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Effects of pH on antioxidant and prooxidant properties of common medicinal herbs

DOI 10.1515/biol-2016-0040
Received May 4, 2016; accepted October 6, 2016

Abstract: *Background*: We studied *in vitro* and *vivo* antioxidant and prooxidant abilities of aqueous extracts from *Rosa canina* L., *Rhodiola rosea* L., *Hypericum perforatum* L., and *Gentiana lutea* L. *Methodology*: Total antioxidant capacity was measured by four assays (phosphomolybdate method, Fe^{3+}-reducing activity, $ABTS^{\bullet+}$ scavenging, H_2O_2 scavenging). Prooxidant activity was estimated by H_2O_2 production. Yeast viability in the presence of H_2O_2 and/or plant extracts was determined by plating or by counting live cells' number. *Results*: Plant extracts differed in the total phenolic content (*R. canina* > *R. rosea* > *H. perforatum* > *G. lutea)* which clearly correlated with their $ABTS^{\bullet+}$ scavenging activity (R^2 = 0.963). H_2O_2 scavenging activity was not clearly associated with plant phenol levels and was significantly higher in acidic, than in alkaline medium. In line with this, plant extracts effectively protected yeast *S. cereviasiae* against H_2O_2 and stimulated reproductive ability of yeast cells at acidic but not at alkaline pH. At alkaline pH, plant extracts produced certain amounts of H_2O_2 which were related to their phenolic content. *Conclusion*: The antioxidant activity of plant extracts is decreased at alkaline pH with an increase in the prooxidant activity. It reduces protective capacity of plant extracts against oxidative and other stresses *in vivo*.

Keywords: phenol content, *Rhodiola rosea, Rosa canina, Hypericum perforatum, Gentiana lutea, Saccharomyces cerevisiae*, hydrogen peroxide.

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1 Introduction

It has been postulated that oxidative stress resulting from an imbalance between production and elimination of reactive oxygen species (ROS) plays a pivotal role in aging and the development of many diseases such as cancer, immune-system decline, inflammation, cardiovascular and neurodegenerative disorders [1,2]. Consumption of foods rich in antioxidants was found to prevent ROS-induced oxidative damages. Protective effects have been demonstrated for well-known plant-derived antioxidants such as vitamins C and E, carotenoids, as well as various phenolic compounds [3-6]. Due to the chemical diversity of antioxidant substances and the complexity of their composition in plants, total antioxidant capacity (TAC) of plant extracts is often measured as an integrated index [7,8]. In many studies, positive correlations between the antioxidant capacity of plant extracts and their phenolic content were observed [9-11]. However, the content of phenolic compounds does not always correlate with total antioxidant activity of plant extracts [8].

There are a number of reports suggesting that plant phenols exhibit prooxidant and cytotoxic properties under certain conditions [3,12]. The prooxidant activity of phytophenols is manifested under conditions that favor their autooxidation, for example, the presence of oxygen or transition metal ions, and alkaline pH [3,7,13,14]. Many plant phenols are rather unstable in aqueous liquid extracts where they are rapidly autooxidized [3,15]. The ability of phenols to autooxidize should be taken into account when studying their metabolism and bioavailability in gastrointestinal tract, since pH in small intestine varies from neutral to alkaline. Low ROS levels produced through oxidation of phenols may be beneficial, since, by imposing a mild oxidative stress, the capacity of antioxidant defense and the activity of xenobiotic-metabolizing enzymes might be raised [14], leading to overall cytoprotection in the gastrointestinal tract [3]. However, the highly reactive ROS production by phenolic compounds may be dangerous due to the possible oxidative damage to intestinal mucosa, and

it may be one of mechanisms explaining toxicity of high doses of phenolic compounds. Along with that, strong prooxidant properties of plant phenols may be considered as an additional mechanism of their antimicrobial action against concomitant pathogenic microorganisms in the intestine [16].

Antioxidant activity has been shown for many medicinal plants, and *in vitro* screening of new plant species as a first step has been carried out in the search for sources of new antioxidants [10,17,18]. As a second step, *in vivo* assays are also necessary to have a more precise evaluation of plant extracts as potential antioxidant agents. Yeast *Saccharomyces cerevisiae*, a widely used model in studies of stress responses in eukaryotic cells [19,20], is a convenient organism for *in vivo* screening for natural antioxidants [17,21,22]. In this study, we used *S. cerevisiae* to assess antioxidant and prooxidant properties of four medicinal plants growing in Ukrainian Carpathian Mountains, *Rhodiola rosea* L., *Gentiana lutea L.*, *Rosa canina* L., and *Hyperycum perforatum* L., which are also distributed in other temperate areas of Europe. A number of studies have reported on antioxidant activities of *R. rosea* [23-26], *R. canina* [9,27-29], *H. perforatum* [11,30,31], and *G. lutea* [18,32,33]. However, little attention has paid to prooxidant properties of these plants, and the findings are focused mainly on reduction of antioxidant activity of plant extracts with increasing their doses [9]. Here, we tested the effects of pH on *in vitro* H_2O_2 scavenging and H_2O_2 producing activities of the extracts prepared from the above plants. We asked the question if these properties could modulate the ability of plant extracts to protect *S. cerevisiae* cells against oxidative stress *in vivo*. In addition, relationships between the plant phenolic content and the antioxidant and prooxidant activities were assessed.

