was done in order to harvest the yeast cells which were then washed three times with 20 mM Tris-HCl buffer (pH 7.5) and put back into suspension in the lysis buffer that contains 50 mM Tris-HCl buffer (pH 7.5), 1 mM ethylenediamine tetraacetic acid (EDTA), 10 mM 2-β- mercaptoethanol, 1 mM phenylmethylsulfony fluoride (PMSF), and 1% (v/v) glycerol at a ratio of 3 mL/g (wet weight). While disturbing the cells with cold, A Bandelin Sonopuls Sonifier (90%, 20 s, 12 ×) was then used, followed by a centrifugation (15,000g, 45 min at 4 °C) using a Sigma 2–16 K refrigerated centrifuge. The obtained supernatant was later on used for all enzyme activity assays. According to the Bradford procedure, the protein content was determined using bovine serum albumin (BSA) as standard (Bradford, 1976).

2.9.2. Detection of superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) activity measurement was based on the determination of the ability of enzyme extract to inhibit the oxidation of NADH caused by superoxide radicals that produced in a chemical system according to (Paoletti et al., 1986), using 5 mM EDTA, 2.5 mM $MgCl_2$, 3.9 mM 2-mercaptoethanol, 0.27 mM NADH in 50 mM potassium phosphate buffer (pH 7) and 50 µL enzyme extract. The reaction kinetics was measured at a wavelength of 340 nm. The SOD activity unit is the amount of enzyme required to inhibit the oxidation of the initial rate of NADH by 50%, expressed in U/mg of protein.

2.9.3. Determination of catalase (CAT) activity

The CAT activity was determined by following the consumption of H_2O_2. The reaction mixture contained 7.5 mM H_2O_2 in 50 mM sodium phosphate buffer (pH 7.0) and 50 µL of the enzyme extract. The kinetics was measured at 240 nm (Aebi, 1984). The molar extinction coefficient of H_2O_2 (0.0394 $mM^{-1}cm^{-1}$) was used to calculate the CAT activity. It is defined as µmol H_2O_2 consumption/min/mg of protein.

2.9.4. Determination of glutathione reductase (GR) activity

The GR activity was determined based on monitoring at 340 nm the decrease in absorbance due to the oxidation of NADPH as described by (Di Ilio et al., 1983). For this purpose, a reaction mixture was used containing: 1 mM EDTA, 0.5 mM GSSG, 50 mM potassium phosphate buffer (pH 7.4) and 50 µL of enzyme extract, all incubated for 2 min at 37 °C, then 100 µL of NADPH (0.1 mM) was added. The kinetics of the activity was measured at a wavelength of 340 nm. The unit of the activity was expressed as nmol NADPH oxidized per min per mg of protein.

2.9.5. Determination of lipid peroxidation

The determination of lipid peroxidation was based on the ability of the extracts to inhibit the formation of malondialdehyde using thiobarbituric acid reactive substances (TBARS) as reported elsewhere (Samokyszyn and Marnett, 1990). The method consisted of transferring 1 mL of extract into 1 mL solution of 0.375% thiobarbituric acid and 15% trichloracetic acid in 0.25 M hydrochloric acid. The mixture was heated to 100 °C for 15 min, then quickly cooled using ice in order to stop the reaction. Centrifugation is then carried out at 1,000 g for 10 min. The absorbance of supernatant was measured at 535 nm and the results were defined as nmoles MDA equivalents per mg protein.

2.9.6. Determination of protein carbonyl content (PCO)

The level of carbonyl group was determined according to Levine's method (Levine, 2002). Concisely, 100 µL of the simple was mixed with 400 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.5 M HCl. After incubation for 60 min at room temperature, proteins were then precipitated using 500 µL of 20% (w/v) trichloroacetic acid (TCA), left on ice for 5 min, and centrifuged at 10,000g for 10 min at 4 °C. The protein pellet was washed 3 times by 500 µL of 1:1 (*v/v*) ethanol: ethyl acetate solution. The final protein pellet was resuspended in 250 µL of 6 M guanidine hydrochloride. The absorbance was read at 370 nm. The protein carbonyl group of each sample was calculated by using absorption coefficient (ε = 22,000 $M^{-1}cm^{-1}$). The protein carbonyl content was expressed as nmol/mg of protein.

2.9.7. Succinate dehydrogenase (SDH) activity assay

The measurement of the SDH activity was done according to (King, 1967), based on the reduction of Dichlorophenolindophenol (DCIP) which is known as a chemical compound used as redox dye by the change of its blue color. The activity was measured at a wavelength of 625 nm, using 0.053 mM DCIP, 0.3 mM EDTA in 100 mM potassium phosphate buffer (pH 7.4) and 50 µL enzyme extract. The reaction mixture was incubated at 25 °C for 10 min, after that 50 µL of KCN-Succinate (3.25 mg/mL of KCN in 0.5 M succinate) was added. The unit (µmol DCIP reduced/min/mg protein) was determined using molar extinction coefficient of DCIP (19,100 M^{-1} cm^{-1}).

2.10. Statistical analysis

The data are represented as the mean ± standard deviation (SD) of at least three independent experiments. Statistical analyses were made using one-way analysis of variance (ANOVA) and Tukey's post-hoc test using the Prism 7 software for Windows (GraphPad Software Inc., San Diego, CA, USA). The probability value of $p < 0.05$ was considered to denote a statistical significance difference.

3. Results

3.1. Chemical composition of EOs

The GC/MS analysis has allowed the determination of the chemical composition of EOs. Results in Table 2 below present the percentage composition of the compounds of all the essential oils used, showing the identification of a total of 16 components for eucalyptus (*E. globulus*), 19 for rosemary (*R. officinalis*) and both of them had cineole (eucalyptol) (46.84%, 66.32%) and α-Pinene (12.58%, 13.95%) respectively as main components. For the other oils, 22 components were determined for thyme (*T. vulgaris*), 33 for oregano (*O. compactum*) and 40 for basil (*O. basilicum).* The major components were linalool (63.95%) in *O. basilicum,* carvacrol (48.16%) in *O. compactum* and thymol (76.66%) in *T. vulgaris* (Table 3*).*

The quantification of the total phenolic content of each EO using the Folin-Ciocalteau method presented in Fig. 1A showed that *O. compactum* had the highest amount of total phenols (85.19 mg GAE/g) followed by *T. vulgaris* with a total phenolic content of (84.36 mg GAE/g) and *O. basilicum* with an amount of (73.03 mg GAE/g). The results obtained are in accordance with the chemical composition of the oils since carvacrol and thymol are the main phenolic constituents according to their chemical structures. However, the essential oils of *E. globulus* and *R. officinalis* had lower amounts of total phenols 43.64 and 43.28 mg GAE/g respectively.

