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Research Article

In vitro evaluation of the effects of flavonoids and saponins from *Chenopodium bonus-henricus* L. on non-enzyme-induced lipid peroxidation in isolated rat liver microsomes

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Abstract

The aim of this study was to evaluate the effects of 15 saponins and flavonoids from the roots and aerial parts of *C. bonus-henricus* L. on non-enzymatic (Fe^{2+}/AA)-induced lipid peroxidation in isolated rat liver microsomes. The production of MDA was used as a marker of lipid peroxidation. All saponins exhibited pronounced antioxidant activity (reducing MDA production by 17–23%) comparable to that of silymarin (20%), a well-established antioxidant and hepatoprotective agent. The bayogenin (**ChBh-04**) and 2β-hydroxygypsogenin saponins showed the strongest effects. Flavonoids demonstrated significant antioxidant activity in microsomes from old rats, whereas their effects were less pronounced in microsomes from young rats. The highest reduction in MDA levels, exceeding that of silibinin (61%), was achieved by 6-methoxykaempferol glycoside (62%, **ChBhnf-09**), likely due to the ferulic acid in its moiety. These findings suggest that saponins and flavonoids from wild spinach may play a significant role in protecting liver cells from oxidative stress.

Keywords

Antioxidant activity, *Chenopodium bonus-henricus*, flavonoids, liver microsomes, lipid peroxidation, saponins

Introduction

The liver performs key functions in the organism, including regulating glucose metabolism, synthesizing plasma proteins, and participating in the detoxification of endogenous and exogenous substances. Numerous xenobiotics, including pharmaceuticals, may impair hepatic function either through direct mechanisms or via reactive metabolites formed during biotransformation (Mahajan et al. 2024). In the field of experimental toxicology, comprehensive characterization of synthetic and naturally derived compounds with respect to their hepatotoxic potential and metabolic profile is of critical importance. Widely utilized *in vitro* models for such investigations include isolated rat liver microsomes and hepatocytes (Soldatow et al. 2013; Fukunaga and Takebe 2025). Liver microsomes are subcellular fractions commonly used to evaluate the metabolic stability of xenobiotics and drug

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candidates. These preparations are rich in enzymes from the cytochrome P450 (CYP) family, which play a central role in oxidative metabolism, as well as flavin-dependent monooxygenases, contributing to the biotransformation of various compounds. Both groups of enzymes are situated in the smooth endoplasmic reticulum (Nnane and Tao 2005). Microsomes are subcellular *in vitro* systems rich in enzymes involved in phase I biotransformation, as well as UDP-glucuronosyltransferase associated with phase II. They can also be used as a model of the lipid membrane for studying lipid peroxidation and for evaluating the antioxidant activity of naturally derived bioactive compounds (Zaidi et al. 1993).

Chenopodium bonus-henricus L. (Amaranthaceae), also known as Good King Henry, is a perennial herbaceous plant distributed across the mountainous regions of Bulgaria (Grozeva 2011). Its leaves and flowering tops are consumed as a vegetable, similar to spinach, while root decoctions are used in the food industry for the production of traditional products such as tahini and white halva (Kokanova-Nedialkova and Nedialkov 2017) (Fig. 1).



Figure 1. *Chenopodium bonus-henricus* L. aerial parts and roots.

Nine di-, tri-, and acylated glycosides of 6-methoxykaempferol, spinacetin, and patuletin with hepatoprotective activity were isolated from the aerial parts of *C. bonus-henricus*. All flavonoids (100 μM) significantly reduced CCl_4-induced cellular damage in rat hepatocytes compared to the positive control, silybin, preserving cell viability and GSH levels, as well as decreasing LDH release and lipid peroxidation. Even at high concentrations, the tested compounds exhibited marginal or no cytotoxicity toward the HepG2 cell line (Kokanova-Nedialkova et al. 2017).