2 Materials and Methods

2.1 Chemicals

Ethylenediamine-tetraacetic acid (EDTA), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), xylenol orange, sorbitol, ferrous sulfate, peptone, and yeast extract were obtained from Sigma-Aldrich Corporation (USA); quercetin was from Reanal (Budapest, Hungary). All other reagents were from local suppliers (Biochimpharma, Ivano-Frankivsk, Ukraine) and they were of analytical grade of higher purity.

2.2 Preparation of plant aqueous extracts

The dried *R. rosea* and *G. lutea* rhizomes, *R. canina* fruit hips, and *H. perforatum* herb, collected in Ukrainian Carpathians, were used in the work. *R. rosea* and *G. lutea* rhizomes were obtained from Fitoapteka "Physalis" (Ivano-Frankivsk, Ukraine), *R. canina* fruit hips and *H. perforatum* herb were from PJSC Pharmaceutical Factory "Viola" (Zaporizhzhia, Ukraine). The herbal raw materials were homogenized to obtain particle fraction of 1-2 mm in size. The aqueous extraction was performed on the boiling water-bath in the ratio of 1:20 (w/v) (herbal dried homogenized material: distilled water) for 30 min. Liquid extracts were filtered, sterilized by boiling for 20 min, and kept at 4°C for no more than 24 hours.

2.3 Determination of total phenols and flavonoids

2.3.1 Total phenols

The total phenolic content in plant extracts was determined by the Folin-Ciocalteu method [34]. Briefly, 5 µl of plant extract sample was mixed with 0.2 ml of distilled water and 1 ml of diluted Folin-Ciocalteu reagent (1:10) (v/v). After 8 min incubation at room temperature, 0.8 ml of 7.5% sodium carbonate solution was added. The samples were then incubated at 45°C for 15 min and the absorbance was measured using spectrophotometer Spekol 211 (Carl Zeiss, Jena, Germany) at 765 nm. Calibration curve was constructed using gallic acid standards (5-50 µg/ml). The total phenol content was expressed as micrograms of gallic acid equivalents per milliliter of plant extract (µg GAE/ml).

2.3.2 Flavonoids

Flavonoid content was measured by aluminum chloride colorimetric assay [35]. To 50 µl of plant extract, 950 µl distilled water and 60 µl of 5% $NaNO_2$ were added; 60 µl of 10% $AlCl_3$ were added 5 min later. After 6 min incubation, 400 µl of 1 M NaOH were added and the total volume was adjusted to 2 ml with distilled water and the absorbance was measured after 15 min of reaction at 510 nm. Standard solutions of quercetin in a range from 2.5 to 50 µg/ml were used to build a calibration curve. The results were expressed as micrograms of quercetin equivalents per milliliter of plant aqueous extract (µg QE/ml).

2.4 *In vitro* antioxidant activity of plant aqueous extracts

2.4.1 Total antioxidant capacity by phosphomolybdate method

The total antioxidant capacity (TAC) of plant extracts was evaluated by the phosphomolybdate method [36]. Briefly, 5 µl of plant extracts were mixed with 2 ml of reagent solution (0.6 M sulfuric acid, 28 mM potassium phosphate and 4 mM ammonium molybdate). The tubes containing reaction mixtures were incubated at 95°C for 90 min. Absorbance of the mixtures was then measured at 695 nm after cooling to room temperature. A calibration curve was built with ascorbic acid as a standard in a range from 5 to 60 µg/ml and the results were expressed as milligrams of ascorbic acid equivalents per milliliter of plant extract (mg AAE/ ml).

2.4.2 Reducing power ability

The ability of plant aqueous extracts to reduce ferric ions was determined by the formation of Perl's Prussian blue [35]. For this, 5 µl of plant extracts were mixed with 0.5 ml of 200 mM potassium phosphate buffer (KPi, pH 6.6) and 0.5 ml of 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] followed by incubation at 50°C in water bath for 30 min. After incubation, 0.5 ml of 10% trichloracetic acid was added to stop the reaction. The upper portion of the solution (0.5 ml) was mixed with 1 ml distilled water, and 0.1 ml of 0.01% $FeCl_3$ solution was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm. Solutions containing 5-200 µg/ml ascorbic acid were used as standards, and the results were expressed as milligrams of ascorbic acid equivalents per milliliter of plant extract (mg AAE/ml).

2.4.3 ABTS•+ scavenging activity

The ability of plant extracts to scavenge $ABTS^{\bullet+}$ radical cation was compared to Trolox standard [37,38]. The $ABTS^{\bullet+}$ radical cation was pre-generated by dissolving 11 mg ABTS in 2 ml of solution of 2 mM H_2O_2 in 30 mM acetate buffer (pH 3.6) to final ABTS concentration of 10 mM and incubating for 1 h in dark at room temperature until characteristic color of $ABTS^{\bullet+}$ appeared. The incubation samples were prepared by mixing 150 µl of 0.4 M acetate buffer solution (pH 5.8), 15 µl of 10 mM $ABTS^{\bullet+}$ solution and 10 µl of plant extract. The rate at which absorbance of $ABTS^{\bullet+}$ cation radical at 414 nm dropped in the presence of plant extracts was monitored using Multiskan MCC/340 (Labsystem, Helsinki, Finland). Solutions of Trolox within a content range from 0.4 to 4 µg/ml were used as standards to build a calibration curve. The results were expressed as micrograms of Trolox equivalents per milliliter of plant extract (µg Trolox/ml).