3.2. In vitro antioxidant activity of EOs

In this study, the antioxidant activity of the EOs was carried out using three tests; ABTS, DPPH free radical scavenging and reducing power. Results showed that basil, thyme and oregano had a high antioxidant activity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals and were also characterized by a high reducing power. However, rosemary and eucalyptus showed a low antioxidant activity and very low RP compared to the antioxidant activity of ascorbic acid used as control (Fig. 1). When it comes to DPPH free radicals scavenging, basil had the highest antioxidant activity in a dose–dependent manner with a percentage of inhibition of free radicals near values of ascorbic acid at (10 mg/mL) followed by thyme then oregano (Fig. 1B). As for ABTS free radicals scavenging (Fig. 1C), oregano presented a very high activity similar to the activity of ascorbic acid at (10 mg/mL) followed by basil and thyme.

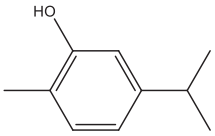
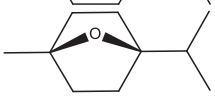
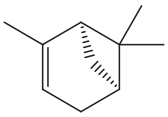
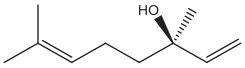
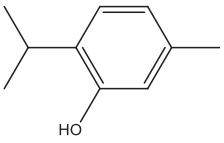
3.3. Sensitivity of S. Cerevisiae to EOs

To evaluate the cytotoxicity of the EOs on S. cerevisiae, a range of concentrations of all EOs (6,25–100 µg/mL) was tested. Results

Table 2
Chemical composition of five essential oils.

Compounds	Kovàts Index	*O.Basilicum* (Area %)	*O.compactum* (Area%)	*T. vulgaris* (Area%)	*R. officinalis* (Area%)	*E. globulus* (Area%)
α-Pinene	0932	0.31	0.60	0.53	12.58	13.95
(-)-β- Pinene	0974	0.42			2.62	
β-Myrcene	0988	0.32	1.14	1.07	0.43	
3-Octanone	0979		0.07			
Octodrine			0.08		0.19	
Octane	0800		0.03			
1-Octen-3-ol	0974		0.03	0.27		
o-Cymene	1022	0.30	12.21	5.51	2.17	1.28
Cymen-8-ol	1176		0.08			
trans-1,2-bis-(1-methylethenyl) cyclobutane		0.39			2.00	3.13
1,8-Cineole	1026	4.68	0.07	0.09	46.84	66.32
Tricyclene	0921				0.15	
β-*cis*-Ocimene	1017	0.64	0.03			
γ-Terpinene	1054	0.15	14.34	2.03		
δ-2-Carene	1001	0.19				
Linalool	1095	63.95	1.22	2.19	0.90	
α-Phellandrene	1002		0.14	0.14		
Camphene	0946		0.09	0.09	4.24	
Camphor	1141	0.38			16.30	
Borneol	1165	0.30	0.20	0.33	2.73	
Menthol	1167	0.57				
d-Alaninol						0.74
Terpinen-4-ol	1174	1.65	0.57	0.81	0.39	
α-Terpineol	1186	0.52	0.18	0.09	1.74	0.37
Anisole, p-allyl-		0.33				
2,4-Dimethylheptane						0.55
(R)-Citronellol	1223	0.17				
cis-Geraniol	1249	4.44				
α-Citral	1264	0.20				
2,5-Dimethylstyrene	1099		0.11			
Isothymol methyl ether	1232		0.17			
Thymol	1289		15.68	0.42		
Bornyl, acetate	1284	1.14			0.53	
3-Allylguaiacol		3.15				
Neryl acetate	1359	0.63				
l-Verbenone	1204				0.26	
β-Bourbonene	1387	0.19				
β-Elemene	1389	1.04				
Caryophyllene	1417	0.38	1.19	4.07	1.25	
Trans-α-Bergamotene	1432	3.53				
Carvacrol	1298		48.22	76.66	0.14	
α-Guaiene	1437	0.33				
α-Humulene	1452	0.44		0.15		
trans-Muurola-4(14),5-diene	1493	0.28				
trans-Pinocarveol	1135					1.70
D-Germacrene	1484	1.84				
β-Sesquiphellandrene	1521	0.17				
γ-Elemene	1434	0.20				
Caryophyllene oxide	1582		0.44	0.58	1.28	
α-Bulnesene	1509	0.50				
γ-Cadinene	1513	1.81				
δ-Cadinene	1522	0.39	0.05			
Nerolidol	1561	0.22				
α-Pinocarvone	1160					0.62
Diethyl phthalate	1590	1.28	0.69			5.17
Cubenol	1645	0.32				
γ -Muurolene	1478	2.03				
Ageratochromene	1658	0.22				
α-Thujene	0924		0.10			
Neryl propanoate	1452		0.16			
3-Carene	1008		0.07			
(+)-4-Carene			1.38	0.94		
(+)-Sylvestrene	1025		0.38	0.36		
cis-Sabinene hydrate	1065		0.05			
4a-Methyl 1,2,3,4,4a,5,6,7octahydro naphthalene			0.09	0.28		
2,6-Dimethyl-6-heptafluorobutyryloxyoctane						1.24
α-Terpinyl acetate	1346					1.03
Spathulenol	1577					1.51
Viridiflorol	1592					0.87
Bisphenol indane						0.42

Table 3
Chemical structure of main compounds identified in the EOs studied.

Plants	Main compound	Structure
O. compactum	Carvacrol	
E. globulus *R. officinalis*	Cineole	
	α-Pinene	
O. basilicum	Linalool	
T. vulgaris	Thymol	

showed toxicity at 50 and 100 µg/mL (data not shown), as for concentrations 6.25–25 µg/mL EOs did not show any toxicity effect (Fig. 2A). However, the nontoxic concentrations 6.25–25 µg/mL were chosen to be used later on for the determination of the protective effect of EOs.

3.4. Effect of EOs on cytotoxicity induced by H_2O_2

In order to investigate the effect of EOs on cytotoxicity induced by H_2O_2, a pretreatment of the cells with EOs was carried out. Results presented in (Fig. 2B) showed compared to the control cells, a significant decrease of viability to 54%± 2.01 ($P < 0.001$) when exposured to H_2O_2. The viability has been restored remarkably after pretreatment with the following EOs: basil (*O. basilicum*), thyme (*T. vulgaris*) and oregano (*O. compactum*) at different concentrations (6.25–25 µg/mL) by 11–26%, 13–30%, 16–47% respectively. As for rosemary (*R. officinalis*) and eucalyptus (*E. globulus*) the viability was slightly ameliorated at (25 µg/mL) by 10–11% compared to H_2O_2 alone treated cells.

3.5. Antioxidant enzymes activity

The investigation of the effect of H_2O_2 and the EOs on the activity of antioxidant enzymes in *S. cerevisiae* showed (Fig. 3) a significant increase in the activity after the exposure of *S. cerevisiae* cells to H_2O_2 compared to the control sample. Thus, the activity of CAT, SOD and GR were higher by ~ 281%, ~166% and ~ 146%, ($P < 0.001$) respectively. After the pretreatment with the EOs (6.25–25 µg/mL); thyme (*T. vulgaris*), oregano (*O. compactum*) and basil (*O. basilicum*) and the exposure to H_2O_2, the activity of the antioxidant enzymes CAT, SOD and GR decreased to be comparable to levels of control sample with the increase in dose of the essential oils. However, it was observed that the decrease of the activity after the treatment with rosemary (*R. officinalis*) and eucalyptus (*E. globulus*) was not significant.