Phytochemical studies of *C. bonus-henricus* roots have led to the isolation of six saponins derived from phytolaccagenin, 2β-hydroxyoleanoic acid, bayogenin, 2β-hydroxygypsogenin, and medicagenic acid. Both the methanolic extract and the isolated saponins exhibited moderate to weak cytotoxic activity against five human leukemic cell lines (HL-60, SKW-3, Jurkat E6-1, BV-173, and K-562) and enhanced interleukin-2 (IL-2) production in PHA/PMA-stimulated Jurkat E6-1 cells (Kokanova-Nedialkova et al. 2019a). Additionally, both the purified methanolic extract and the isolated saponins demonstrated hepatoprotective and antioxidant activities in *in vivo* and *in vitro* models of CCl_4-induced liver damage, with effects comparable to those of silymarin (Kokanova-Nedialkova et al. 2019). As a continuation of our research on the phytochemistry and pharmacology of *C. bonus-henricus* L., this paper examines the effects of the 15 flavonoids and saponins mentioned above on non–enzyme-induced lipid peroxidation in liver microsomes.

Material and methods

Materials and chemicals

Six saponins (purity 95–96%) previously isolated from the roots of *C. bonus-henricus* L. were investigated. The tested compounds were di- and triglycosides of five sapogenins as follows: 3-O-β-D-glucuronopyranosyl-medicagenic acid-28-O-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl ester (**ChBh-01**); Bonushenricoside A (3-O-α-L-arabinopyranosyl-phytolaccagenin-28-O-β-D-glucopyranosyl ester) (**ChBh-03**); 3-O-β-D-glucuronopyranosyl-bayogenin-28-O-β-D-glucopyranosyl ester (**ChBh-04**); 3-O-α-L-arabinopyranosyl-bayogenin-28-O-β-D-glucopyranosyl ester (**ChBh-05**); 3-O-β-D-glucuronopyranosyl-2β-hydroxygypsogenin-28-O-β-D-glucopyranosyl ester (**ChBh-06**); Bonushenricoside B (3-O-β-D-glucuronopyranosyl-2β-hydroxyoleanoic acid-28-O-β-D-glucopyranosyl ester) (**ChBh-07**) (Kokanova-Nedialkova et al. 2019a). In the present study, the nine investigated flavonoids (purity 95–96%)—patuletin-3-O-[β-apiofuranosyl(1→2)]-β-glucopyranosyl(1→6)-β-glucopyranoside (**ChBhnf-01**), patuletin-3-O-gentiobioside (**ChBhnf-02**), 6-methoxykaempferol-3-O-[β-apiofuranosyl(1→2)]-β-glucopyranosyl(1→6)-β-glucopyranoside (**ChBhnf-03**), spinacetin-3-O-[β-apiofuranosyl(1→2)]-β-glucopyranosyl(1→6)-β-glucopyranoside (**ChBhnf-04**), spinacetin-3-O-gentiobioside (**ChBhnf-06**), patuletin-3-O-(5‴-O-E-feruloyl)-β-D-apiofuranosyl(1→2)[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (**ChBhnf-07**), spinacetin-3-O-(5‴-O-E-feruloyl)-β-D-apiofuranosyl(1→2)[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (**ChBhnf-08**), and 6-methoxykaempferol-3-O-(5‴-O-E-feruloyl)-β-D-apiofuranosyl(1→2)[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (**ChBhnf-09**) were previously isolated from the aerial parts of *C. bonus-henricus* L. (Kokanova-Nedialkova et al. 2017). The chemicals Na_2CO_3, NaOH, potassium sodium tartrate, $CuSO_4$, Folin reagent, $KHSO_4$, TRIS-HCl, glycerol, KCl, $FeSO_4$, and ascorbic acid were obtained from Merck (Germany), while thiobarbituric acid, silymarin, and silibinin were purchased from Sigma-Aldrich (Germany).