2.5 H_2O_2 scavenging and H_2O_2 producing activities of plant extracts

For determination of H_2O_2-scavenging activity, mixture containing 10 mM H_2O_2, 50 mM KPi, pH 6.0 or 7.8, and 100 µl of plant extract in a final volume of 2 ml was incubated for 30 min at room temperature. As a control, 10 mM H_2O_2 in 50 mM KPi (without plant extracts) was incubated under the same conditions. After incubation, the samples were 100-fold diluted and 200 µl of the samples were mixed with 1.8 ml of FOX reagent (250 µM $FeSO_4$, 25 mM H_2SO_4, 100 µM xylenol orange, and 100 mM sorbitol) according to previously described FOX (ferrous ion oxidation xylenol orange) method [39]. The reaction mixture was then incubated at room temperature for 30 min. The absorbance of the ferric-xylenol orange complex was measured at 580 nm. The percentage of hydrogen peroxide scavenged by plant aqueous extracts was calculated as follows: scavenging of H_2O_2 = $[(A_0 - A_X)/A_0]\times100$, where A_0 was the absorbance of the control (without plant extract) and A_X was the absorbance in the presence of respective plant extract.

For estimation of H_2O_2 generating activity, 100 µl of plant extracts were mixed only with 50 mM KPi, pH 6.0 or 7.8, in a final volume of 2 ml and incubated during 30 min at room temperature. After incubation, the concentration of H_2O_2 in samples was determined by FOX method as described above. Solutions of H_2O_2 of known concentrations (1-100 micromoles per ml) were used as standards.

2.6 Estimation of reproductive ability of yeast cells

The *Saccharomyces cerevisiae* strain YPH250 (wide type, *MAT*a *trp1-Δ1 his3-Δ200 lys2-801 leu2-Δ1 ade2-101 ura3-52*) was used in this study. Yeast cultures were grown in liquid medium YPD (1% yeast extract, 2% peptone, and 2% glucose) using an orbital shaker at 28°C and 175 rpm. The cells from experimental cultures were collected after 15 h growth by centrifugation (5 min, 3000 *g*), washed

twice with sterile distilled water and resuspended in fresh medium (pH 6.0) containing 1% glucose [14].The resulting cell suspensions were treated with respective plant extracts for 2 h at 28 °C. The plant liquid extracts were added to a concentration of 50 µl/ml of culture medium, and the control yeast suspension was incubated without plant extracts. Reproductive ability was evaluated as an ability of yeast cells to form colonies by plating in duplicate on solid YPD medium after proper dilution. Plates were incubated at 28°C for 72 h and the colony-forming unit (CFU) numbers were counted. Yeast colony growth was expressed as percentage of CFUs; calculated as the ratio between the CFU number in experimental cultures to the CFU number in respective control cultures after plating on YPD agar [20].

2.7 Yeast oxidative stress assay

Exponential-phase cells were harvested after cultivation for 15 h and resuspended in 50 mM KPi, pH 6.0 or 7.8. Then, the aliquots of experimental suspensions were exposed to 10 mM H_2O_2 in the absence or the presence of respective plant extracts (50 µl/ml) followed by their incubation at 28°C for 30 min. Control samples were incubated under the same conditions but without H_2O_2. Cell survival after stress exposure was monitored by counting dead cells stained by methylene blue [40]. Tolerance was expressed as percentages of survival relative to control viability.

2.8 Statistical analysis

Experimental data are expressed as the mean value of 4-6 independent experiments ± the standard error of the mean (SEM). Comparison between means was performed in MYNOVA program using a two-tailed Student's t-test or analysis of variance (ANOVA) followed by the two-tailed Dunnett's test or Student-Newman-Keuls (SNK) test.

3 Results and Discussion

3.1 Total antioxidant capacity of plant extracts depends on phenol levels

At the first stage, we examined the antioxidant capacity of prepared plant extracts by three widely used methods, namely phosphomolybdate method, reducing power and $ABTS^{\bullet+}$ scavenging assays. The plant extracts differed in their TAC measured by phosphomolybdate method with using ascorbic acid as a standard (Fig. 1A). The *R. rosea* aqueous extract had the highest TAC which amounted to 3.66 ± 0.24 mg AAE/ml. It was ~1.8-, 4.3- and 2.8-fold higher than values for *R. canina*, *H. perforatum,* and *G. lutea* extracts, respectively (Fig. 1A). The ferric ion reducing ability of plant extract is demonstrated in Fig. 1B. In this assay, the reducing agents (i.e. antioxidants) reduce the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). As in the case of phosphomolybdate method, the highest reducing ability was observed for *R. rosea* extract (2.84 ± 0.26 mg AAE/ml). In general, reducing ability of *R. rosea* extract was 1.9-, 2.4- and 13.8-fold higher than ones for *R. canina*, *H. perforatum* and *G. lutea* extracts, respectively. Unlike TAC measured by phosphomolybdate and reducing ability assays, the highest ability to scavenge $ABTS^{\bullet+}$ radical was observed for *R. canina* extract and it was equivalent to 1328 ± 83 µg Trolox/ml (Fig. 1C). *R. rosea* extract had 20% lower $ABTS^{\bullet+}$ scavenging activity, than *R. canina* extract. The lowest ability to reduce $ABTS^{\bullet+}$ was shown for *G. lutea* extract which has 86% lower $ABTS^{\bullet+}$ scavenging activity than *R. canina* extract.

