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Lifespan-extending Effects of *Prunus mahaleb* L. Extracts in a Yeast Model: Comparison with Extreme-Caloric Restriction

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HIGHLIGHTS

- Mahaleb extracts exhibited hormetic effects in yeast growth.
- Mahaleb extracts showed phase-dependent effects in proliferating and resting yeast cells.
- Water and methanol extracts of mahaleb significantly extended yeast lifespan.
- Polar extracts outperformed E-CR and willow bark in extending chronological lifespan.

Abstract: Plant-derived natural products have attracted considerable interest for their potential to promote longevity and healthy aging through bioactive phytochemicals. This study evaluated the effects of *Prunus mahaleb* L. leaf and seed extracts on the chronological lifespan of *Saccharomyces cerevisiae*. Extracts of *Salix alba* L. bark, a known geroprotective plant, were used as a comparative control. Various solvent-based extracts were prepared and tested for cytotoxicity, growth modulation, and longevity-enhancing effects. Extreme-calorie restriction (E-CR) conditions were employed as a reference intervention. The cytotoxicity of extracts varied by solvent polarity, with methanol and water extracts showing low toxicity at concentrations up to 10 mg/mL, while hexane extracts, especially from mahaleb leaves, exhibited high toxicity with LC_{50} values as low as 0.54 mg/mL. Acetone extracts displayed intermediate toxicity profiles. In lifespan assays, polar extracts from both leaves and seeds significantly extended lifespan, exceeding the effects of both E-CR and *S. alba* extracts. Acetone extracts also improved survival at low doses, while hexane extracts showed limited or no benefit. GC-MS profiling identified coumarin, squalene, and phytol among the potentially active compounds. These findings demonstrate that *P. mahaleb* extracts can enhance yeast lifespan under nutrient-deprived conditions without inducing cytotoxicity and support their potential as plant-based candidates for future geroprotective strategies. Further studies are needed to elucidate the underlying molecular mechanisms and to evaluate their effects in higher organisms.

Keywords: Bioactive compounds; Chronological lifespan; Geroprotectors; *Prunus mahaleb*; *Salix alba.*

INTRODUCTION

Aging is a progressive and complex biological process regulated by genetic, metabolic, and environmental factors. With increasing global life expectancy, the need for safe and effective interventions that promote healthy aging has gained significant attention. One promising avenue is the use of plant-derived natural compounds, which have been shown to delay aging and reduce age-related cellular decline by modulating oxidative stress, inflammation, and metabolic pathways.

Natural phytochemicals with reported antioxidant, anti-inflammatory, and metabolic regulatory properties have been shown to influence aging pathways and extend lifespan in various model organisms. Among these, *Saccharomyces cerevisiae* serves as a widely used model due to its well-characterized aging pathways, genetic tractability, and conserved molecular mechanisms of aging. Yeast aging can be studied using complementary models: the replicative lifespan (RLS), which measures the number of daughter cells produced by a mother cell, and the chronological lifespan (CLS), which reflects the survival of non-dividing, stationary-phase cells. Key signaling pathways regulate yeast aging, including the Ras/PKA and TOR/Sch9 pathways [1,2]. Caloric restriction (CR) is another factor known to enhance yeast longevity, sometimes exhibiting effects comparable to or even surpassing those of genetic modifications [3]. After undergoing a diauxic shift, yeast cells enter the stationary phase (G_0 quiescent state), a condition similar to CR, where chronological aging begins. Transferring yeast cells from a non-CR condition to water (a hypometabolic state), a process termed 'extreme CR', has been observed to significantly extend CLS in multiple yeast strains [4-6]. CR mimetics (CRMs), such as rapamycin and resveratrol, imitate CR effects and thereby contribute to lifespan extension [7,8].

Geroprotectors are compounds that modulate aging mechanisms, slowing age-related deteriorations, and enhancing lifespan [9]. Phytochemicals, including polyphenols, flavonoids, and coumarins, act as geroprotectors by promoting longevity and age-related diseases [10,11]. Databases such as Geroprotectors.org, Aging Atlas, and DrugAge catalog lifespan-extending chemicals in model organisms, including *S. cerevisiae* [12,13]. Notably, these databases include thirty-seven compounds, such as caffeine, resveratrol, and rapamycin, that have been associated with yeast lifespan modulation. The anti-aging effects of plant-derived compounds have been extensively studied and reviewed [14,15]. Oxidative stress, primarily induced by excessive reactive oxygen species (ROS), triggers the upregulation of antioxidant proteins, that help neutralize ROS and consequently extend lifespan [16]. The antioxidative properties of plant-derived molecules are widely believed to have their anti-aging potential [11,17-19].

Prunus mahaleb L. (mahaleb), a member of the Rosaceae family, is of particular interest due to its rich phytochemical composition and traditional medicinal uses for treating pain, diabetes, kidney stones, and gastrointestinal disorders. The phytochemical content of mahaleb varies depending on the plant part and extraction method, with high concentrations of phenolic compounds such as glycosides, anthocyanins, coumarin, and quercetin derivatives [20-24]. Depending on the part used, mahaleb leaves, seeds, and kernels have demonstrated significant antioxidant and antimicrobial properties [22,25,26]. Recent findings indicate that mahaleb fruit and kernel extracts exhibit dose-dependent growth-inhibitory effects on melanoma cells [25].

Previous studies have shown that coumarin derivatives can extend lifespan in *Caenorhabditis elegans* and modulate aging-related pathways such as sirtuin inhibition in *S. cerevisiae* [27,28]. Although antioxidant, antimicrobial, and anticancer activities of *P. mahaleb* have been reported in other biological systems, its potential effects on aging and longevity have not yet been investigated in any model organism. This study presents the first systematic evaluation of *P. mahaleb* leaf and seed extracts in a yeast CLS model. Solvent-based extracts were assessed for cytotoxicity, growth dynamics, and survival during stationary phase. White willow (*Salix alba* L.) bark, previously shown to prolong lifespan in yeast, was included as a phytochemical comparator. Both *P. mahaleb* and *S. alba* extracts were evaluated under extreme caloric restriction (E-CR) conditions, enabling a direct comparison of their lifespan-extending potential in a nutrient-deprived environment. In addition, GC-MS profiling was performed to identify candidate bioactive compounds that may contribute to the observed biological outcomes. These combined approaches aim to explore the potential of *P. mahaleb* as a novel plant-based modulator of lifespan.

