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FULL COMMUNICATION

Antioxidant, Antimutagenic, and Anticarcinogenic Effects of *Papaver rhoeas* L. Extract on *Saccharomyces cerevisiae*

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ABSTRACT The aim of this work was to analyze the antioxidant and antimutagenic/anticarcinogenic capacity of *Papaver rhoeas* L. water extract against standard mutagen/carcinogen methyl methanesulfonate (MMS) and radiomimetic zeocin (Zeo) on a test system *Saccharomyces cerevisiae*. The following assays were used: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, quantitative determination of superoxide anion (antireactive oxygen species [antiROS test]), DNA topology assay, D7ts1 test—for antimutagenic—and Ty1 transposition test—for anticarcinogenic effects. Strong pro-oxidative capacity of Zeo was shown to correlate with its well-expressed mutagenic and carcinogenic properties. The mutagenic and carcinogenic effects of MMS were also confirmed. Our data concerning the antioxidant activity of *P. rhoeas* L. extract revealed that concentration corresponding to IC50 in the DPPH assay possessed the highest antioxidant activity in the antiROS biological assay. It was also observed that a concentration with 50% scavenging activity expressed the most pronounced antimutagenic properties decreasing Zeo-induced gene conversion twofold, reverse mutation fivefold, and total aberrations fourfold. The same concentration possessed well-expressed anticarcinogenic properties measured as reduction of MMS-induced Ty1 transposition rate fivefold and fourfold when Zeo was used as an inductor. Based on the well-expressed antioxidant, antimutagenic, and anticarcinogenic properties obtained in this work, the *P. rhoeas* L. extract could be recommended for further investigations and possible use as a food additive.

KEY WORDS: • ***antiROS*** • ***corn poppy*** • ***D7*** • ***DNA topology*** • ***DPPH*** • ***MMS*** • ***scavenging activity*** • ***Ty1 transposition*** • ***yeast*** • ***zeocin***

INTRODUCTION

REACTIVE OXYGEN SPECIES (ROS) are found to play a key role in more than 100 diseases (cardiovascular, neurological, endocrine, respiratory, immune, ischemia, gastric disorders, tumor progression, and carcinogenesis).[1] These data focus the attention of many research groups on identification of natural antioxidants for prevention and treatment of diseases, maintenance of human health,[2] and formation of antimutagenic and anticarcinogenic beneficial effects.[3]

A large number of plants, known from folk medicine, have been screened for various biological activities as an additional strategy for pharmacological use.[4–7] Chemical analysis, purification, and investigation of plant constituents such as flavonoids (quercetin, kaempferol, luteolin) and polyphenols have already revealed not only their antioxidant properties[8–12] but also the DNA protective effects through stimulation of DNA repair processes[8,13] and antimutagenic activity.[3,9–12] On the other hand, the antioxidant activity of an individual compound does not necessarily indicate the true overall antioxidant capacity and may result in misleading conclusions.[14] For that reason, some authors speculate that the biological activities and health-promoting effects of some extracts and food products, for example, *Ginkgo biloba* leaves and buckwheat could be related to synergistic effects among constituents.[13,15] Moreover, in traditional medicine, plants, including members of the *Papaveraceae*,[16,17] are largely utilized as crude extracts in the form of herbal remedies.[18] The anticarcinogenic potential of *Papaver somniferum* extract[19,20] and the antioxidant activity of its polyphenolic extract[21] have already been defined. With regard to the *Papaver rhoeas* L. extract, the data on its antioxidant, antimicrobial, and antigenotoxic activities are scarce[5,22–24] and its antimutagenic and anticarcinogenic effects have not been confirmed so far.