In total, the results of three methods used for determination of total antioxidant capacity shows that the aqueous extracts from *R. rosea* and *R. canina* are powerful scavengers of free radicals and reductants of oxidized molecules. *H. perforatum* extract takes an intermediate place, and *G. lutea* extract possesses the lowest antioxidant capacity. Our results are in agreement with other studies that have demonstrated a high antioxidant potential for extracts of *R. rosea* [23-26], *R. canina* [9,27-29] and *H. perforatum* [11,30,31]. Extracts from *G. lutea* rhizomes were shown to have no such powerful antiradical activity *in vitro* as plants rich in polyphenols [18,32] which is consistent with our results.

To elucidate the contribution of phenolic compounds to antioxidant properties of the plant extracts, we measured levels of total phenols and flavonoids, one of the most abundant groups of plant phenols. The *R. canina* extract contained the highest content of phenols (1437 ± 172 µg GAE/ml) while the extract from *G. lutea* rhizomes had the lowest (231 ± 31 µg GAE/ml) among the studied extracts (Fig. 2A). The *R. rosea* and *H. perforatum* extracts possessed 20% and 47% lower content of phenols, respectively, than *R. canina* extract. *R. canina* hips is known to contain large amounts of ascorbic acid which can interfere with phenols in the Folin-Ciocalteu assay [29]. Measurement of ascorbic acid content found only trace amounts of ascorbic acid in the prepared plant extracts (data not shown). It was expected, because ascorbic acid is heat sensitive and seems to be rapidly destroyed under extraction procedure with boiling water-bath.