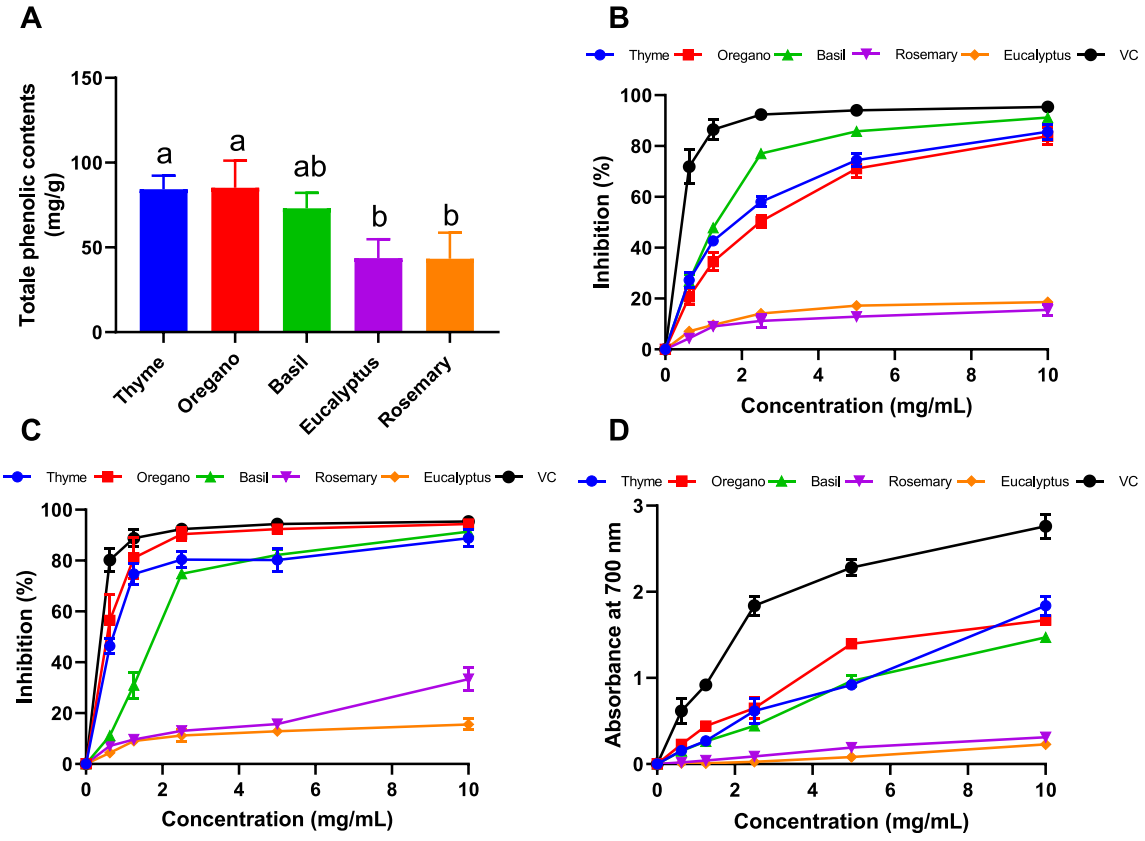


Fig.1. (A) Total phenol content (mg/g) and *in vitro* antioxidant activity of the EOs by (B) DPPH free radicals scavenging (C) ABTS free radicals scavenging and (D) Reducing power. Values are means ± SD of three independent experiments performed in triplicate. [a-b] Means without a common superscript letter differ ($p < 0.05$), as analyzed by one-way ANOVA.

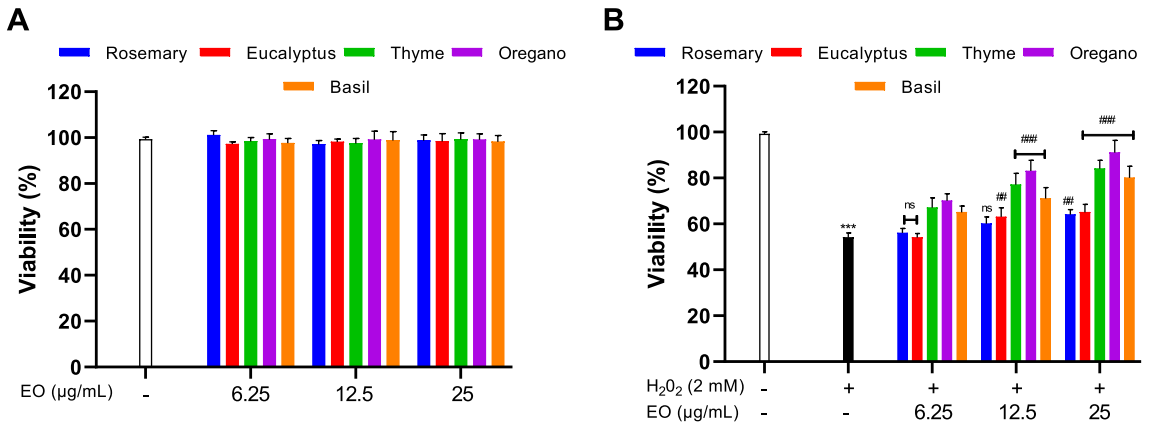


Fig. 2. (A) Dose response effect of the EOs on the viability of *S. cerevisiae* cells after 72 h of incubation; (B) Effect of EOs treatment on cellular survival after exposure to H_2O_2. *S. cerevisiae* cells were pretreated with the essential oils at different concentrations (6.25–100 µg/mL) for 1 h, followed by incubation with H_2O_2 for another hour. Data for 50 and 100 ug/ml were not shown. The data represent the mean of three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001, compared with untreated control cells, #P < 0.05, ##P < 0.01 and ###P < 0.001, compared with cells treated with H_2O_2 only.