P. rhoeas L. (corn poppy), an annual plant, widely spread in regions with a temperate climate, is used in folk medicine for treatment of respiratory problems, asthma, hay fever, insomnia, urinary irritations, and other conditions.[17,25]

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The phytochemical studies of corn poppy extract have revealed the presence of compounds, such as alkaloids rhoeadine, rhoeadic acid, allocryptopine, protopine, coulteropine, berberine, coptisine, sinactine, (+)-isocorydine, (+)-roemerine,[26] papaveric acid,[25] rhoeagenine,[27] and anthocyanins.[28] The flavonoids kaempferol, quercetin, luteolin, and hypolaetin with their glycosides 3-O-β-D-glucopyranosyl quercetin (isoquercetin), 3-O-β-D-glucopyranosyl kaempferol (astragaline), 3-O-beta-D-galactopyranosyl quercetin (hyperoside) have also been found.[29]

The aim of this work was to analyze the antioxidant and antimutagenic/anticarcinogenic capacity of *P. rhoeas* L. water extract against standard mutagen/carcinogen methyl methanesulfonate (MMS) and radiomimetic zeocin (Zeo) on a test system *Saccharomyces cerevisiae*.

The genotoxicity methods based on yeast cells are recommended by the Organization for Economic Cooperation and Development[30] and Environmental Protection Agency[31] for several reasons such as follows.

- The entirely sequenced *S. cerevisiae* genome reveals around 31% similarity to the human genome[32]; a result that can be extrapolated at the human level.[33,34]
- The discovery of quite a large number of homologous proteins, including cytochromes, secretory proteins, heat-shock proteins, transcription factors, G-proteins, and oncogenes in yeasts and humans.[35]
- The availability of a stable haploid and diploid phase facilitates classical mutational analysis.[36]
- The simple growth conditions and short reproduction time[37] allow to get relatively fast inside the mechanisms of different challenges such as oxidative stress and the related consequences associated with several human pathologies, including cancer, neurological disorders, and cardiovascular diseases.[38,39]

These advantages allow medicine-related research[40] and investigation of bioactivity and mode of action of bioactive compounds such as novel drugs and natural compounds.[41,42]

MATERIALS AND METHODS

Chemicals

MMS, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and pBR322 were from Sigma-Aldrich (FOT Ltd.). Zeo was from Invitrogen. 2,3-bis (2-methoxy-nitro-5-sulfophenyl)-5-[(phenylamino)-arbonyl]-2H-tetrazolium hydroxide (XTT)—from BioShop Canada, Inc. Nutritional components for yeast media preparation were from Difco Chem. Co. Chemicals and reagents were of analytical grade.

Plant material and extraction procedure

P. rhoeas plants were collected around Banska Bystrica, Slovakia in May 2008. The air-dried plant material from capsule was preextracted three times with hexane to remove chlorophyll. After filtration, the material was transferred into 100 mL of distilled water at 50°C and the procedure was repeated five times. The water was then evaporated by distillation on a vacuum rotary evaporator (20 Torr, 50°C). Subsequently, the extract was filtered and traces of water were removed by azeotropic distillation with toluene. Plant extract was stored at 4°C. Appropriate concentrations of the extract were prepared before every experiment from a stock solution of 10 mg/mL.

Tests used

Five tests were applied to evaluate the protective effect of *P. rhoeas* L.; two for the measurement of antioxidant and three for the antimutagenic and anticarcinogenic potential of this extract.

DPPH radical scavenging activity

The DPPH assay, based on a color reduction of DPPH hydrate from purple to yellow, was applied according to Sharma and Bhat[43] with slight modifications. The color change was monitored at 517 nm wavelength after 20 min of incubation in the dark at 4°C. The scavenging activity was calculated by the following equation:

Percentage inhibition $= [(A_A - A_B)/A_A] \times 100$, where A_A is the absorbance of DPPH solution and A_B is the absorbance of tested extract solution. Ascorbic acid was used as a standard.