Animals

A total of 15 male white Wistar rats were used in the experiments, comprising young (3–4 months of age) and old animals (1.5–2 years of age). The animals were obtained from the National Breeding Center of the Bulgarian Academy of Sciences (Slivnitsa, Bulgaria) and maintained under standard laboratory conditions in plexiglass cages with unrestricted access to food and water, a 12 h light/12 h dark cycle, and an ambient temperature maintained at 20–25 °C. Before the experiments, the rats were acclimated for at least 7 days. Animal health was routinely monitored by a veterinarian. Food was withheld for 12 hours before each experimental procedure. The experiments were conducted in accordance with Ordinance No. 15 on the Minimum Requirements for the Protection and Welfare of Experimental Animals (SG No. 17, 2006), the applicable European regulations concerning the handling of experimental animals, and Permit No. 304, issued by the Bulgarian Food Safety Agency and valid until 28 June 2026.

Isolation of liver microsomes by the method of Guengerich et al. (1982)

The liver was removed and weighed, after which it was perfused with 1.15% KCl. A 25% liver homogenate containing 0.1 M TRIS–potassium phosphate buffer at pH 7.4 was prepared. The resulting homogenate was centrifuged in a refrigerated Janetzki K-24 centrifuge for 30 min at 10,000 × *g*. Microsomes were obtained by differential centrifugation of the supernatant for 60 min at 106,000 × *g* in a refrigerated Beckman-Coulter ultracentrifuge. The supernatant (cytosol) was discarded, and the microsomal precipitate was frozen in 0.1 M potassium phosphate buffer (pH 7.5) containing 20% glycerol. Before the experiment, the microsomes were resuspended in this buffer to a protein concentration of 1 mg/mL. All stages of liver fraction preparation were carried out at 0 °C. The resuspended microsomes were stored at –25 °C. Microsomes were incubated with the tested substances at concentrations of 1 mg/mL for saponins and 100 μM for flavonoids.

Determination of microsomal protein by the method of Lowry et al. (1957)

To determine the microsomal protein content, a previously prepared standard calibration curve with concentrations of 50, 100, 150, and 200 μg/mL was employed. The development of a blue color was observed upon the addition of the Folin reagent. Absorbance was measured spectrophotometrically at 580 nm, and the results were presented graphically.

Induction of non-enzymatic lipid peroxidation

Lipid peroxidation was induced by the addition of 20 μL $FeSO_4$ (40 μM) and 50 μL ascorbic acid (500 μM).

Determination of malondialdehyde (Deby and Goutier 1990)

The reaction was stopped by the addition of 1 mL of both 25% trichloroacetic acid and 0.67% thiobarbituric acid. Samples were heated at 100 °C for 20 min and centrifuged at 2000 rpm for 10 min. Absorbance was measured at 535 nm, and the MDA concentration was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (for the malondialdehyde–thiobarbituric acid complex), expressed as nmol/mg protein.

Statistical analysis

MEDCALC software was used to perform statistical analyses. Each experiment was performed in triplicate, and values are expressed as the mean of six ($n = 6$). The significance of the data was assessed using the nonparametric Mann–Whitney *U* test. Values of $p < 0.05$, $p < 0.01$, and $p < 0.001$ were considered statistically significant.

Results and discussion

In vitro evaluation of saponins from the roots of *C. bonus-henricus* L. on non-enzyme-induced lipid peroxidation in isolated rat liver microsomes

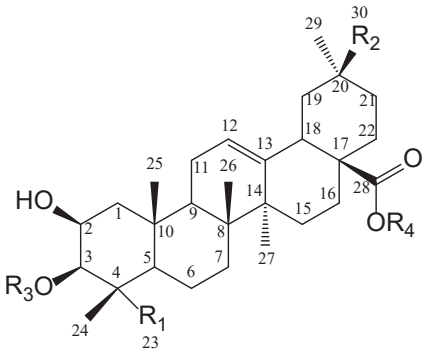
The effects of six saponins previously isolated from the roots of *C. bonus-henricus* L. were evaluated on non-enzyme-induced lipid peroxidation in isolated rat liver microsomes (Kokanova-Nedialkova et al. 2019a) (Fig. 2).