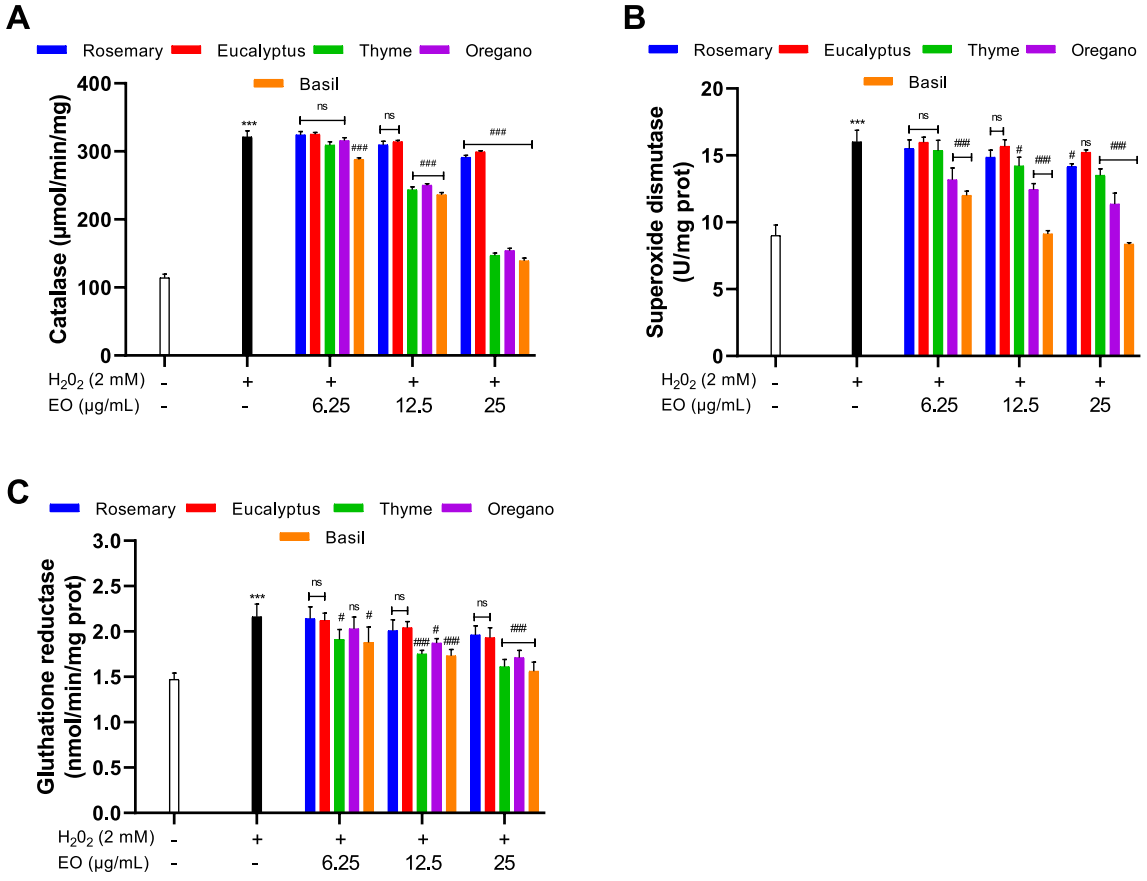


Fig.3. The effect of the essential oils on the activity of the antioxidant enzymes CAT (A), SOD (B) and GR (C) in *S. cerevisiae*. Yeast cells were pretreated with different concentrations of EOs (6.25–25 µg/ml) for 1 h, followed by incubation with H_2O_2 for another 1 h. The data represent the mean of three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001, compared with untreated control cells, #P < 0.05, ##P < 0.01 and ###P < 0.001, compared with cells treated with H_2O_2 only.

3.6. Lipid peroxidation

One of the consequences of oxidative stress is the damage of the intracellular components such as lipids. In this study, in order to investigate this intracellular damage, level of lipid peroxidation (LPO) was used as a marker (Fig. 4A). Thus, LPO was determined using the level of TBA-MDA adduct that showed a level of 0.61 ± 0 .070 nmol/mg of protein in the control sample. However, When the cells were exposed to H_2O_2 This level increased significantly to 1. 14 ± 0.075 nmol/mg of protein (P < 0.001), that to say 1.86-fold.

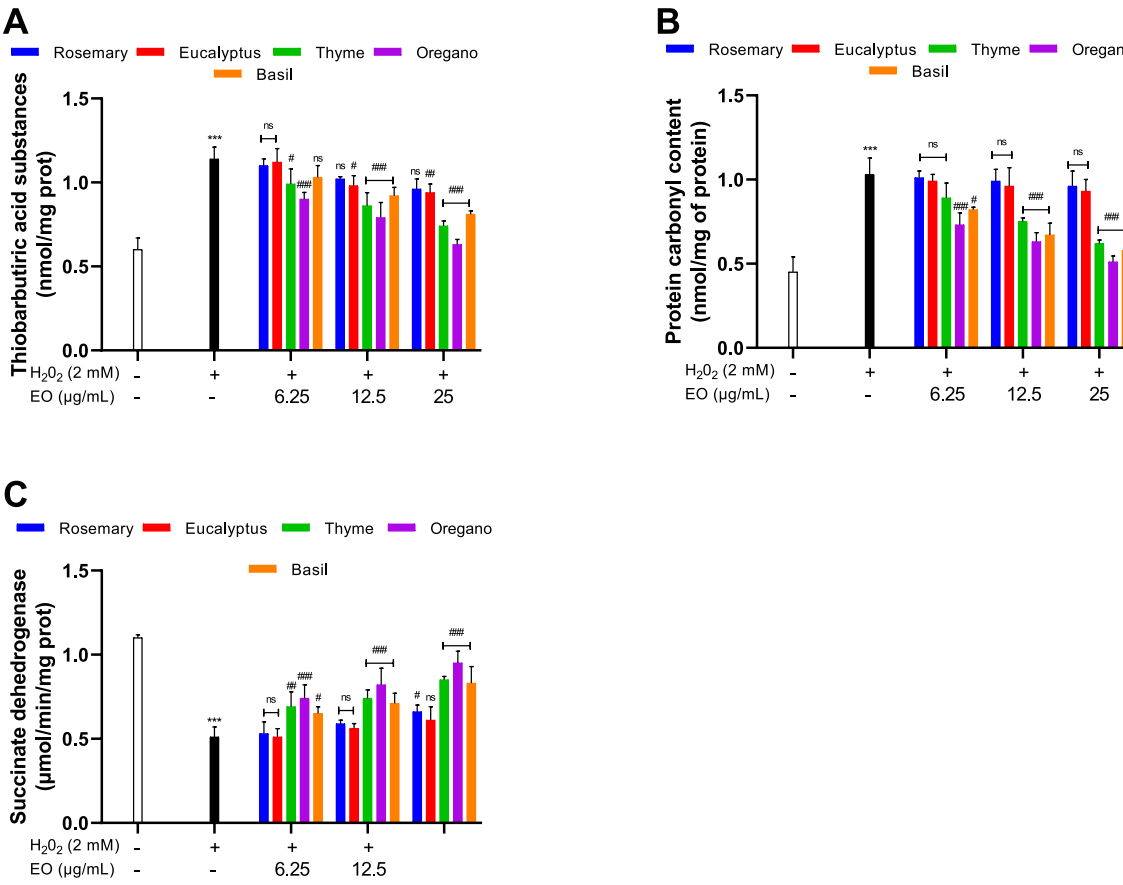


Fig.4. The effect of EOs on lipid peroxidation(A), PCO content (B) and (C) SDH activity in yeast cells treated with H_2O_2.The data represent the mean of three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001, compared with untreated control cells, #P < 0.05, ##P < 0.01 and ###P < 0.001, compared with cells treated with H_2O_2 only.

As for the pretreatment with EOs (6.25–25 µg/mL) showed that rosemary and eucalyptus decreased slightly at (25 µg/mL) the TBARS level by 1.18-fold (0.96 ± 0.066 nmol/mg of protein), and 1.21-fold (0.94 ± 0.050 nmol/mg of protein) respectively. Although, the treatment with the other EOs at (6.25–25 µg/mL); thyme, oregano and basil decreased significantly the TBARS level by 1.15–1.54-fold (0.99 ± 0.091–0.74 ± 0.031 nmol/mg of protein, P < 0.001), 1.26–1.80-fold (0.90 ± 0.04 – 0.63 ± 0.032 nmol/mg of protein, P < 0.001) and by 1.10–1.40-fold (1.03 ± 0.07 – 0.81 ± 0. 02 nmol/mg of protein, P < 0.001) respectively, compared to H_2O_2 treated cells.