Quantitative assay for superoxide anions (antiROS test)

The assay based on reduction of the tetrazolium dye XTT by superoxide anions ($O_2^{\bullet -}$) to water-soluble orange-colored formazans was adapted for *S. cerevisiae* cells.[44]

The *S. cerevisiae* strain D7ts1, grown in YEPD media to the end of exponential and the beginning of stationary phase (~ 4–$5/10^7$ cells/mL), was harvested and resuspended in phosphate-buffered saline (PBS). Cells were pretreated with several concentrations of *P. rhoeas* extract in the range 0.25–10 mg/mL for 1 h at 30°C with aeration. After incubation, cell suspensions were washed and 0.1 mg/mL Zeo was added as an inducer of $O_2^{\bullet -}$ (1 min on ice). After removing of Zeo, cells were washed in PBS and 125 μM XTT was added. The aliquots were incubated for 6 h at $t = 30$°C on a rotary shaker, spun, and the absorbance of supernatant was measured at 470 nm. The number of living cells was determined as colony-forming units and results were presented as pM $O_2^{\bullet -}$/cell ± standard deviation.

DNA topology assay

DNA topology assay was applied according to Čipák *et al.*[45] The transformation of supercoiled pBR322 DNA to a relaxed circular form was visually detected using agarose gel electrophoresis. The structural modifications were photographed with UV transillumination using G: BOX (Syngene). As a positive control 0.08 mM Fe^{2+} was used.

Antimutagenic activity of P. rhoeas *extract in* S. cerevisiae *strain D7ts1*

Zimmermann's[46] test with diploid strain D7ts1 (*MATa/α ade2-119/ade2-40 trp5-27/trp5-12 ilv1-92/ilv1-92 ts1/ts1*)

was applied. The D7ts1 strain provides simultaneous detection of mitotic gene conversion at the *trp-5* locus, reversion mutations in the *ilv1* locus, and mitotic crossing over between the centromere and *ade2* allele.[47] All genetic events linked with the *ADE2* locus are classified as total aberrations.[48,49] Zeo (0.1 mg/mL) was used as a positive control.

Cells, grown in YEPD media to the end of exponential and the beginning of stationary phase (~4–5/10^7 cells/mL), were harvested and resuspended in PBS. Different concentrations of corn poppy extract were added and cells were incubated for 1h at $t = 30°C$ on a rotary shaker. The extract was removed by centrifugation (825 *g*) and the pellet was washed with PBS and treated with Zeo for 1 min on ice during centrifugation. Appropriate dilutions of cells were plated on a solid complete medium for survival and total abberants. Gene conversion was detected on selective media lacking tryptophan, and selective media lacking isoleucine were used for reverse mutations. Five plates in each category were incubated for 5–7 days at $t = 30°C$. Yeast media were prepared, as described by Zimmermann *et al.*[50]

Anticarcinogenic activity of P. rhoeas *extract in* S. cerevisiae *strain 551rho$^+$*

The Ty1 transposition assay was performed, as described by Pesheva *et al.*[51] and Dimitrov *et al.*[52] The strain 551 (DG1141ts1) with genotype *MATα ura3-167 his3Δ200::TymHIS3AI sec53 rho$^+$* (National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria, Cat no. 8719) was used.[51] The strain has a Ty1 element with an inserted indicator gene *HIS3AI*, designed by Curcio and Garfinkel.[53] The indicator gene, containing an artificial intron (AI), inserted in antisense orientation, allows following of Ty1 transposition in the genome as a whole. Every transposition of the marked Ty1 element by integration of Ty1-cDNA into a new place in the genome leads to a histidine prototrophic (His$^+$) colony on a minimal medium lacking histidine.