MATERIAL AND METHODS

Yeast strains and growth conditions

Saccharomyces cerevisiae BY4741 strain (*MATa, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) (EUROSCARF, Frankfurt, Germany) was used. Yeast cells were cultured at 30°C in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) or in minimal YNBD medium (0.67% Yeast nitrogen base without amino acids, 0.5% ammonium sulfate, 2% dextrose) supplemented with the appropriate amino acids.

Plant materials and extraction

Prunus mahaleb samples (leaves and seeds) were collected from Tokat, Turkey in July 2021 (GPS coordinates: 40° 12' 34.0344" North and 36° 28' 51.0456" East). *S. alba* samples were collected from Bursa, Turkey in June 2021 (GPS coordinates: 40° 40° 01' 01.3" North and 28° 15' 24.7" East). The samples were dried and powdered. Sequential extraction was performed using Soxhlet apparatus with n-hexane, acetone, methanol, and distilled water (8 h/solvent). Extracts were filtered, concentrated using a rotary evaporator, dissolved in dimethyl sulfoxide (DMSO), and stored at 4°C. Working concentrations of plant extracts (PE) were prepared by DMSO dilution.

Cytotoxicity assays

Cell viability

Yeast cells were grown to mid-log phase (OD_{600}≈0.6) and treated with extracts at 0.2-10.0 mg/mL. Cell viability was assessed using methylene blue staining [30]. Methylene blue (MB) is commonly used to distinguish live and dead cells: viable cells either exclude MB or reduce it to a colorless form, whereas non-viable cells retain the blue stain. All cultures were incubated at 30°C with shaking at 120 rpm for 24 hours. Live and dead cells were counted at 12 and 24 hours using a hemocytometer under a microscope. Cell viability percentage was calculated for each concentration using the formula: Cell viability % = (Number of live cells / Total number of cells) × 100.

Mortality and LC_{50} quantification

The mortality percentage and lethal concentration (LC_{50}) of PE on yeast growth were determined using the live-dead cell counting method [30]. Mortality percentage at each concentration was calculated using the formula: Mortality (%)=(Number of dead cell / Total number of cell) × 100. These mortality values were used to generate probit scores, and regression analysis was performed to determine the LC_{50} values of the extracts. The LC_{50} represents the concentration required to cause 50% mortality in the yeast population.

Growth inhibition assay

The inhibitory effects of extracts on yeast growth were evaluated based on previously reported protocols [18,29]. Yeast cultures in log-phase were diluted to OD_{600}≈0.01 and incubated with extracts in a 96-well plate (MultiskanTM FC Microplate Photometer) at 27°C. OD_{600} was measured every 30 minutes for 24 hours. Growth inhibition was calculated based on area under the curve (AUC) relative to untreated control: Growth (%) = ($AUC_{treated}$ / $AUC_{control}$) × 100.

Long-term toxicity assay

To assess chronic toxicity, stationary-phase yeast cells were treated with non-toxic extract concentrations (based on LC_{50} and viability assays). Cells were incubated at 30°C with constant shaking for 8 days. Samples were collected every 2 days, stained with MB, and examined under a microscope using a hemocytometer to count live and dead cells. The toxicity percentage (T%) was calculated as: T (%) = (Number of dead cells / Total number of cells) × 100. Subsequently, the toxicity index (TI) was calculated for each extract concentration used in the CLS assay using formula: $TI=T_{treated} / T_{control}$.

Chronological lifespan assay

The effect of PE on yeast aging was evaluated using an outgrowth-based CLS assay, adopted from previously described microplate- and bottle-based methods [4,31]. Yeast cultures were grown to stationary phase and transferred into sterile distilled water containing appropriate concentration of extracts. This time point was designated as Day 0 of chronological aging, and viability at this time point was defined as 100%. At each subsequent time point, 60 µL of the aging culture was transferred into 540 µL of fresh YPD medium (distributed as 200 µL/well) and recording OD_{600} every 30 minutes for 24 hours. The resulting data were normalized and used to generate outgrowth curves which were used to generate survival curves and computing survival integrals (SI), representing the area under the curve, as previously described [11]. The survival percentage (S_n) on each aging day was calculated as the time delay (Δt_n) for the aged culture to reach OD_{600}=0.3, normalized by the doubling time (δ) of the corresponding Day 0 outgrowth curve, using the formula: S_n =½ × (Δt_n / δ) × 100. Doubling time (δ) was calculated from the exponential phase of the Day 0 outgrowth curve by evaluating consecutive OD_{600} readings between 0.2-0.5. It was computed using the

formula: $\delta = \ln(2) / [(\ln(OD_2) - \ln(OD_1)) / (t_2 - t_1)]$, where OD_1 and OD_2 are successive absorbance readings and t_1 and t_2 are the corresponding time points. Multiple δ values were calculated per replicate, and the mean value was used. Survival percentages were averaged across replicates and plotted as survival curves over the aging period. To quantify cumulative viability, the survival integral (SI) was calculated using the trapezoidal rule as follows: $SI = \Sigma [(S_{n-2} + S_n) / 2] \times [(Day_n - Day_{n-2})]$, where the survival values at each aging day (n) and the value two intervals earlier (n-2) were used to estimate the area under the curve between time points. Sampling was conducted every 2 days for the first 16 days, followed by every 6 days thereafter, until the viability declined below 5-10% in at least one extract-treated or control culture.

GC-MS analysis of extracts

The Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the bioactive compounds in PE that may have the potential to affect yeast viability and aging. GC-MS analysis was performed using the Shimadzu GC-MS QP 2010 Ultra Gas Chromatography Mass Spectrometry (Kyoto, Japan). A Restek RXI-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm) was employed as the analytical column. Helium was used as a carrier gas at a constant flow rate of 1.5 mL/min. A 1 µL aliquot of each PE sample was injected in split mode. The injection port temperature was set to 250°C, while the ion source and interface temperatures were maintained at 200°C and 250°C, respectively. The oven temperature was initially set at 40ºC for 3 min, then gradually increased to 240°C at a rate of 4ºC/min. The total analysis time was 53 min. Compound identification was carried out using the Wiley W9N11 mass spectral library and by comparing retention indices.

Statistics

All assays were performed in biological triplicates. Data were analyzed using one-way-ANOVA with Tamhane's T2 post-hoc test in SPSS 22. Results were presented as mean ± SD. Differences were considered significant at $p<0.05$.