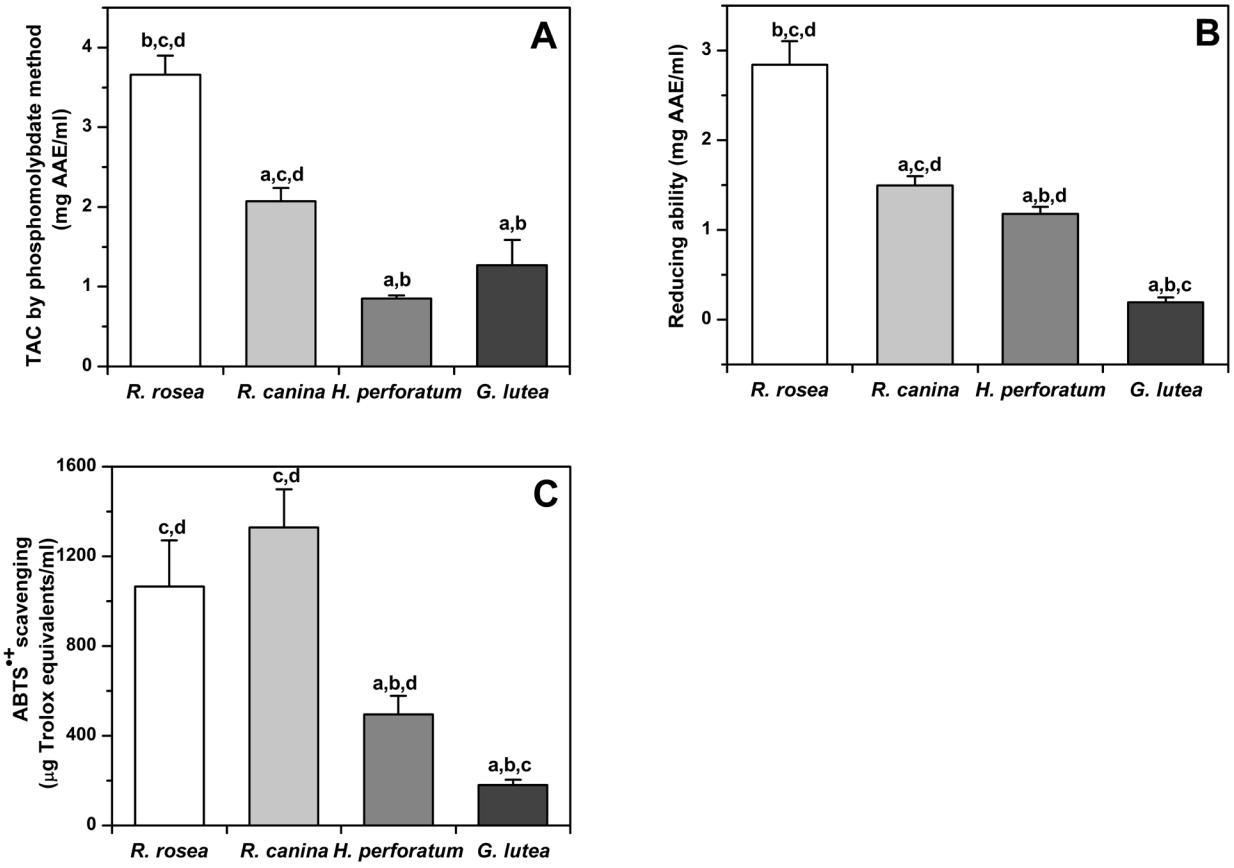

Figure 1. Total antioxidant capacity of aqueous extracts from four Ukrainian medicinal herbs. (A) TAC measured by phosphomolybdate method; (B) reducing power measured as the ability to reduce ferric ions with formation of Perl's Prussian blue; (C) $ABTS^{\bullet+}$ scavenging activity. [a]Significantly different from values of *R. rosea* extract, [b]from values of *R. canina* extract, [c]from values of *H. perforatum* extract, and [d]from values of *G. lutea* extract with $P < 0.05$ as determined by ANOVA and the post-hoc Student-Newman-Keuls test. Data are means ± SEM, n = 5-6.

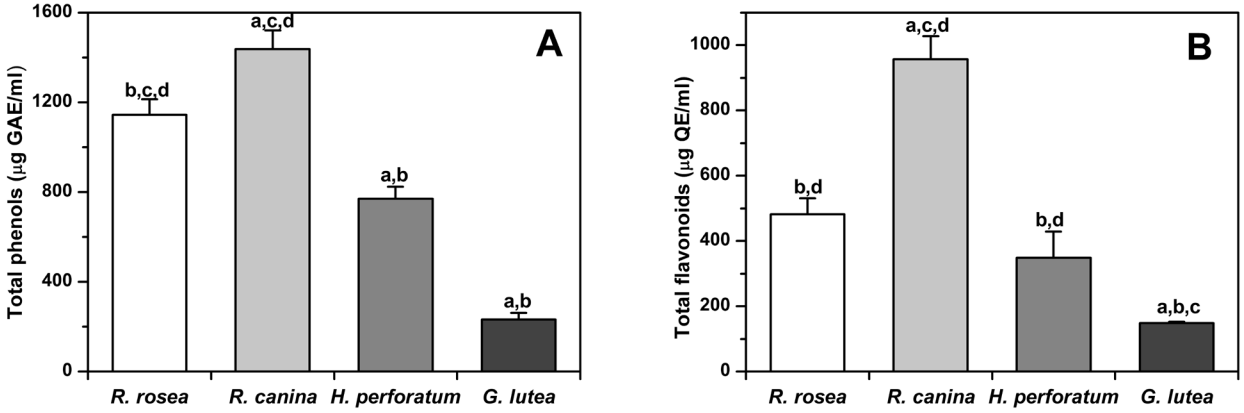

Figure 2. The concentrations of total phenols (A) and total flavonoids (B) in aqueous extracts from four Ukrainian medicinal herbs. [a]Significantly different from values of *R. rosea* extract, [b]from values of *R. canina* extract, [c]from values of *H. perforatum* extract, and [d]from values of *G. lutea* extract with $P < 0.05$ as determined by ANOVA and the post-hoc Student-Newman-Keuls test. Data are means ± SEM, n = 6.

As in the case of total phenols, *R. canina* and *G. lutea* extracts had the highest and the lowest flavonoid contents, respectively (957 ± 71 versus 148 ± 4 μg QE/ml), and moderate flavonoid levels were determined in *R. rosea* and *H. perforatum* extracts (Fig. 2B). A strong positive correlation revealed between total phenols and total flavonoids ($R^2 = 0.852$, $P < 0.05$) suggests, that flavonoids are the important group of polyphenols in the plant

species studied. Our results are in good agreement with levels of phenolic compounds measured previously in *R. canina* [9,28,29], *R. rosea* [25], and *H. perforatum* [11,31]. At the same time, terpenoids, but not phenolic compounds, are key constituents of *G. lutea* rhizomes [32,33,41].

The clear correlations between phenolic content and TAC measured by phosphomolybdate or reducing power assays were not found. However, the total antioxidant capacity measured by $ABTS^{\bullet+}$ scavenging assay correlated strongly with total phenolic content ($R^2 = 0.963$, $P < 0.025$) and flavonoid content ($R^2 = 0.852$, $P < 0.05$) in the plant extracts. This suggests that free radical scavenging activity of the tested plant extracts may be attributed mainly to their phenolic compounds. Furthermore, $ABTS^{\bullet+}$ scavenging assay may be more informative that other used here in the context of reliable assessment antioxidant activity of plants rich in phenolics.