3.7. Protein carbonyl content (PCO)

Results showed that the concentration of protein carbonyls was significantly higher by 2.3 fold (1.03 ± 0.1 nmol/mg of protein; P < 0. 001) in H_2O_2 treated cells compared to the control sample (0.452 ± 0.09 nmol/mg of protein). Levels of protein carbonyls decreased significantly in pretreated cells with EOs of oregano, basil and thyme at (6.25–25 µg/mL) in a dose dependent manner by 1.41–2.01fold (0.73 ± 0.072 – 0.51 ± 0.035 nmol/mg of protein), 1.25–1.77 fold (0.82 ± 0.015 – 0.58 ± 0.05 nmol/mg of protein) and by 1.15–1.66 fold (0.89 ± 0.084 – 0.62 ± 0.021 nmol/mg of protein) respectively, compared to H_2O_2 treated cells. Whilst, the pretreatment with EOs of rosemary and eucalyptus decreased protein carbonyls levels slightly at (25 µg/mL) by 1.07-fold (0.96 ± 0.09 n mol/mg of protein) and 1.10-fold (0.93 ± 0.07 nmol/ mg of protein) respectively, compared to H_2O_2 treated cells (Fig. 4B).

3.8. Metabolic enzyme activity (SDH)

In this work, the effect of H_2O_2 induced oxidative stress and EOs on the activity of succinate dehydrogenase (SDH) which is considered as a metabolic enzyme was also investigated (Fig. 4C). It was observed, compared to the control sample, that the activity decreased remarkably to 46.36% (P < 0.01) after the exposure to H_2O_2. The treatment with the EOs rosemary and eucalyptus showed a very slight increase in the activity of SDH at (12.5–25 µg/mL) by 1.04–1.29-fold (0.53 ± 0.07–0.66 ± 0.04 µmol/min/mg of protein) and 1–1.19-fold (0.51–0.61 µmol/min/mg of protein) respectively. However, the treatment with thyme, oregano and basil increased significantly the activity of SDH in a dose dependent manner to reach a value comparable to the control sample at (25 µg/mL).

4. Discussion

Excessive production of reactive oxygen species (ROS) can exceed the ability of the natural antioxidant defense system of the cells and, in long term, lead to the development of various oxidative stress-associated diseases such as cardiovascular diseases, diabetes, cancer, Alzheimer's disease (Huang et al., 2006; Martins et al., 2015; Valko et al., 2007). Therefore, antioxidants supplementation can help to maintain an optimal biological system by removing excessive concentrations of free radicals (Deetae et al., 2012; Guenaou et al., 2021b). In recent years, there has been a great interest in the use of phenolic compounds including phenolic acids and flavonoids derived from plants which are naturals, safe and powerful antioxidant molecules that exert their action through a variety of mechanisms including increasing antioxidant enzymes activity, chelating ions, eliminating ROS and inhibiting lipid peroxidation (Kumar Ganesan and Baojun Xu, 2017; Nakagawa et al., 2002). Hence, the aim of this study was to investigate the efficacy of five different essential oils in protecting against H_2O_2-induced oxidative stress in *S. cerevisiae* cells used as a cellular model.

Firstly, the results of GC/MS showed a chemical variability within tested EOs. It has been determined that the major chemical components of rosemary and eucalyptus were Cineole/ Eucalyptol and α-Pinene respectively. Linalool was the main component in basil, Carvacrol was the most present component in oregano as well as thymol, cymene and terpinene. As for thyme, the main components were thymol and caryophyllene. All of these components are behind many biological activities of EOs including antioxidant activity (Bhavaniramya et al., 2019; Dhifi et al., 2016). Also, we suggest that the higher content of phenolic molecules contribute greatly to the antioxidant activity observed, particularly, the main constituents such as carvacrol and thymol. In addition, it is admitted that the OH group found in linalool, thymol and carvacrol exhibited and showed very high antioxidant activity (Friedman, 2014). The investigation of the total phenolic content quantified using the Folin-Ciocalteau method showed high amounts were present in Oregano, basil and thyme. While for rosemary and eucalyptus, low amounts of phenols have been observed. It has been reported in previous studies that phenolic compounds were shown to have various biological effects such as antioxidant activity (Sanchez-Moreno, 2002). Furthermore, *in vitro* assays (ABTS, DPPH and reducing power) carried out to evaluate the antioxidant activity specifically free radicals scavenging ability of the EOs also indicated a significant and important antioxidant activity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals and a high reducing power in thyme, basil and oregano. Whereas, rosemary and eucalyptus were showed to be less capable of scavenging $ABTS^{\bullet+}$and $DPPH^{\bullet}$ free radicals and a low reducing power comparing to ascorbic acid used as standard sample. Therefore, these obtained results are in line with results reported in many previous studies in which cells were treated adding phenolic compounds and using different pro-oxidants, all showing a lower production of ROS than cells treated with pro-oxidants only (Flora et al., 2013; Meng et al., 2017). Phenolic compounds were reported to be involved in in neutralizing free radicals (Sanchez-Moreno, 2002).

A preliminary study on the cytotoxic effect of all the EOs on *S. cerevisiae* showed no cytotoxic effect at a range of concentrations (6.25–25 μg/mL), but at higher concentrations EOs were toxic (50 μg/mL and above). This may be explained, as a reversed effect of phenolic compounds that goes from being beneficial to lethal for cells due to excessive doses (McGaw et al., 2014). Also, the damages of H_2O_2 induced stress such as cell death have been prevented after the pretreatment with EOs showing a clearly restored cell viability after the use of EOs compared to cells treated with H_2O_2 only.

In order to control the ROS generation and protect cells from their oxidative damage, an enzymatic defense system where antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) work all together in a cooperative way. Hence, SOD catalyzes the conversion of superoxide anions to hydrogen peroxide (H_2O_2). CAT, efficient in scavenging H_2O_2, will later on catalyze the conversion of H_2O_2 to H_2O and O_2. As for GR, It is involved in the scavenging of ROS by providing consistently intermediate metabolites for instance reduced glutathione (GSH) that when present, GPx accelerates the reduction of hydrogen peroxide and hydroperoxides (Burke, 2010; Schieber and Chandel, 2014; Tongul and Tarhan, 2016). In our study, a significant increase in the activity of the antioxidant enzymes activity was observed when cells were exposed to H_2O_2 only compared to control. Interestingly, the pretreatment of yeast cells with of *T. vulgaris, O. compactum* and *O. basilicum* decreased in a dose dependent manner the activity of CAT, SOD and GR back to their normal values as compared control. This can be attributed to the phytochemical composition of EOs rich in phenolic compounds that are known to scavenge ROS (Sanchez-Moreno, 2002).