RESULTS

Effect of mahaleb extracts on cell viability

Viability of yeast cells exposed to increasing concentrations (0.2-80 mg/mL) of *P. mahaleb* and *S. alba* extracts were assessed at 12 and 24 hours using MB staining. Hexane extract of mahaleb leaves (ML-H) showed high toxicity, with viability dropping from 85% to 1% at 5 mg/mL and becoming undetectable at higher doses. In contrast, methanol (ML-M) and water (ML-W) extracts preserved >90% viability up to 10 mg/mL. Acetone extracts (ML-A) displayed moderate toxicity, decreasing viability to 24% at 10 mg/mL. (Figure 1a). A comparable pattern was observed with mahaleb seed extracts. Acetone extracts (MS-A) displayed the highest toxicity, while the hexane extract (MS-H) was better tolerated, maintaining moderate viability even at elevated concentrations. Methanol (MS-M) and water (MS-W) extracts preserved high cell viability up to 10 mg/mL, although MS-H yielded slightly higher survival than MS-W at equivalent doses (Figure 1b). For *S. alba* extracts, the water extract (WB-W) showed strong cytotoxicity, reducing viability to below 10% at 10 mg/mL, while the methanol extract (WB-M) retained moderate viability, remaining above 60%. In contrast, both hexane (WB-H) and acetone (WB-A) extracts exhibited dose-dependent cytotoxicity, with viability dropping significantly above 5 mg/mL (Figure 1c).

Mortality assessment and LC_{50} estimation

To compare relative toxicity among the tested extracts, LC_{50} values were used as reference points. Among mahaleb leaf extracts, ML-H was the most cytotoxic (LC_{50}=0.54 mg/mL), while ML-M exhibited the lowest toxicity (LC_{50}=2456.6 mg/mL) (Table 1). This solvent-dependent pattern was also observed in seed extracts, where MS-A showed higher toxicity than MS-M. A similar trend emerged for willow bark extracts, with the water extract demonstrating higher toxicity than the methanol extracts. These results collectively suggest that extract polarity plays a key role in determining cytotoxicity profiles in yeast cells.

Growth inhibition profiles

The growth inhibition assay was performed by monitoring OD_{600} for 24 hours to evaluate how plant extracts affect yeast proliferation over time. ML-H inhibited yeast growth in a dose-dependent manner, reducing growth by 70% at 1 mg/mL. ML-M and ML-W showed growth stimulation at low concentrations (up

to 52% and 26%, respectively), followed by moderate inhibition at higher doses. ML-A showed biphasic behavior: stimulation at ≤5 mg/mL, and inhibition at 10 mg/mL (Figure 2a). Similar patterns were observed with seed extracts: MS-W and MS-M strongly promoted growth at low doses (up to 66% and 51% stimulation, respectively), while higher concentrations suppressed growth (Figure 2b). Willow extracts showed comparable trends; WB-M and WB-W promoted growth at ≤5 mg/mL but were inhibitory above 10 mg/mL (Figure 2c). Overall, methanol and water extracts exhibited hormetic effects which stimulate at low concentrations and inhibiting at high doses.

Table 1. Comparative LC_{50} Values of mahaleb and willow extracts using various solvents.

Plant Sample	**LC_{50} (mg/mL)**
Mahaleb leaf-Hexane (ML-H)	0.54
Mahaleb leaf-Acetone (ML-A)	5.65
Mahaleb leaf-Methanol (ML-M)	2456.64
Mahaleb leaf-Water (ML-W)	36.81
Mahaleb seed-Hexane (MS-H)	23.08
Mahaleb seed-Acetone (MS-A)	2.44
Mahaleb seed-Methanol (MS-M)	197.62
Mahaleb seed-Water (MS-W)	22.31
Willow bark-Hexane (WB-H)	22.23
Willow bark-Acetone (WB-A)	17.30
Willow bark-Methanol (WB-M)	41.49
Willow bark-Water (WB-W)	6.72