3.2 Alkaline pH decreases *in vitro* H_2O_2 scavenging activity with increasing H_2O_2 producing activity of plant extracts

The using an acidic incubation medium to determine TAC of plant extracts is a distinctive feature of all used above methods. At the same time, plant extracts are able to produce ROS as a result of autooxidation at pH higher than 7 [3,6,13-15]. It can be assumed that this prooxidant ability can modulate the antioxidant capacity of plant extracts. Determination of H_2O_2 concentration seems to be useful for assessment of plant prooxidant/oxidant properties, since the H_2O_2 can be measured after the incubation of the plant extracts alone or with H_2O_2 in the medium at different pH [35]. Therefore, we compared H_2O_2 scavenging and H_2O_2 producing activity of the plant extracts under acidic and alkaline pH (Fig. 3). For assay of H_2O_2 scavenging activity, fresh plant extracts were mixed with 50 mM KPi, pH 6.0 or 7.8, at the ratio 1:20 (v/v) and incubated for 30 min in the presence of 10 mM H_2O_2. All studied plant extracts showed the ability to scavenge H_2O_2 at pH 6.0 and 7.8, but the percentage of H_2O_2 inhibition was higher in the incubation medium with acidic pH (Fig. 3A). Among plant extracts, *R. rosea* extract was the most effective H_2O_2-scavenger with H_2O_2 decomposition percentage of 89 ± 3 versus 63 ± 4 at pH 6.0 and 7.8, respectively. H_2O_2 scavenging activity of other plant extracts was in range from 38 to 55% at pH 6.0 and from 30 to 36% at pH 7.8. Hence, plant aqueous extracts are able to scavenge H_2O_2 more effectively at acidic pH, than at alkaline one. The effectiveness of H_2O_2 scavenging at pH 6.0 was 1.4-fold higher for *R. rosea* and *G. lutea* extracts and 1.2-fold higher for *R. canina* and *H. perforatum* extracts. For *R. canina*, *H. perforatum* and *G. lutea* extracts, H_2O_2 scavenging activity correlated with their phenolic content ($R^2 = 0.973$, at pH 6.0), but it was not observed for *R. rosea* extract. It suggests that other phytochemicals than phenols could be also responsible for H_2O_2-detoxificating ability of plant extracts. Decreased H_2O_2 scavenging at pH 7.8 could be connected with the instability of many phenolic compounds at alkaline pH [15] due to which their antioxidant activity might be decreased.

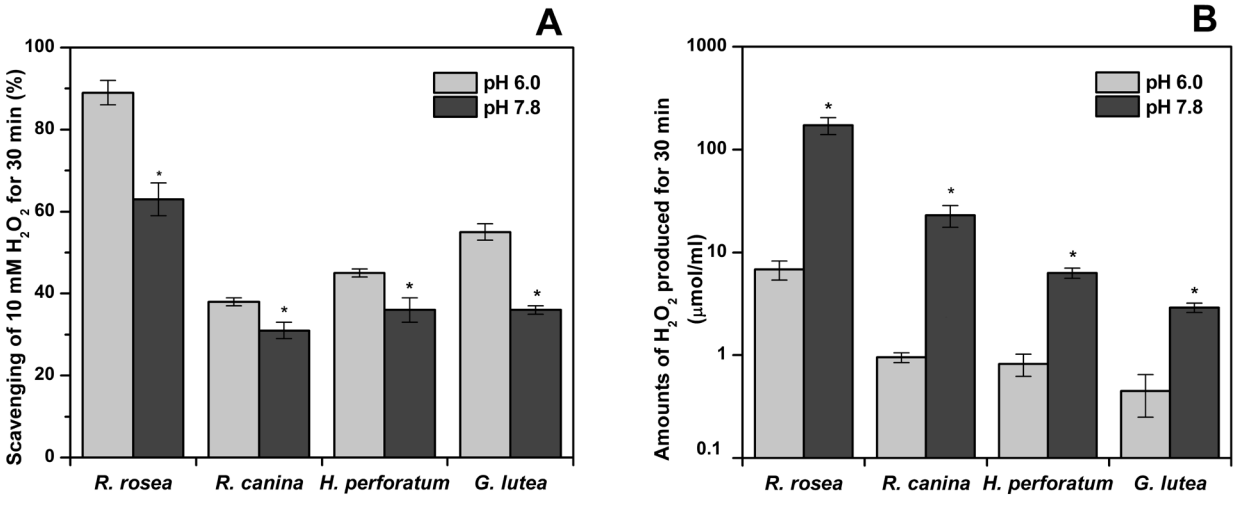

Figure 3. H_2O_2 scavenging (A) and H_2O_2 producing (B) activities of plant extracts (50 µl/ml) at different pHs. H_2O_2 scavenging activity was expressed as percentages of scavenged H_2O_2 and H_2O_2 producing activity (in log scale) was expressed as micromoles of H_2O_2 produced by plant extract in 1 ml of medium for 30 min. *Significantly different from respective values at pH 6.0 with $P < 0.05$ using Student's t-test. Data are means ± SEM, n = 5-6.