Cell suspensions, grown to the end of exponential and the beginning of stationary phase, were treated with different concentrations of *P. rhoeas* extract (for 1 h at 30°C with aeration). The anticarcinogenic activity of *P. rhoeas* extract was evaluated after combined treatment—pretreatment with different concentrations *P. rhoeas* extract (1 h at 30°C on a rotary shaker) and subsequent treatment with Zeo (for 1 min on ice) or MMS (for 30 min at 30°C with aeration) without intertreatment time. After washing with the YEPD medium, cells were cultivated at $t = 20°C$ (optimal conditions for Ty1 transposition) for 24 h. Appropriate dilutions of cells were plated on YEPD to evaluate survival and on selective medium lacking histidine for His$^+$ transposants. MMS and Zeo were used as positive controls. Yeast media were prepared as described by Sherman *et al.*[54]

Mean transposition rates were determined and results presented as fold increase of Ty1 transposition rate related to control sample, taken as 1.00. A fold increase in treated cultures equal or higher than 2.00 is considered as a positive response of the Ty1 assay.[51]

The yeast strains 551 and D7ts1 have the temperature-sensitive allele *ts1*, leading to increased cellular permeability to different substances, including mutagens/carcinogens.[48,51]

Statistical analysis

At least three independent experiments were performed for each test. The significance in differences between tested concentrations and the positive controls was calculated by one-way analysis of variances (ANOVA) with Dunnett's multiple comparisons test. Correlation coefficient was established by linear regression analysis to determine the IC50 in the DPPH assay. Calculations were done with the GraphPad Prism program, version 6.04. Asterisks provide information for the significance in the differences where $*P < .05$; $**P < .01$; $***P < .001$.

RESULTS AND DISCUSSION

Antioxidant activity of P. rhoeas *L. extract measured with DPPH (chemical) and antiROS (biological) assays*

Two tests were conducted to evaluate the antioxidant activity of different concentrations and to clarify the correspondence between data obtained by chemical and biological assays.

The DPPH assay showed an increase of radical scavenging activity of extract in a dose-dependent manner, $r^2 = 0.9711$ (Fig. 1a). The IC50 of *P. rhoeas* L. extract was

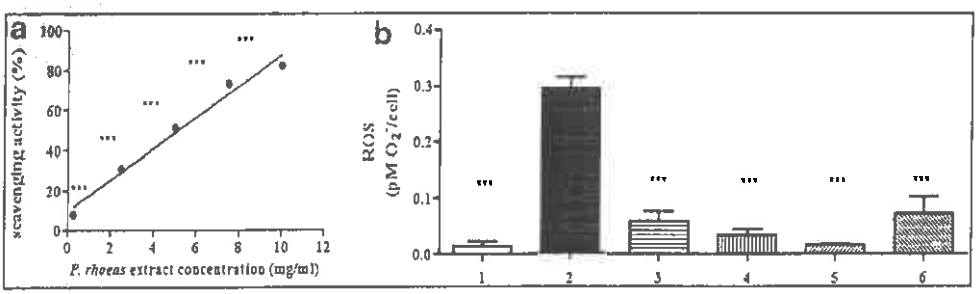

FIG. 1. Antioxidant activity of *P. rhoeas* extract measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) **(a)** and antireactive oxygen species (antiROS) **(b)** assay. **(a)** Scavenging activity of *P. rhoeas* extract at the tested concentrations. **(b)** Levels of superoxide anion: 1: control untreated cells; 2: zeocin (Zeo) treatment; 3: combined treatment 0.25 mg/mL *P. rhoeas* extract and Zeo; 4: 2.5 mg/mL *P. rhoeas* extract and Zeo; 5: 5 mg/mL *P. rhoeas* extract and Zeo, 6: 10 mg/mL *P. rhoeas* extract and Zeo. Where no error bars are evident, errors were equal or smaller than the symbols ($***P < .001$).

found to be 4.81 ± 0.11 mg/mL, corresponding to 0.054 ± 0.008 mg/mL ascorbic acid.

On the other hand, antiROS results demonstrated 15-fold higher induction of $O_2^{\bullet -}$ by Zeo (Fig. 1b) compared to the levels measured in untreated cells (negative control).

Pretreatment with *P. rhoeas* L. extract at concentrations up to 5 mg/mL led to diminishing ROS in a dose-dependent manner (Fig. 1b). The most pronounced effect was measured when IC50 was applied ($O_2^{\bullet -}$ was around ninefold lower). Concentrations above this one showed fivefold higher levels of ROS ($P < .001$) compared to negative control.