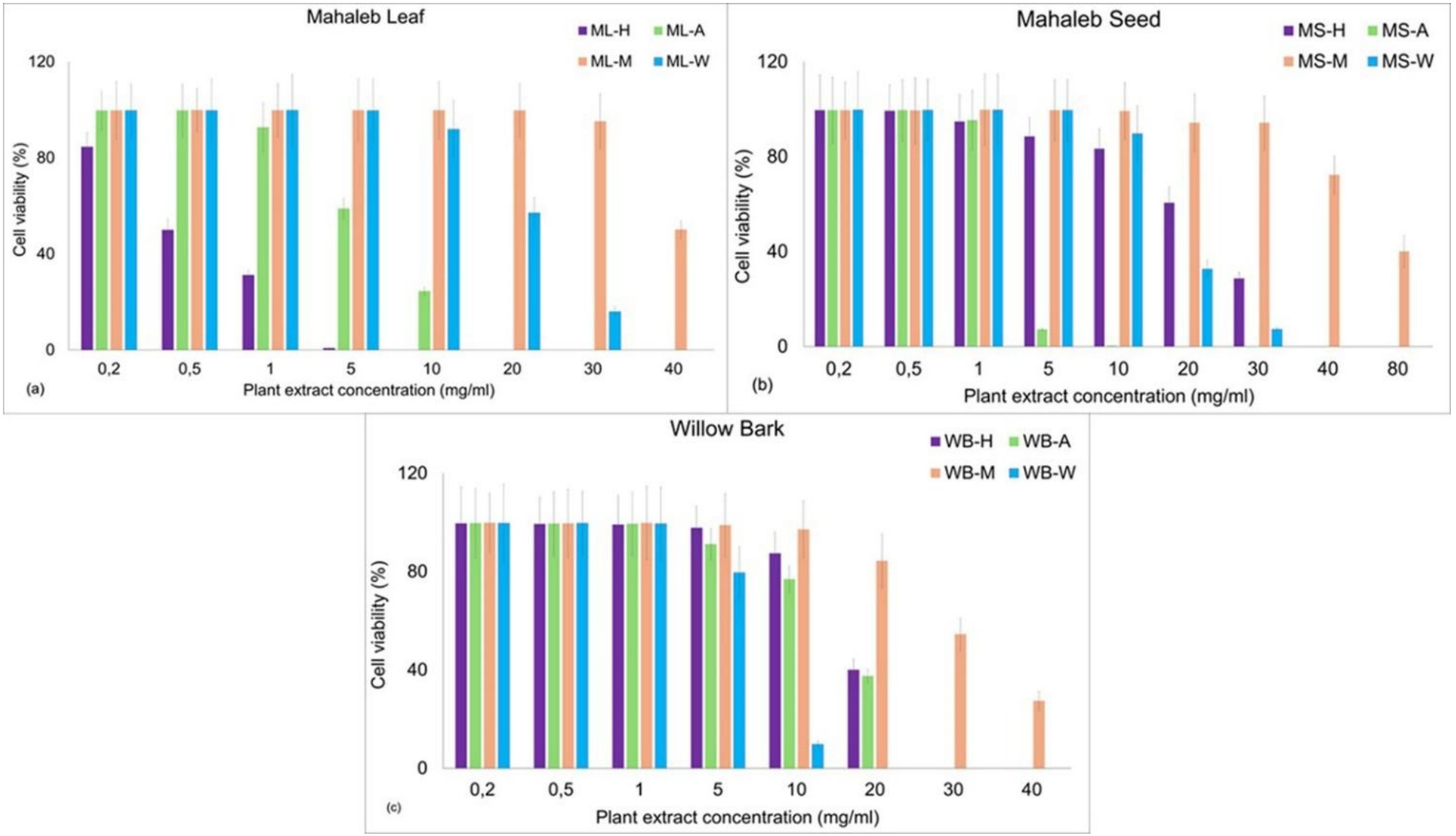

Figure 1. The viability of yeast cells under varying concentrations of mahaleb and willow extracts, expressed as a percentage relative to the control (100%). (a) Mahaleb leaf (ML) (b) mahaleb seed (MS) (c) willow bark (WB) extracts. H: hexane; A: Acetone; M: Methanol; W: Water. Error bars represent standard deviation (SD) values.

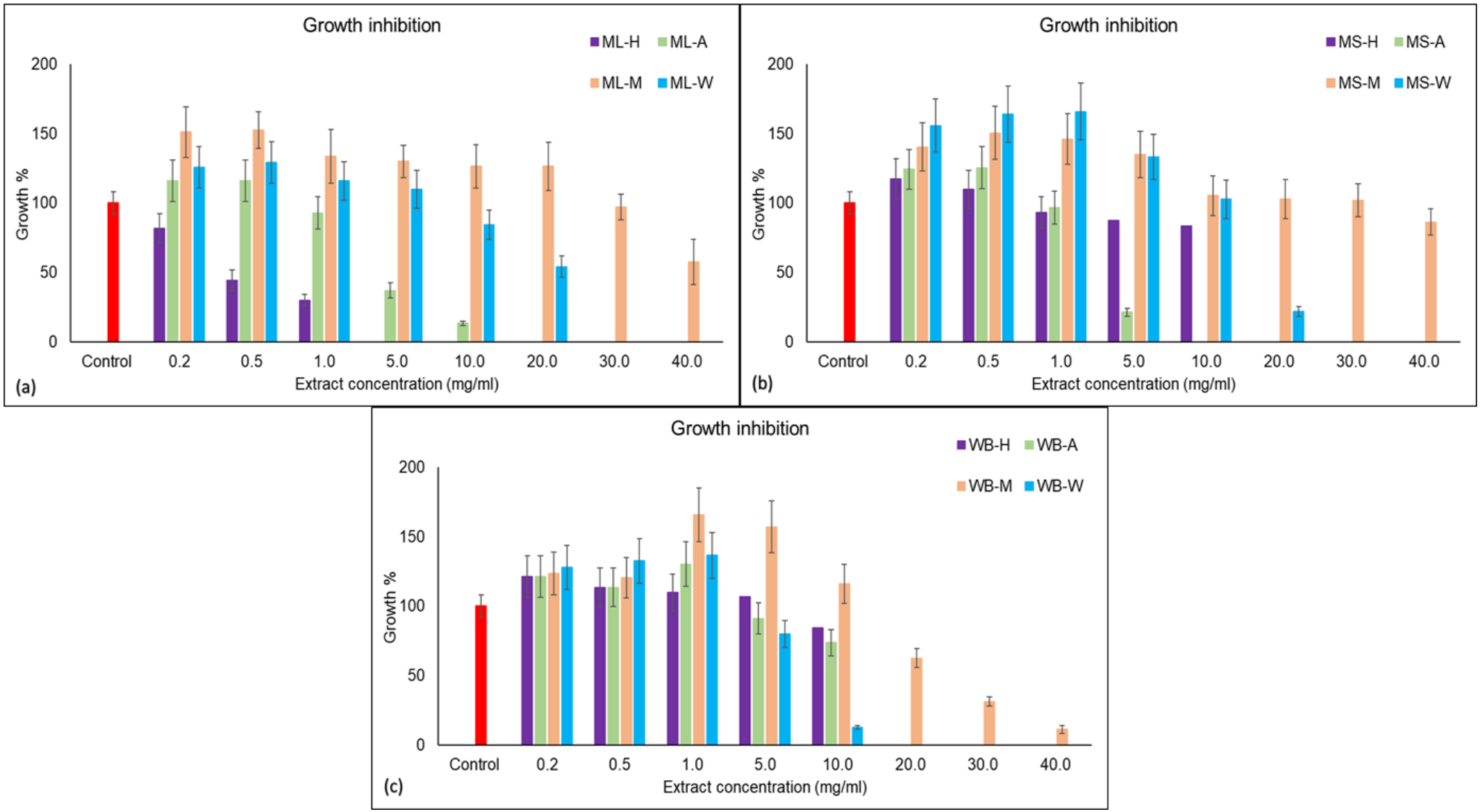

Figure 2. The inhibitory effects of mahaleb and willow extracts on cell proliferation, expressed as a percentage relative to the control (100%). (a) Mahaleb leaf (ML) (b) mahaleb seed (MS) (c) willow bark (WB) extracts. H: Hexane; A: Acetone; M: Methanol; W: Water. Error bars represent standard deviation (SD) values.