Lipid peroxidation which is caused by an excess of ROS that react with polyunsaturated fatty acids in cell membranes, leading to the production of malondialdehyde (MDA) that can eventually be used to determine the level of LPO in biological models (Jia et al., 2019; Parthasarathy et al., 1999). Enhanced MDA formation was previously observed in *S. cerevisiae* following oxidative stress induction (Meng et al., 2017; Piechowiak and Balawejder, 2019). The present study showed that the level of MDA increased significantly after H_2O_2 exposure compared to the control. The pretreatment with rosemary and eucalyptus had a very slight and non-significant effect on the level of TBA-MDA except at (25 μg/mL). Contrariwise, the pretreatment with the remaining EOs (oregano, basil and thyme) significantly reduced the level of LPO ($p < 0.001$) in a dose dependent manner near their normal level. This effect of EOs can be explained by the presence of phenolic compounds. It has been shown, that phenolic compounds are able to inhibit LPO in cells by producing stable phenoxy radical species that are not capable of any other radical reaction (Meng et al., 2017; Yan et al., 2020). They are able to inhibit LPO at all stages of the cycle, hence the reduction of the degree of the oxidative damage caused in cells constituents (Piechowiak and Balawejder, 2019). Also, this effect was noticed in treated yeast with propolis and quecertin in H_2O_2 induced stress (de Sá et al. 2013).

Protein carbonylation is associated with oxidative stress related damages. It is irreversible and is considered as one of the most damaging oxidative protein modifications. Protein carbonyl can be formed after oxidative modifications on arginine, proline, histidine and lysine residues, as well as by oxidative cleavage of the peptide chain. The measurement of protein carbonyl content is used to evaluate the degree of the cellular damages related to oxidative stress (Fedorova et al., 2014; Pirinccioglu et al., 2010). In our study, PCO level was remarkably higher in H_2O_2-treated cells compared to the control sample. Pretreatment with EOs (oregano, basil and thyme) showed a significant protective effect against oxidative damage and stabilizes the protein against oxidation. This can be explained by the antioxidant potential of phenolic compounds found in the EOs to suppress protein carbonylation (Kızıl et al., 2011).

Succinate dehydrogenase (SDH), is an enzyme of the respiratory chain present in the mitochondria of yeast cells. It catalyzes reactions leading to the oxidation of succinate to fumarate in Krebs cycle and the reduction of ubiquinone using the cofactor FAD (Robinson and Lemire, 1996; Rustin, 2002; Sadowska-Bartosz et al., 2013). SDH is considered as a metabolic marker used to evaluate oxidative stress (Mountassif et al., 2007). The expression of

SDH is important to produce energy in cells (Guenaou et al., 2021a; Rustin et al., 2002). Moreover, It has been reported in previous studies that H_2O_2 can inactivate and disable enzymes that contribute to the production of cellular energy (Tretter and Adam-Vizi, 2000). In the present study, a remarkable decrease in the SDH activity was observed in H_2O_2 stressed cells. The supplementation with EOs especially (oregano, basil and thyme) restored the activity of SDH which displays their antioxidant activity. This results suggest the contribution again of phenolic compounds present in the essential oils in this antioxidant defense since as It has been shown, phenolic compounds are involved in scavenging free radicals and decomposing peroxides... (Dai and Mumper, 2010; Meng et al., 2017; Piechowiak and Balawejder, 2019; Sanchez-Moreno, 2002).

5. Conclusion

We can conclude that the oxidative stress induced in yeast cells by using H_2O_2 has caused so many damages which was manifested in a high antioxidant enzymes activity in addition of lipid peroxidation and damages of cellular components. Yet the use of the essential oils of *T. vulgaris, O. compactum* and *O. basilicum* as a natural source of antioxydants displayed a protective potential against the oxidative stress in yeast cells. Eventually, after the pretreatment with EOs, the activity of antioxidant and metabolic enzymes was brought back to normal values as well as a reduced lipid peroxidation and protein carbonylation. To sum up, further research could be conducted on these efficient oils that can be of great use to protect organisms against the serious damages and diseases associated with oxidative stress.

CRediT authorship contribution statement

Khadija Ridaoui: Conceptualization, Writing – original draft. **Ismail Guenaou:** Conceptualization, Formal analysis. **Ikram Taouam:** Conceptualization. **Mounia Cherki:** Conceptualization. **Noureddine Bourhim:** Conceptualization. **Abdelaziz Elamrani:** Methodology, Investigation. **Mostafa Kabine:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are very grateful for the Naturactive Laboratoires Pierre Fabre France, for the made available the precious essential oils. The support from the National Center for Scientific and Technical Research (CNRST) within the Research Excellence Scholarship Program is gratefully acknowledged.

Consent to Publish We confirm that the manuscript has been read and approved and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved.

References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectorscopy. Allured Pub. Corp, Carol Stream, Ill.

Aebi, H., 1984. Catalase in vitro, in: Methods in Enzymology. Elsevier, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Allan Butterfield, D., 2002. Amyloid β-peptide (1–42)-induced Oxidative Stress and Neurotoxicity: Implications for Neurodegeneration in Alzheimer's Disease Brain. A Review. Free Radical Res. 36 (12), 1307–1313. https://doi.org/10.1080/1071576021000049890.

Aouacheri, O., Saka, S., Krim, M., Messaadia, A., Maidi, I., 2015. The Investigation of the Oxidative Stress-Related Parameters in Type 2 Diabetes Mellitus. Can. J. Diabetes 39, 44–49. https://doi.org/10.1016/j.jcjd.2014.03.002.

Bahoran, T., Soobrattee, M.A., Luximon-Ramma, V., Aruoma, O.I., 2007. Free Radicals and Antioxidants in Cardiovascular Health and Disease. Internet J. Med. Update – J. 1. https://doi.org/10.4314/ijmu.v1i2.39839.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M.S., Vijayakumar, R., Baskaran, D., 2019. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain Oil Sci. Technol. 2 (2), 49–55. https://doi.org/10.1016/j.gaost.2019.03.001.

Bouyahya, A., Jamal, A., 2016. Origanum compactum Benth: A Review on Phytochemistry and Pharmacological Properties. Med. Aromatic Plants 05 (04). https://doi.org/10.4172/2167-041210.4172/2167-0412.1000252.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1-2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Burke, K.E., 2010. Photoaging: the role of oxidative stress. G Ital Dermatol Venereol 145, 445–459.

Caramori, G., 2004. Oxidants and asthma. Thorax 59 (2), 170–173. https://doi.org/10.1136/thorax.2002.002477.

Castro, F.A.V., Herdeiro, R.S., Panek, A.D., Eleutherio, E.C.A., Pereira, M.D., 2007. Menadione stress in Saccharomyces cerevisiae strains deficient in the glutathione transferases. Biochimica et Biophysica Acta (BBA) - General Subjects 1770, 213–220. https://doi.org/10.1016/j.bbagen.2006.10.013.

Dai, J., Mumper, R.J., 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 15, 7313–7352. https://doi.org/10.3390/molecules15107313.

Dani, C., Bonatto, D., Salvador, M., Pereira, M.D., Henriques, J.A.P., Eleutherio, E., 2008. Antioxidant Protection of Resveratrol and Catechin in *Saccharomyces cerevisiae*. J. Agric. Food. Chem. 56 (11), 4268–4272. https://doi.org/10.1021/jf800752s.