Certain levels of ROS, such as superoxide anion radical and hydrogen peroxide, can be produced as a result of autooxidation of plant extracts [3,14]. In order to check this for our plant extracts, we detected H_2O_2 production by the extracts in 50 mM KPi at pH 6.0 and 7.8. Fig. 3B demonstrates the limited ability to generate H_2O_2 at acidic pH for extracts of *R. canina*, *H. perforatum* and *G. lutea*: the amounts of H_2O_2 produced per 1 ml of medium by 50 µl of these extracts did not exceed 1 µmole. Larger amounts of H_2O_2 were produced by 50 µl of *R. rosea* extract (6.8 ± 1.4 µmoles H_2O_2 /ml). Interestingly, the production of H_2O_2 was significantly higher in 50 mM KPi with pH 7.8: the increase was ~ 25-, 21-, 8- and 6-fold for *R. rosea, R. canina*, *H. perforatum* and *G. lutea* extracts, respectively (Fig. 3B). It seems that deprotonation of phenols provides higher capability to enter autoxidation: the acid dissociation of the phenolic group has a stronger electrondonating property than the undissociated one [15]. No clear linear correlation between total phenolic content and H_2O_2 producing activity of plant extracts was observed (R^2 = 0.175, at pH 7.8). However, plant extracts with higher phenolic contents, namely *R. rosea* and *R. canina* extracts, produced more H_2O_2 than extracts of *H. perforatum* and *G. lutea*, which have lower total phenolic content. High prooxidant ability of plant extracts at alkaline pH could explain their lower H_2O_2 scavenging activity in similar environment.

3.3 Plant extracts protect yeast cells against H_2O_2 at acidic but not at alkaline pH

As it was shown above, the tested plant aqueous extracts possess pH-dependent H_2O_2 scavenging and H_2O_2 producing activities. These facts directed us to explore the antioxidant effectiveness of plant extracts *in vivo* depending on pH medium. To achieve this aim, yeast *S. cerevisiae* YPH250 cells were treated with 10 mM H_2O_2 in 50 mM KPi buffer, pH 6.0 or 7.8, and plant extracts at concentration of 50 µl/ml were immediately added. After 30-min period of treatment, the number of live cells in experimental cultures was counted. In control cultures (without plant extracts) treated with H_2O_2, number of viable cells was 67% lower at pH 6.0 and only 3.2 ± 1.2% of cells survived at pH 7.8 (Fig. 4). Yeast *S. cerevisiae* belongs to acidophilic organisms which grow better at acidic than at neutral or alkaline pHs [42]. Moreover, alkalinization of the medium represents a stress condition for the budding yeast [42,43]. Therefore, we supposed that the combined action of two stressors, alkaline pH and hydrogen peroxide, could sensitize yeast cells to H_2O_2 leading to rapid cell death.

The incubation of yeast cells with H_2O_2 in the presence of plant extracts significantly alleviated toxic action of this oxidant at pH 6.0 (Fig. 4). Under combined treatment with H_2O_2 and plant extracts, viability of yeast cells was 2-2.7-fold higher than of control ones treated with H_2O_2 only. The *R. canina* extract demonstrated the highest protective effect with increasing yeast survival up to 90%. Previous reports demonstrated protective effects against H_2O_2 in cell cultures for extracts or isolated compounds from *H. perforatum* [44] and *R. rosea* [21,45]. At the same time, the plant extracts tested did not significantly affect yeast survival under H_2O_2 exposure at pH 7.8. Thus, we concluded that the plant extracts were able to protect yeast cells against oxidative treatment at acidic pH but not at alkaline pH. These data are in good agreement with our results regarding pH-sensitivity of the antioxidant activity of plant extracts: plant extracts were better H_2O_2-scavengers at acidic, than at alkaline pH. Increase in pH medium led to increase in ROS-generating capacity of plant extracts. Since sensitivity to H_2O_2 in yeast cells was increased at alkaline pH, the interfering of antioxidant activity with prooxidant activity did not allow the plant extracts to act as stress-protectants under such conditions. In addition, no relationship between yeast stress survival and H_2O_2-scavenging activity of the plant extracts was observed. At the same time, viability of yeast cells treated with H_2O_2 in acidic pH in the presence of plant extracts positively correlated with total phenolic content (R^2 = 0.656) and $ABTS^{\bullet+}$ scavenging activity of these extracts (R^2 = 0.772). These results suggest that protective effects of plant extracts against H_2O_2 exposure are determined to a great extent by antioxidant properties of the phenolic compounds. The antioxidant properties include not only H_2O_2 scavenging ability but also the ability to scavenge

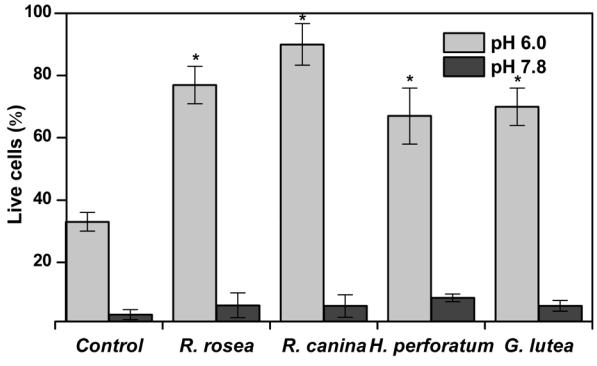

Figure 4. Viability of *S. cerevisiae* YPH250 cells treated with 10 mM H_2O_2 for 30 min in the presence of 50 µl/ml of plant extracts. *Significantly different from respective control values (without plant extracts) at pH 6.0 with $P < 0.05$ using Dunnett's test. Data are means ± SEM, n = 5.

other ROS which can be formed as a result of metabolism of H_2O_2 inside the cells.