Long-term toxicity of extracts

While cytotoxicity assays were conducted on exponentially growing yeast cells, the CLS assay focused on stationary-phase cells, which are generally more sensitive to toxic stress and exhibited reduced proliferative activity. The long-term toxicity was tested in non-dividing cells over 8 days. Based on viability, growth inhibition, and LC_{50} analyses, 0.2 mg/mL was selected as the working concentrations for all hexane extracts due to their toxicity, while 0.5 mg/mL was used for acetone, methanol, and water extracts, which exhibited minimal cytotoxicity at that dose. Hexane extracts showed variable long-term toxicity; ML-H and ML-A exhibited slightly elevated cell death by day 8, exceeding the control but remaining below 20% (Figure 3a). In contrast, methanol and water extracts of both mahaleb leaf and seeds exhibited minimal toxicity, comparable to or below the control (11.4% by day 8) (Figure 3b). Among willow extracts, WB-H was the most toxic, with toxicity rising to 24.9% by day 8, whereas WB-M and WB-W maintained low toxicity levels of 5.4% and 7.4%, respectively, which were below that of the control (Figure 3c).

Enhancing yeast CLS: Effects of mahaleb and willow extracts

CLS assay was performed by transferring stationary phase cells into distilled water containing plant extracts. Viability was measured over 64-day period by monitoring outgrowth in fresh YPD media. ML-W was the most effective among leaf extracts, maintaining 35.9% viability on day 50. ML-A retained 29.6%, ML-M 18.0%, and ML-H 11.7% viability (Figure 4a). All ML-treated groups outperformed the control, which exhibited complete loss of viability by day 50. Similarly, MS-W emerged as the most potent among all tested extracts, preserving 39.3% viability at day 50 and 21.4% at day 64. MS-M maintained 25.3% viability at day 50, while MS-A and MS-H showed moderate protection (Figure 4b). WB extracts extended viability modestly; WB-W and WB-M outperformed control but did not match the efficacy of ML or MS extracts (Figure 4c).

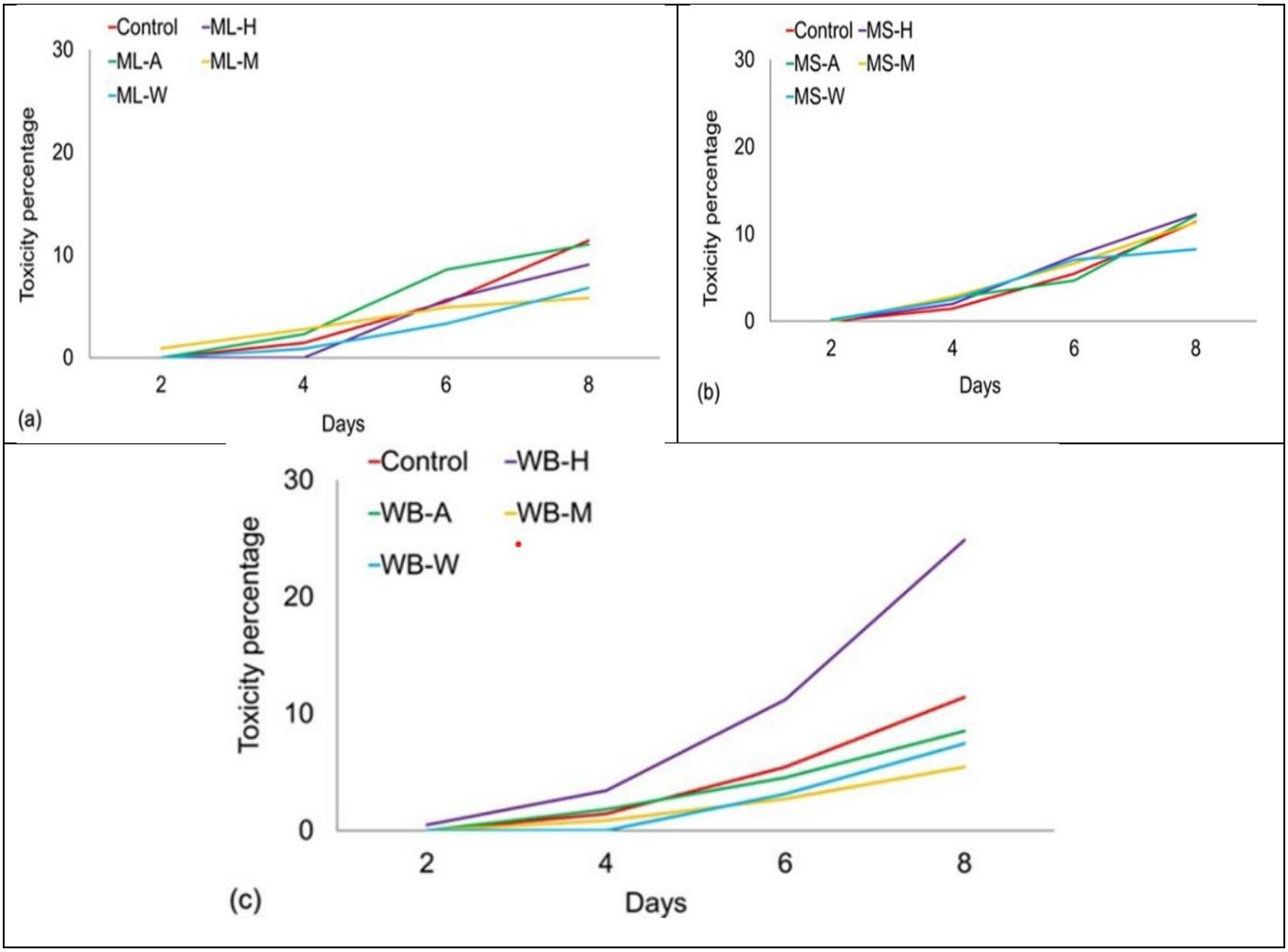

Figure 3. The toxicity percentage of plant extracts compared to control. (a) mahaleb leaf extracts (b) mahaleb seed extracts (c) willow bark extracts. Hexane extract concentration: 0.2 mg/mL; Acetone, methanol and water extract concentrations: 0.5 mg/mL; Control: no extract. Error bars represent standard deviation (SD) values.