Deetae, P., Parichanon, P., Trakunleewatthana, P., Chanseetis, C., Lertsiri, S., 2012. Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas. Food Chem. 133 (3), 953–959. https://doi.org/10.1016/j.foodchem.2012.02.012.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., Mnif, W., 2016. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines 3, 25. https://doi.org/10.3390/medicines3040025.

Di Ilio, C., Polidoro, G., Arduini, A., Muccini, A., Federici, G., 1983. Glutathione peroxidase, glutathione reductase, glutathione S-transferase, and γ-glutamyltranspeptidase activities in the human early pregnancy placenta. Biochem. Med. 29 (2), 143–148. https://doi.org/10.1016/0006-2944(83)90034-0.

Dorman, H.J.D., Bachmayer, O., Kosar, M., Hiltunen, R., 2004. Antioxidant Properties of Aqueous Extracts from Selected Lamiaceae Species Grown in Turkey. J. Agric. Food. Chem. 52 (4), 762–770. https://doi.org/10.1021/jf034908v.

Dröge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82 (1), 47–95. https://doi.org/10.1152/physrev.00018.2001.

El-Nekeety, A.A., Mohamed, S.R., Hathout, A.S., Hassan, N.S., Aly, S.E., Abdel-Wahhab, M.A., 2011. Antioxidant properties of Thymus vulgaris oil against aflatoxin-induce oxidative stress in male rats. Toxicon 57 (7-8), 984–991. https://doi.org/10.1016/j.toxicon.2011.03.021.

Fedorova, M., Bollineni, R.C., Hoffmann, R., 2014. Protein carbonylation as a major hallmark of oxidative damage: Update of analytical strategies: PROTEIN CARBONYLATION: AN ANALYTICAL UPDATE. Mass Spec. Rev. 33, 79–97. https://doi.org/10.1002/mas.21381.

Flora, S.J.S., Shrivastava, R., Mittal, M., 2013. Chemistry and Pharmacological Properties of Some Natural and Synthetic Antioxidants for Heavy Metal Toxicity. Curr. Med. Chem. 20, 4540–4574. https://doi.org/10.2174/09298673113209990146.

Friedman, M., 2014. Chemistry and Multibeneficial Bioactivities of Carvacrol (4-Isopropyl-2-methylphenol), a Component of Essential Oils Produced by Aromatic Plants and Spices. J. Agric. Food. Chem. 62 (31), 7652–7670. https://doi.org/10.1021/jf5023862.

Galle, J., 2001. Oxidative stress in chronic renal failure. Nephrol. Dial. Transplant. 16, 2135–2137. https://doi.org/10.1093/ndt/16.11.2135.

Guenaou, I., Hmimid, F., Lahlou, F.A., Errami, A., Irahal, I.N., Fahde, S., Ouafik, '., Bourhim, N., 2021a. Cytoprotective effect of ethyl acetate fraction from Ephedra fragilis on H2O2-induced oxidative damage in Tetrahymena pyriformis. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 239, 108899. https://doi.org/10.1016/j.cbpc.2020.108899.

Guenaou, I., Nait Irahal, I., Errami, A., Lahlou, F.A., Hmimid, F., Bourhim, N., 2021b. Bioactive Compounds from Ephedra fragilis: Extraction Optimization, Chemical Characterization, Antioxidant and AntiGlycation Activities. Molecules 26, 5998. https://doi.org/10.3390/molecules26195998.

Halliwell, B., 2008. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch. Biochem. Biophys., Highlight Issue: Polyphenol Health 476 (2), 107–112. https://doi.org/10.1016/j.abb.2008.01.028.

Huang, Y.-H., Shih, C.-M., Huang, C.-J., Lin, C.-M., Chou, C.-M., Tsai, M.-L., Liu, T.P., Chiu, J.-F., Chen, C.-T., 2006. Effects of cadmium on structure and enzymatic

activity of Cu, Zn-SOD and oxidative status in neural cells. J. Cell. Biochem. 98 (3), 577–589. https://doi.org/10.1002/(ISSN)1097-464410.1002/jcb.v98:310.1002/jcb.20772.

Jamieson, D.J., 1998. Oxidative stress responses of the yeastSaccharomyces cerevisiae. Yeast 14, 1511–1527. https://doi.org/10.1002/(SICI)1097-0061(199812)14:16<1511::AID-YEA356>3.0.CO;2-S.

Jia, R., Li, Y., Cao, L., Du, J., Zheng, T., Qian, H., Gu, Z., Jeney, G., Xu, P., Yin, G., 2019. Antioxidative, anti-inflammatory and hepatoprotective effects of resveratrol on oxidative stress-induced liver damage in tilapia (Oreochromis niloticus). Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 215, 56–66. https://doi.org/10.1016/j.cbpc.2018.10.002.

Kaurinovic, B., Popovic, M., Vlaisavljevic, S., Trivic, S., 2011. Antioxidant Capacity of Ocimum basilicum L. and Origanum vulgare L. Extracts. Molecules 16, 7401–7414. https://doi.org/10.3390/molecules16097401.

King, T.E., 1967. Preparations of succinate—cytochrome c reductase and the cytochrome b-c1 particle, and reconstitution of succinate-cytochrome c reductase. Methods Enzymol. Elsevier, 216–225.

Kızıl, G., Kızıl, M., Çeken, B., Yavuz, M., Demir, H., 2011. Protective Ability of Ethanol Extracts of *Hypericum Scabrum* L. and *Hypericum Retusum* Aucher Against the Protein Oxidation and DNA Damage. Int. J. Food Prop. 14 (4), 926–940. https://doi.org/10.1080/10942910903491181.

Ganesan, K., Baojun, X.u., 2017. A Critical Review on Polyphenols and Health Benefits of Black Soybeans. Nutrients 9, 455. https://doi.org/10.3390/nu9050455.

Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 15, 71.

Levine, R.L., 2002. Carbonyl modified proteins in cellular regulation, aging, and disease2,3 2Guest Editor: Earl Stadtman 3This article is part of a series of reviews on "Oxidatively Modified Proteins in Aging and Disease". The full list of papers may be found on the homepage of the journal. Free Radical Biol. Med. 32 (9), 790–796. https://doi.org/10.1016/S0891-5849(02)00765-7.

Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M., Ferreira, I.C.F.R., 2015. Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. Food Chem. 167, 131–137. https://doi.org/10.1016/j.foodchem.2014.06.094.

McGaw, L.J., Elgorashi, E.E., Eloff, J.N., 2014. Cytotoxicity of African medicinal plants against normal animal and human cells. In: Toxicological Survey of African Medicinal Plants. Elsevier, pp. 181–233. https://doi.org/10.1016/B978-0-12-800018-2.00008-X.

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

Mountassif, D., Kabine, M., Manar, R., Bourhim, N., Zaroual, Z., Latruffe, N., El Kebbaj, M.S., 2007. Physiological, morphological and metabolic changes in Tetrahymena pyriformis for the in vivo cytotoxicity assessment of metallic pollution: Impact on d-β-hydroxybutyrate dehydrogenase. Ecol. Ind. 7 (4), 882–894.