It is already known that many beneficial properties of extracts as antigenotoxic and antimutagenic could be partially attributed to their antioxidant activity.[13,55] The *P. rhoeas* extract has already been studied for antioxidant activity with a battery of chemical tests.[5,24] Until now, the antioxidant activity has not been evaluated *in vivo*. In this work, for the first time, a correspondence between DPPH and antiROS (*in vivo*) data is revealed. Our results demonstrate that concentrations forming IC50 in DPPH assay possess the highest antioxidant activity in the antiROS biological test. The elevation of ROS levels is measured using concentrations higher than this one.

Based on these findings, further experiments were carried out with concentrations in the range of 0.25–5 mg/mL.

DNA protective activity of P. rhoeas *extract measured by pBR322 DNA topology assay*

The topological changes of pBR322 DNA were visually detected as lines using agarose gel electrophoresis.

Results presented (Fig. 2a) show that any of the tested concentrations of *P. rhoeas* L. extract did not damage plasmid DNA (supercoiled DNA was the only presented form).

On the other hand, concentrations possessing 31% (2.5 mg/mL) and 51% (5 mg/mL) scavenging activity partially protected plasmid DNA from the damaging effect of Fe^{3+} (Fig. 2b, lanes 9 and 10).

Mutagenic/antimutagenic activity against Zeo in S. cerevisiae *D7ts1*

In this work, an attempt was made to clarify the relationship between the antioxidant and antimutagenic effects of *P. rhoeas* L. extract against the harmful effects of radiomimetic Zeo on a test system *S. cerevisiae* strain D7ts1.

Our pilot experiments (data not shown) revealed no statistically significant mutagenic effect of *P. rhoeas* L. extract up to concentrations 5 mg/mL (determining the IC50 scavenging activity). An increase of genetic events was found after the treatment with concentrations higher than this one. Further, all experiments were performed with concentrations up to 5 mg/mL.

The frequencies of three types of mutations in nuclear DNA caused by Zeo are presented in Table 1. Several genetic events were induced by Zeo: gene conversion, 4-fold ($P < .001$); reverse mutation, 10-fold ($P < .05$); and total aberrant, 7-fold ($P < .001$).

It is already shown that Zeo binds to DNA, leading to the induction of oxidative stress[8] and double-strand breaks (DSB),[56,57] as well as base losses (AP sites). It also possesses clastogenic DNA damaging and genotoxic properties.[6,56–58] The copper-chelated form of Zeo is inactive. When the antibiotic enters the cell, the copper cation is reduced from Cu^{2+} to Cu^{1+} and removed by sulfhydryl compounds in the cell. Upon copper removal, Zeo is activated, and binds and cleaves DNA, causing cell death.[59] This mechanism therefore does not require metabolic activation. In this work for the first time, it is shown that mutations in nuclear DNA such as gene conversion, reverse mutation, and mitotic crossing over could be induced by Zeo in *S. cerevisiae*. As the high levels of gene conversion and mitotic crossing over (Table 1) corresponded well with the elevated levels of ROS (Fig. 1b), we can speculate that this observed recombinogenic effect could be related to the Zeo capability to induce oxidative stress similarly to ionizing radiation, leading to DSB. The presence of low levels of DSB activates different repair processes[56] and thus could be considered as an initiating step for such recombinogenic events. The high levels of reverse mutations, induced by Zeo, confirmed the results obtained for bleomycin on *S. cerevisiae* strain D7 by Hoffmann *et al.*,[60] who proposed that imperfect repair of DNA breaks might be responsible for this point mutation.