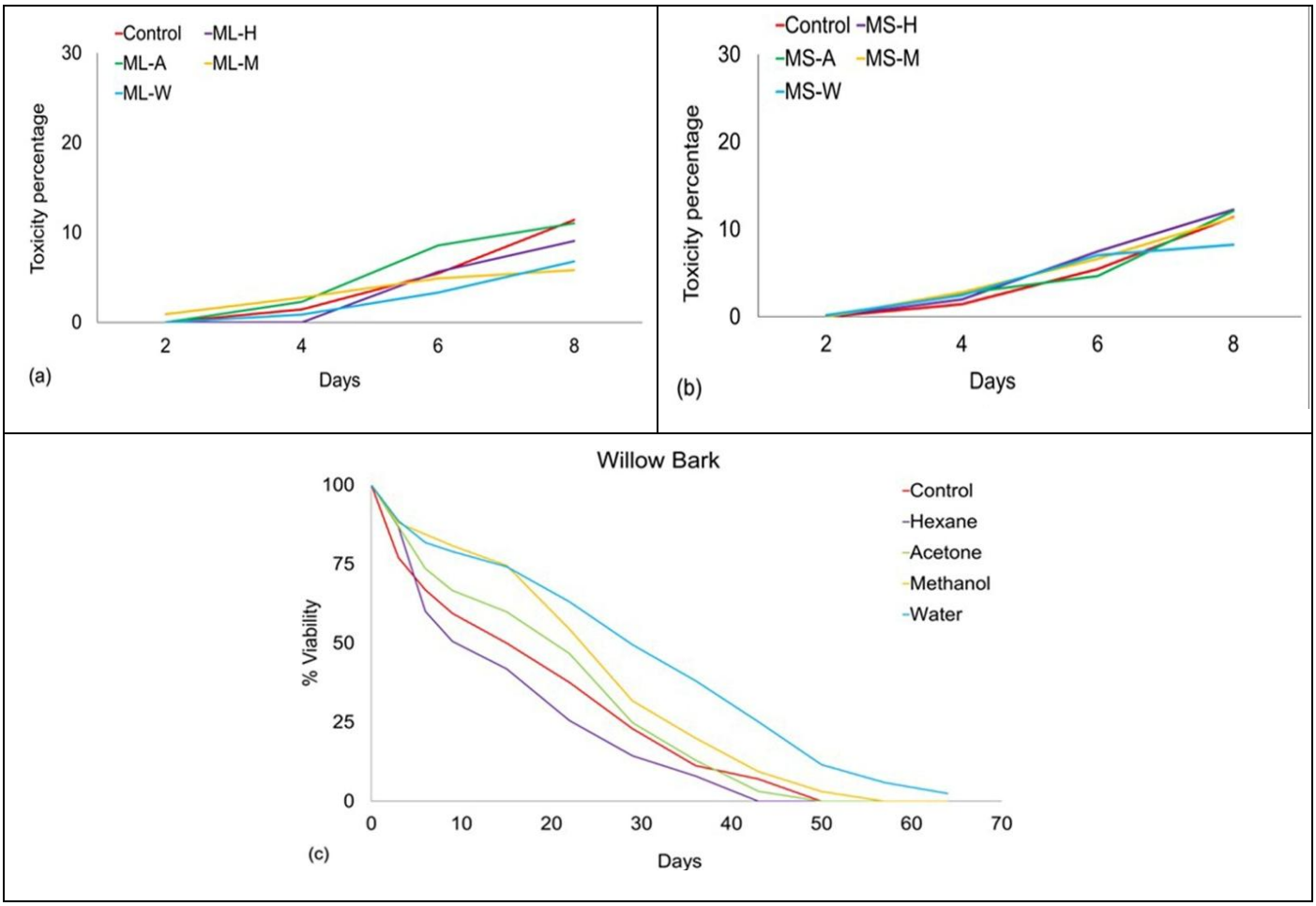

Figure 4. The effect of mahaleb leaf extracts to yeast survival during stationary phase. (a) Mahaleb leaf (b) mahaleb seed extracts (c) willow bark extracts. Control: no extract. To avoid overcrowding, SD values were not displayed in the line graph but remained below 15%.

While viability data offers momentary insights into cell survival, the survival integral (SI) provides a more comprehensive evaluation by quantifying the area under the viability curve over time. To assess the cumulative effects of extracts, SI values calculated and normalized to the control group (assigned a value of 100), allowing for direct comparison of the lifespan-extending potential of mahaleb and willow bark extracts. Among all tested extracts, the highest SI values were observed in ML-W and MS-W, both exceeding 200%, while WB-H yielded the lowest (82%) (Figure 5). Interestingly, despite its moderate toxicity, ML-A also resulted in a substantial increase in SI, suggesting a potential hormetic or compensatory effect. In contrast, WB-H, although highly toxic, was included in the CLS assay purely out of scientific curiosity, and as expected, it showed poor performance in cumulative survival. Notably, SI values for methanol and water extracts of *P. mahaleb* were significantly higher than those of *S. alba* bark extracts ($p<0.05$), indicating a stronger and more consistent lifespan-extending effect. These findings suggest that polar extracts from mahaleb samples are more effective in promoting cumulative survival in yeast under nutrient-depleted conditions compared to those from willow bark.

Phytochemical content of plant extracts

GC-MS profiling provided insights into the chemical constituents potentially responsible for the observed biological effects. The identified compounds have been deposited on the Zenodo digital platform, accompanied by DOI references and listed in Supplementary Table. The detected compounds were categorized into four chemical groups. Group I compounds (organic acids and derivatives) predominated in water extracts such as MS-W and ML-W. Group II included hydroxylated compounds, sugars, and phenolic alcohols, detected across all polar extracts. Group III compounds, comprising hydrocarbons and aromatics, featured prominently in ML-M, ML-A, and MS-M. Group IV included ketones and steroid derivatives, found in moderate quantities in ML-A, WB-A, and MS-H (Figure 6).