Nait Irahal, I., azzahra Lahlou, F., Hmimid, F., Errami, A., Guenaou, I., Diawara, I., Kettani-Halabi, M., Fahde, S., Ouafik, L., Bourhim, N., 2021. Identification of the chemical composition of six essential oils with mass spectroscopy and evaluation of their antibacterial and antioxidant potential. Flavour Fragr J 36 (4), 465–476. https://doi.org/10.1002/ffj.v36.410.1002/ffj.3657.

Nait Irahal, I., Hmimid, F., Lahlou, F., Errami, A., Guenaou, I., Diawara, I., Kettani-Halabi, M., Fahd, S., Ouafik, L., Bourhim, N., 2020. Chemical composition, antibacterial and antioxidant activities of some essential oils against multidrug resistant bacteria. Eur. J. Integrat. Med. 35, 101074. https://doi.org/10.1016/j.eujim.2020.101074.

Nakagawa, T., Yokozawa, T., Terasawa, K., Shu, S., Juneja, L.R., 2002. Protective Activity of Green Tea against Free Radical- and Glucose-Mediated Protein Damage. J. Agric. Food. Chem. 50 (8), 2418–2422. https://doi.org/10.1021/jf011339n.

Oyaizu, M., 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Japan. J. Nutr. Dietetics 44 (6), 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.

Paoletti, F., Aldinucci, D., Mocali, A., Caparrini, A., 1986. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal. Biochem. 154 (2), 536–541.

Parthasarathy, S., Santanam, N., Ramachandran, S., Meilhac, O., 1999. Oxidants and antioxidants in atherogenesis: an appraisal. J. Lipid Res. 40 (12), 2143–2157. https://doi.org/10.1016/S0022-2275(20)32089-7.

Piechowiak, T., Balawejder, M., 2019. Onion skin extract as a protective agent against oxidative stress in *Saccharomyces cerevisiae* induced by cadmium. J. Food Biochem. 43 (7). https://doi.org/10.1111/jfbc.2019.43.issue-710.1111/jfbc.12872.

Pirinccioglu, A.G., Gökalp, D., Pirinccioglu, M., Kizil, G., Kizil, M., 2010. Malondialdehyde (MDA) and protein carbonyl (PCO) levels as biomarkers of oxidative stress in subjects with familial hypercholesterolemia. Clin. Biochem. 43 (15), 1220–1224. https://doi.org/10.1016/j.clinbiochem.2010.07.022.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., 2017. Oxidative Stress: Harms and Benefits for Human Health. Oxid. Med. Cell. Longevity 2017, 1–13. https://doi.org/10.1155/2017/8416763.

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., Gnanadhas, E.N., Lakshminarasaiah, U., Gopas, J., Nishigaki, I., 2014. Antioxidants and human diseases. Clin. Chim. Acta 436, 332–347. https://doi.org/10.1016/j.cca.2014.06.004.

Robinson, K.M., Lemire, B.D., 1996. Covalent Attachment of FAD to the Yeast Succinate Dehydrogenase Flavoprotein Requires Import into Mitochondria, Presequence Removal, and Folding. J. Biol. Chem. 271 (8), 4055–4060. https://doi.org/10.1074/jbc.271.8.4055.

Rustin, P., 2002. Mitochondria, from cell death to proliferation. Nat. Genet. 30 (4), 352–353. https://doi.org/10.1038/ng0402-352.

Rustin, P., Munnich, A., Rötig, A., 2002. Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. Eur. J. Hum. Genet. 10 (5), 289–291. https://doi.org/10.1038/sj.ejhg.5200793.

de Sá, R.A., de Castro, F.A.V., Eleutherio, E.C.A., de Souza, R.M., da Silva, J.F.M., Pereira, M.D., 2013. Brazilian propolis protects Saccharomyces cerevisiae cells against oxidative stress. Braz. J. Microbiol. 44, 993–1000. https://doi.org/10.1590/S1517-83822013005000062.

Sadowska-Bartosz, I., Pączka, A., Mołoń, M., Bartosz, G., 2013. Dimethyl sulfoxide induces oxidative stress in the yeast "Saccharomyces cerevisiae". FEMS Yeast Res. 13 (8), 820–830. https://doi.org/10.1111/fyr.2013.13.issue-810.1111/1567-1364.12091.

SAMOKYSZYN, V., MARNETT, L., 1990. Inhibition of liver microsomal lipid peroxidation by 13-cis-retinoic acid. Free Radic. Biol. Med. 8 (5), 491–496.

Sanchez-Moreno, C., 2002. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. Food Sci. Technol. Int. 8 (3), 121–137. https://doi.org/10.1106/108201302026770.

Santos-Buelga, C., Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. J. Sci. Food Agric. 80, 1094–1117. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1094::AID-JSFA569>3.0.CO;2-1.

Sato, H., Shibata, M., Shimizu, T., Shibata, S., Toriumi, H., Ebine, T., Kuroi, T., Iwashita, T., Funakubo, M., Kayama, Y., Akazawa, C., Wajima, K., Nakagawa, T., Okano, H., Suzuki, N., 2013. Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. Neuroscience 248, 345–358. https://doi.org/10.1016/j.neuroscience.2013.06.010.

Satyal, P., Jones, T., Lopez, E., McFeeters, R., Ali, N., Mansi, I., Al-kaf, A., Setzer, W., 2017. Chemotypic Characterization and Biological Activity of Rosmarinus officinalis. Foods 6, 20. https://doi.org/10.3390/foods6030020.

Schieber, M., Chandel, N., 2014. ROS Function in Redox Signaling and Oxidative Stress. Curr. Biol. 24 (10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Viticulture 28, 49–55.

Tongul, B., Tarhan, L., 2016. Oxidant and antioxidant status in Saccharomyces cerevisiae exposed to antifungal ketoconazole. Process Biochem. 51 (12), 1984–1991. https://doi.org/10.1016/j.procbio.2016.08.016.

Tran, K., Green, E., 2019. Assessing Yeast Cell Survival Following Hydrogen Peroxide Exposure. BIO-PROTOCOL 9. https://doi.org/10.21769/BioProtoc.3149.

Tretter, L., Adam-Vizi, V., 2000. Inhibition of Krebs cycle enzymes by hydrogen peroxide: a key role of α-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J. Neurosci. 20 (24), 8972–8979.

Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., Telser, J., 2004. Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem. 266 (1/2), 37–56. https://doi.org/10.1023/B:MCBI.0000049134.69131.89.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39 (1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001.

Wu, Z., Tan, B., Liu, Y., Dunn, J., Martorell Guerola, P., Tortajada, M., Cao, Z., Ji, P., 2019. Chemical Composition and Antioxidant Properties of Essential Oils from Peppermint, Native Spearmint and Scotch Spearmint. Molecules 24, 2825. https://doi.org/10.3390/molecules24152825.

Yan, Z., Zhong, Y., Duan, Y., Chen, Q., Li, F., 2020. Antioxidant mechanism of tea polyphenols and its impact on health benefits. Anim. Nutr. 6 (2), 115–123. https://doi.org/10.1016/j.aninu.2020.01.001.