On the other hand, pretreatment with different concentrations of *P. rhoeas* L. extract reduced the frequencies of gene conversion at the *trp-5* locus, reverse mutations in the *ilv1-92* locus, and total aberrants induced by Zeo in a dose-dependent manner ($P < .01$). The most promising results

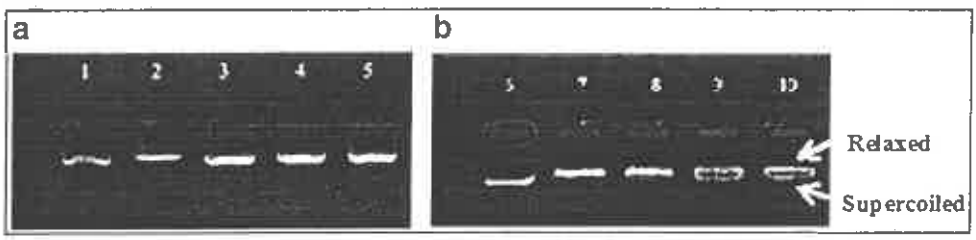

FIG. 2. Agarose gel electrophoretic patterns of plasmid DNA treated with *P. rhoeas* L. extract in the absence or presence of Fe^{3+} ions (0.08 mM). pBR322 DNA (300 ng) was incubated for 30 min at 37°C in the presence of the following additives: **(a)** *lane 1:* DNA control; *lane 2:* 0.08 mM Fe^{3+} without *P. rhoeas* L. extract; *lane 3:* 0.25 mg/mL *P. rhoeas* L. extract; *lane 4:* 2.5 mg/mL *P. rhoeas* L. extract; *lane 5:* 5 mg/mL *P. rhoeas* L. extract. **(b)** *lane 6:* DNA control; *lane 7:* 0.08 mM Fe^{3+} without *P. rhoeas* L. extract; *lane 8:* Fe^{3+} ions (0.08 mM) and 0.25 mg/mL *P. rhoeas* L. extract; *lane 9:* Fe^{3+} ions (0.08 mM) and 2.5 mg/mL *P. rhoeas* L. extract; *lane 10:* Fe^{3+} ions (0.08 mM) and 5 mg/mL *P. rhoeas* L. extract.

TABLE 1. ANTIMUTAGENIC EFFECT OF *P. RHOEAS* L. EXTRACT ON ZEOCIN-INDUCED MUTATIONS IN *S. CEREVISIAE* D7TS1 CELLS

P. rhoeas *L. extract (mg/mL)*	*Zeo (mg/mL)*	*Survival (%)*	*Gene conversion/10^5 cells*	*Reversion/10^4 cells*	*Total aberrants (%)*
0	0	100	1.19 ± 0.12***	0.003 ± 0.0015*	0.68 ± 0.24***
0	0.1	34.18 ± 2.25***	4.55 ± 0.37	0.029 ± 0.019	4.68 ± 1.29
Combined treatment					
0.25	0.1	56.62 ± 1.77***	3.48 ± 1.17^{NS}	0.018 ± 0.017^{NS}	3.08 ± 0.74*
2.5	0.1	49.43 ± 2.02***	3.02 ± 0.77*	0.017 ± 0.016^{NS}	2.58 ± 0.98**
5	0.1	77.73 ± 2.05***	1.96 ± 0.55**	0.006 ± 0.004**	1.13 ± 0.26***

Frequencies are mean ± SD from at least three independent experiments. The significance of differences between positive control (Zeo) and combined treatments—*P. rhoeas* L. extract and Zeo were calculated by ANOVA with a *post hoc* test Dunnett's Multiple Comparison Test (NS: nonsignificant; *$P < .05$; **$P < .01$; ***$P < .001$).

ANOVA, analysis of variances; SD, standard deviation; Zeo, zeocin

were observed using concentrations with a 50% scavenging activity (5 mg/mL). Twofold ($P < .01$) lower gene conversion, around fivefold ($P < .05$) reverse mutation and fourfold ($P < .001$) of total aberrants were measured (Table 1). The frequency of genetic events was comparable with that in control untreated cells. A relationship between the reduction of $O_2^{\bullet -}$ amounts (Fig. 1b) and the protective effect of *P. rhoeas* L. extract was established, suggesting that antimutagenic properties of the extract could be partially related to its ability to scavenge superoxide anions formed by Zeo. In this study, our previous finding about the role of ROS in the induction of mutations in nuclear DNA[61] was confirmed.