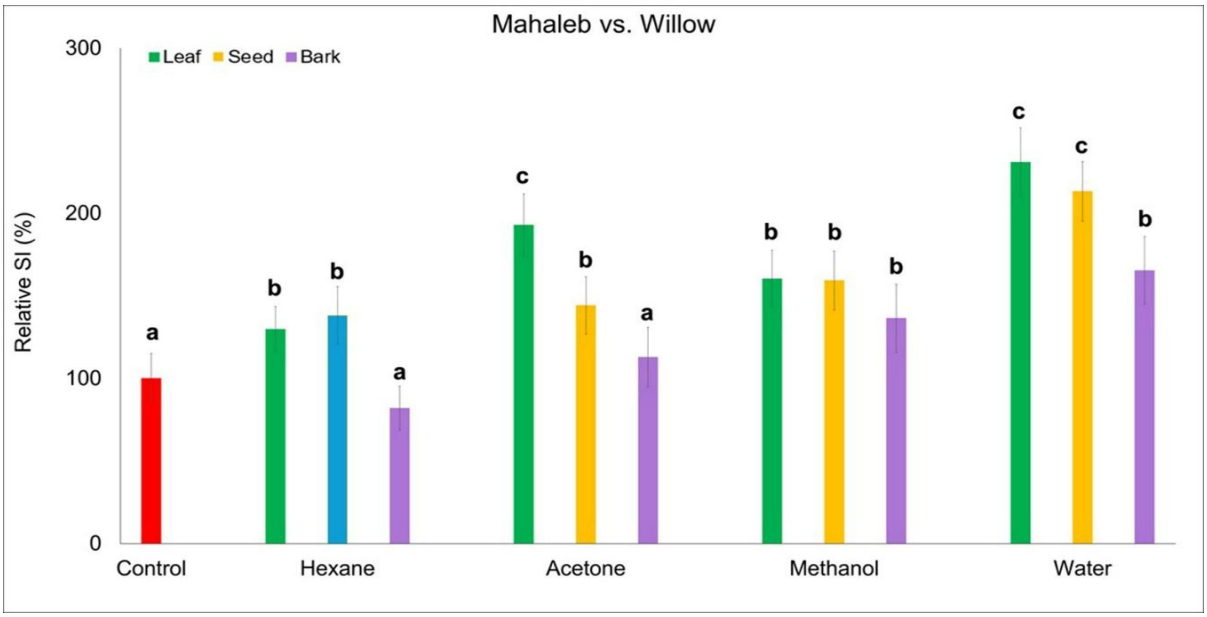

Figure 5. Comparing the effect of mahaleb and willow extracts on yeast CLS. Control: Yeast cells grown in water without plant extract. According to one-way ANOVA and Tamhane's T2 test, mean values labeled with distinct letters denote statistically significant differences (p<0.05) among samples (Confidence Interval: 95%, F: 60.551, dF1:12, dF2: 104, P: 0.000).

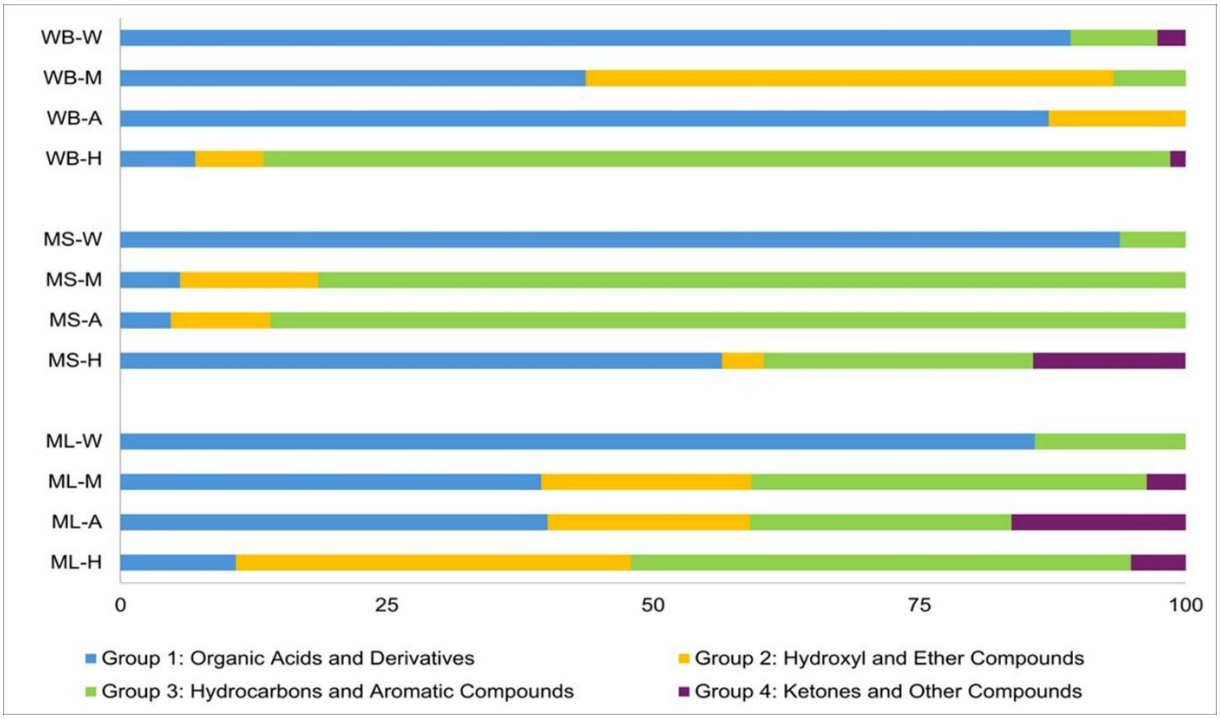

Figure 6. Grouping of compounds identified by GC-MS based on chemical structures and functional groups.

DISCUSSION

The exploration of natural products as geroprotective agents has garnered increasing attention due to their potential to extend lifespan and mitigate aging-associated diseases. *P. mahaleb*, known for its antioxidant and anti-inflammatory properties, is a promising candidate in this field. In this study, the effects of mahaleb extracts were investigated using *S. cerevisiae* in the non-dividing phase, a model relevant to post-mitotic cell aging in higher eukaryotes. The primary objective was to assess whether mahaleb extracts could extend CLS in yeast as effectively as extreme-calorie restriction (E-CR) or known plant-derived geroprotectors such as *S. alba*, which have been previously reported to possess robust anti-aging activities [18,29]. Results showed that mahaleb extracts, particularly those obtained with methanol and water, significantly extended yeast CLS, surpassing the effects of both E-CR and willow bark extracts. Previous

studies have demonstrated that plant-derived compounds can influence longevity through antioxidant and CR-related mechanisms [8,11]. The effects observed in this study may involve similar biological processes, although mechanistic validation remains to be conducted.

Cytotoxicity and growth inhibition assays, performed at extract concentrations ranging from 0.2 to 80 mg/mL, determined non-toxic doses that ensured lifespan extension was not due to acute toxicity or growth suppression. Methanol and aqueous extracts of mahaleb leaves and seed exhibited low toxicity across a broad range, whereas hexane extracts, especially from leaves, were highly toxic even at lower concentrations (LC_{50}~0.54 mg/mL). These observations are consistent with studies showing that solvent polarity impacts the extraction of both beneficial and harmful compounds [32-35].