3.4 Plant extracts stimulate yeast reproductive ability at acidic pH

S. cerevisiae cells are able to grow at relatively broad range of external pH values, but proliferate better at acidic than at neutral or alkaline pH, and in fact, their growth is stopped in a medium with pH 8.2 [42]. Furthermore, it was shown that H_2O_2 at very low concentrations could stimulate reproduction of yeast cells [20,38] and other fungi [46]. Taking into consideration these facts and the showed above H_2O_2 producing ability of the plant extracts at alkaline pH, we addressed the question if plant extracts were able to affect yeast growth in the culture medium with different pHs. The reproductive capability was estimated by an ability of yeast cells to form colonies on agar YPD plates. The results are presented in Fig. 5. The number of colonies formed in control yeast cultures (without plant extracts) was similar upon incubation for 2 h at different pH levels, namely 6.0, 7.0 and 7.8, and it was set to be 100%. The supplementation of the medium with *R. canina* extract did not influence cell reproduction at all pH used. Along with that, the extracts from other plants used (50 µl/ml) stimulated the colony yeast formation by 25-42% in acidic medium (pH 6.0) and had no effects on yeast colony growth at neutral and alkaline pH. Thus, H_2O_2 producing ability of plant extracts at alkaline pH did not improve yeast growth under these conditions.

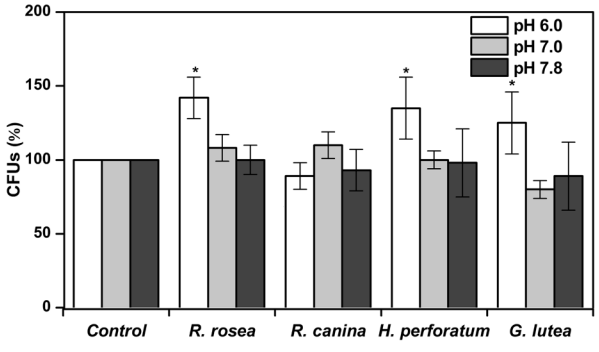

Figure 5. The effects of 2-h treatment with plant extracts (50 µl/ml) on colony-forming ability of *S. cerevisiae* YPH250 at different pHs. Colony-forming ability was assessed as the ability of one cell to form a colony (a colony-forming unit, CFU) after plating on YPD agar. The numbers of CFUs in control cultures (without plant extracts) were set to 100%. *Significantly different from respective control values (without plant extracts) with $P < 0.05$ using Dunnett's test. Data are means ± SEM, n = 4-5.

It is consistent with previous data that *S. ceresiviae* cell differentiation programs and proliferation are under tight control of alkalinization of the medium [43]. Our results suggest that certain phytochemicals present in plant extracts are able to stimulate yeast reproduction at acidic pH. The ability of different herb extracts to activate cell proliferation was demonstrated earlier. Usually, the stimulation effects were observed at low doses of plant preparations with inhibitory effects at higher ones [47,48]. For example, it has been reported that hyperforin from *H. perforatum* can induce keratinocyte differentiation *in vitro* [49]. Stimulation of proliferation was found to be beneficial for normal cells or tissues which require rejuvenation and regeneration and it is undesirable for cancer cells due to promoting cancer malignization [48, 50]. In yeast, stimulation of the reproductive potential seems to be an effective approach to increase biomass and to improve yeast survival under different stresses [38].

The data taken together suggest that beneficial effects of plant aqueous extracts on living organisms with physiological pH range close to neutral can result from their combined antioxidant and prooxidant activities. Mild prooxidant activity can be beneficial as a pre-adaptation mechanism, since it can stimulate endogenous defense system leading to overall cytoprotection and cross-adaptation to followed stresses [3,14,20,38]. Prooxidant activity of plant extracts is also considered as one of the mechanisms of anticancer effects of many phytochemicals [12]. However, overproduction of ROS by phytochemicals may also be dangerous due to possible oxidative damages of intestinal mucosa in animals. In addition, phenolic compounds are metabolized as "typical xenobiotics" by the human body, and such metabolism can influence their antioxidant and prooxidant abilities [3] that can result in oxidative stress of different intensity with respective consequences [1].

4 Conclusions

The results obtained in this study clearly show that: (*i*) *in vitro* and *in vivo* phenolic compounds in plant extracts may demonstrate both antioxidant and prooxidant properties; (*ii*) prooxidant activity is increased, while antioxidant activity of plant extracts is inhibited in alkaline medium; (*iii*) plant extracts may act as effective antioxidants and stimulators of cell growth in yeast *S. cereviase* at acidic pH but not at alkaline one; (*iv*) budding yeast *S. cerevisiae* can be used as a model organism to elucidate mechanisms of protective effects of phytochemicals which can be transferred further with certain precautions to higher eukaryotes.

Acknowledgments. We are grateful to Dr. Yoshiharu Inoue (Kyoto University, Japan) for the providing *S. cerevisiae* strain, our students L.I. Izers'ka and Kh.I. Hryshuk for technical assistance, and Ms. U. Dzaman for English editing of the manuscript.

Conflict of interest: The authors declare nothing to disclose.

Abbreviations

AAE, ascorbic acid equivalent; CFU, colony-forming unit; GAE, gallic acid equivalent; KPi, potassium phosphate buffer; QE, quercetin equivalent; ROS, reactive oxygen species; TAC, total antioxidant capacity; YPD medium, yeast extract peptone dextrose (glucose) medium.

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