Bioactivity of P. rhoeas *L. extract in* S. cerevisiae *551rho$^+$*

S. cerevisiae has transposable elements with structural and functional similarities to retroviruses. They are classified as Ty elements with the most abundant member Ty1. The Ty1 elements replicated through RNA mediate where the new Ty1-cDNA incorporates into new places of the genome leading to DNA damages and rearrangements, similar to those found in mammalian neoplastic cells.[51,62]

Stimulation of cell survival and reduction of Ty1 transposition levels ($P < .001$) in a dose-dependent manner (Table 2) could be considered as informative for the absence of any carcinogenic activity of *P. rhoeas* extract in a concentration range of 0.25–5 mg/mL. This finding provides additional information about the bioactivity of corn poppy extract—no DNA damaging activity in *Chlamydomonas reinhardtii*[63] or toxic and proliferative effects on human lymphocytes and keratinocytes[64] at certain experimental conditions.

TABLE 2. TY1 TRANSPOSITION TEST FOR POTENTIAL CARCINOGENIC EFFECT OF *P. RHOEAS* L. EXTRACT ON *S. CEREVISIAE* 551RHO$^-$

P. rhoeas *L. extract (mg/mL)*	*Survival (%)*[a]	*Fold increase transposition rate*[b,c]
0	100 (1308)	1
0.25	113.71 (1487)	0.71 ± 0.04*
2.5	128.14 (1676)	0.46 ± 0.07***
5	130.22 (1703)	0.32 ± 0.01***

[a]Actual number of colonies is given in parentheses.

[b]Average value ± SD from at least three independent experiments.

[c]The significance in differences between negative control-untreated cells and treatments with different concentrations of *P. rhoeas* extract was calculated by ANOVA with *post hoc* test, Dunnett's Multiple Comparison Test (*$P < .05$, ***$P < .001$).

Anticarcinogenic effect of P. rhoeas *L. extract against MMS in* S. cerevisiae *551rho$^+$*

MMS was used as a standard mutagen and carcinogen, known to interact directly with DNA, without metabolic activation. This statement is pointed out in numerous researches.[65–69] Metabolic activation is required for chemical substances considered as promutagens to become mutagens.[65–69] Furthermore, the metabolic enzymes of bacteria used in the Ames test differ substantially from those in mammals. On the other hand, yeasts have the cytochrome P-450 system able to activate promutagens without adding exogenous activators such as S9 fraction similar to human cells.[70]

Diminution of cell survival to 26% and fivefold elevated levels of Ty1 transposition data induced by MMS are presented in Table 3.

On the contrary, the dose-dependent reduction of Ty1 transposition to the control level and amelioration of cell

TABLE 3. PROTECTIVE EFFECT OF *P. RHOEAS* L. EXTRACT ON TY1 TRANSPOSITION INDUCED BY METHYL METHANESULFONATE IN *S. CEREVISIAE* 551RHO$^-$

P. rhoeas *L. extract (mg/mL)*	*MMS (mg/mL)*	*Survival (%)*[a]	*Fold increase transposition rate*[b,c]
0	0	100 (1308)	1***
0	1.76	26.15 (342)	5.36 ± 0.19
Combined treatments			
0.25	1.76	50.84 (665)	1.44 ± 0.15***
2.5	1.76	80.43 (1052)	1.20 ± 0.15***
5	1.76	96.64 (1264)	0.95 ± 0.05***

[a]Actual number of colonies is given in parentheses.

[b]Average value ± SD from at least three independent experiments.