Hexane and acetone extracts showed strong cytotoxic and inhibitory effects, likely due to membrane-disrupting lipophilic compounds, as evidenced by cell viability assays, and membrane damage markers. This aligns with previous findings that non-polar solvents tend to extract more lipophilic and potentially harmful compounds, leading to toxicity [22]. Such inhibitory properties of plant extracts have been explored in contexts such as antifungal therapies, postharvest protection, and food preservation. Plant extracts, including those from mahaleb, have shown antifungal and antibacterial activity against pathogenic microorganisms, supporting their therapeutic potential [26,36,37].

Plant-derived compounds have also attracted attention for their anticancer properties [38-41]. One hallmark of cancer is uncontrolled proliferation, and in this study, generally the hexane and acetone extracts of mahaleb and willow were found to reduce yeast cell growth and subsequent cell division. Willow (*Salix safsaf* L.) leaf extracts, for instance, have been shown to induce DNA fragmentation similar to that triggered by resveratrol. These bioactive compounds have been shown to promote apoptosis, induce DNA fragmentation, and disrupt cell membrane integrity, all of which are cellular effects often associated with antitumor activity in experimental models [38,42]. Although these mechanisms were not directly assessed in this study, the cytotoxic effects of hexane and acetone extracts may involve pathways reported in other studies, including membrane disruption, as indicated by dye uptake in damaged cells.

Based on toxicity data, optimal concentrations for the lifespan assays were determined to be 0.2-1 mg/mL. These concentrations consistently promoted growth without significant inhibition and maintained high viability (except ML-H). CLS assays were therefore conducted using 0.2 mg/mL for hexane extracts and 0.5 mg/mL for other solvents. The CLS assay, performed over 64-day period, showed that mahaleb leaf and seed extracts, particularly the aqueous extracts, significantly extended yeast lifespan compared to both the control and willow bark. While WB-H exhibited the lowest viability, ML-H was toxic in proliferating cells but extended lifespan in stationary-phase cells. These findings demonstrate that physiological state of cells (log or stationary phase) results in differential responses as indicated previously [43].

Data from the CLS assays confirm that mahaleb extracts can extend lifespan under E-CR conditions in stationary-phase cells. However, the possibility that these effects arise from low-level metabolic support rather than direct modulation of aging pathways was considered. Nonetheless, CLS assays were conducted under nutrient-free conditions, where added nutrients typically reduce, rather than prolong lifespan. Additionally, the effective extract doses were too low to support cell growth, and higher doses were often inhibitory. Thus, the lifespan extension is more likely due to stress modulation or activation of pro-longevity signaling pathways than mere nutritional support.

Methanol extracts from mahaleb seeds yielded the highest survival rates, likely due to elevated levels of coumarin derivatives such as dihydrocoumarin and herniarin, the compounds naturally present in mahaleb fruits, seeds, and other plant parts [20-22]. Benzocoumarin, for example, has been shown to extend lifespan in *C. elegans*, while dihydrocoumarin inhibits Sir2 protein, a histone deacetylase involved in yeast aging mechanisms [27,28]. Although Sir2p generally promotes RLS, most studies suggest it has neutral or even negative effects on CLS [44,45]. Interestingly, CLS extension occurs when *SIR2* deletion is combined with calorie restriction, whereas *SIR2* deletion or CR alone has no effect [45]. Although speculative, it is intriguing to consider that the combined condition of E-CR and coumarin-related Sir2p inhibition may have contributed to lifespan extension. This possibility could be explored in future studies using targeted molecular approaches.

Hexane extracts also contained bioactive compounds such as phytol (in leaves) and squalene (in seeds). These lipophilic compounds may contribute to membrane integrity and antioxidant defense, both of which support cellular longevity. Phytol, a chlorophyll component and precursor α-tocopherol (Vitamin E), has demonstrated antioxidant, anti-cancer, and senescence-delaying effects [46,47]. Remarkably, mahaleb seeds contain extremely high levels of squalane (8317.35 mg/g), far exceeding concentrations found in other *Prunus* species [48,49]. Squalene is a biosynthetic precursor to sterols like cholesterol, which decline with age [50]. While direct evidence of lifespan extension is lacking, squalene has been shown to reduce

symptoms of age-related disorders, slow physiological changes, and inhibit cancer development [51,52]. Future studies should explore the potential contribution of these compounds to cellular longevity and elucidate their underlying mechanisms through targeted biochemical and genetic approaches.

While this study focused on phenotypic lifespan extension in a yeast model, certain scope-related boundaries should be acknowledged. Molecular and antioxidant assays were not included, as they fell beyond the primary aim of this exploratory screen; however, preliminary DPPH and FRAP tests (data not shown) indicated notable radical-scavenging capacity, particularly in methanol and water extracts. Given the unicellular nature of *S. cerevisiae*, caution is warranted when extrapolating these findings to higher organisms. Future studies may expand upon this work by exploring transcriptomic and metabolomic responses under calorie restriction or in long-lived yeast mutants (e.g., *tor1Δ*, *sch9Δ*, *sod2Δ*) to uncover conserved longevity pathways. Moreover, testing these extracts in mammalian cells such as fibroblast or SH SY5Y lines under stress conditions, along with pharmacokinetic profiling including intestinal absorption and microsomal stability, would help assess their translational relevance and potential biotechnological applications.

CONCLUSION

In summary, the methanol and water extracts of *P. mahaleb* leaves and seeds effectively enhanced the CLS of *S. cerevisiae* surpassing both E-CR and *S. alba* bark extracts in phenotypic performance. These polar extracts displayed low toxicity and were enriched with phytochemicals such as coumarin derivatives, phytol, and squalene, which may underlie the observed longevity promoting effects. Given these properties *P. mahaleb* extracts hold promise as plant-derived candidates in the development of geroprotective formulations including functional foods, natural anti-aging cosmetics, and probiotic systems. Meanwhile, cytotoxic and membrane-disruptive characteristics observed in non-polar extracts suggest potential roles in antimicrobial applications such as food preservation and postharvest protection. Future research should investigate the underlying molecular mechanisms, including nutrient-sensing pathways and epigenetic regulation, and evaluate efficacy in higher model organisms to advance the translational utility of these findings.

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