[c]The significance in differences between positive control (MMS) and combined treatments—different concentrations of *P. rhoeas* extract and MMS was calculated by ANOVA with *post hoc* test Dunnett's Multiple Comparison Test (***$P < .001$).

MMS, methyl methanesulfonate.

TABLE 4. PROTECTIVE EFFECT OF *P. RHOEAS* L. EXTRACT ON TY1 TRANSPOSITION INDUCED BY ZEOCIN IN *S. CEREVISIAE* 551RHO$^+$

P. rhoeas *L. extract (mg/mL)*	*Zeo (mg/mL)*	*Survival (%)*[a]	*Fold increase transposition rate*[b,c]
0	0	100 (1308)	1***
0	0.1	38.07 (498)	3.59 ± 0.39
Combined treatments			
0.25	0.1	47.86 (626)	1.90 ± 0.05***
2.5	0.1	58.41 (764)	1.77 ± 0.24***
5	0.1	93.20 (1219)	0.83 ± 0.03***

[a]Actual number of colonies is given in parentheses

[b]Average value ± standard deviation from at least three independent experiments

[c]The significance in differences between the positive control treatment with Zeo and combined treatments—different concentrations of *P. rhoeas* L. extract and Zeo were calculated by ANOVA with *post hoc* test Dunnett's Multiple Comparison Test (***$P < .001$).

survival to 97% were scored after the combined treatment. It should be noted that concentrations corresponding to IC50 in DPPH possessed the most pronounced anticarcinogenic effects against MMS (Table 3).

Anticarcinogenic effect of P. rhoeas *L. extract against Zeo in* S. cerevisiae *551rho$^+$*

Data, presented in Table 4, are the first experimental evidence that Zeo can induce Ty1 transposition. Zeo treatment led to fourfold higher levels of Ty1 transposition compared to those in untreated control and 38% cell survival. As it is already known, Ty1 transposon mobility could be activated through overproduction of ROS generated only by carcinogens.[44,69,71] Therefore, the high levels of Ty1 transposition (Table 4) and strong induction of $O_2^{\bullet -}$ (Fig. 1b) measured after Zeo treatment provide new information about its carcinogenic potential in *S. cerevisiae*.

The protective capacity of *P. rhoeas* L. extract, observed as a dose-dependent reduction of Ty1 transposition, is well expressed when the combined treatment is performed. The concentration corresponding to IC50 was the most promising, leading to levels of Ty1 transposition comparable with those in negative control and cell survival up to 90% (Table 4).

Our results demonstrate that *P. rhoeas* L. extract at concentrations corresponding to IC50 in DPPH assay can protect cells from the carcinogenic effects of some standard chemicals (MMS and Zeo). Having in mind the fact that MMS[52,72] and Zeo (Fig. 1b) are powerful generators of ROS, this high anticarcinogenic activity of a plant extract can be partially related to the antioxidant activity mainly because of the presented phytochemicals and their synergistic effect.[73]

It is also revealed that doses of *P. rhoeas* L extract lower than 5 mg/mL do not possess any antimutagenic or anticarcinogenic effect against Zeo and MMS. This corresponds well with their lower antioxidant activity.

SUMMARY

In summary, for the first time, a relationship between chemical and biological tests for measuring the *P. rhoeas* L. antioxidant activity is established. The concentration corresponding to IC50 in the DPPH assay possesses the highest antioxidant activity in the antiROS assay.

A strong relationship between the antioxidant and antimutagenic/anticarcinogenic properties of *P. rhoeas* extract is found. The most pronounced antimutagenic and anticarcinogenic effects against the damaging action of MMS and Zeo are obtained for concentrations possessing 50% scavenging activity.

On the other hand, new evidences for the mutagenic and carcinogenic properties of Zeo in D7ts1 and 551rho$^+$ *S. cerevisiae* are presented.

Based on the well-expressed antioxidant, antimutagenic, and anticarcinogenic properties, established in this study, *P. rhoeas* L. extract could be recommended for further investigations and possible use as a food additive.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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