The induction of non-enzymatic lipid peroxidation in microsomes was achieved by introducing the pro-oxidant combination of ferrous sulfate and ascorbic acid. The presence of iron, a metal with variable valence, facilitates the Fenton reaction, which contributes to the generation of free radicals. Ascorbic acid acts as a pro-oxidant at low concentrations and as an antioxidant at high concentrations. It can exist in two forms—oxidized and reduced.

Microsomes were used as a model membrane of a lipid substrate. When administered alone, the six saponins (**ChBh-01**, **ChBh-03**–**ChBh-07**) isolated from the roots of *Chenopodium bonus-henricus* and silymarin (at a concentration of 1 mg/mL) did not exhibit a statistically significant toxic effect on isolated rat liver microsomes. No statistically significant change in malondialdehyde (MDA) levels was observed (Fig. 3).

Administered alone, the combination of ferrous sulfate and ascorbic acid (Fe^{2+}/AA) increased statistically significant MDA production by 28% compared to the control (untreated microsomes).

Under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation, saponins exhibited a statistically significant antioxidant effect comparable to that of silymarin (Fig. 4).



ChBh-01 R_1= COOH $R_2 = CH_3$ R_3 = GluA R_4 = Xyl(1→4)Rham(1→2)Ara
ChBh-03 R_1= CH_2OH $R_2 = COOCH_3$ R_3 = Ara R_4 = Glu
ChBh-04 R_1= CH_2OH $R_2 = CH_3$ R_3 = GluA R_4 = Glu
ChBh-05 R_1= CH_2OH $R_2 = CH_3$ R_3 = Ara R_4 = Glu
ChBh-06 R_1= CHO $R_2 = CH_3$ R_3 = GluA R_4 = Glu
ChBh-07 R_1= CH_3 $R_2 = CH_3$ R_3 = GluA R_4 = Glu

Figure 2. Saponins from the roots of *C. bonus-henricus* L.

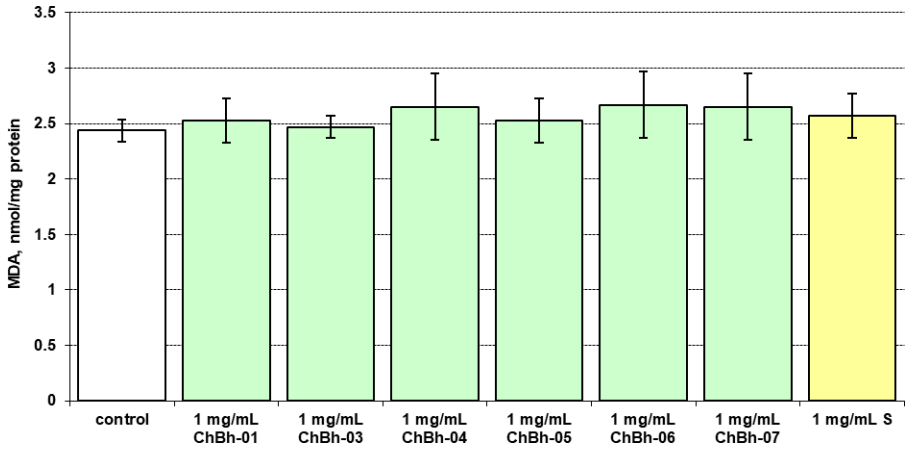


Figure 3. Effects of saponins (**ChBh-01**, **ChBh-03**–**ChBh-07**), isolated from the roots of *C. bonus-henricus*, and silymarin (S) (at a concentration of 1 mg/mL), administered alone to isolated rat liver microsomes.

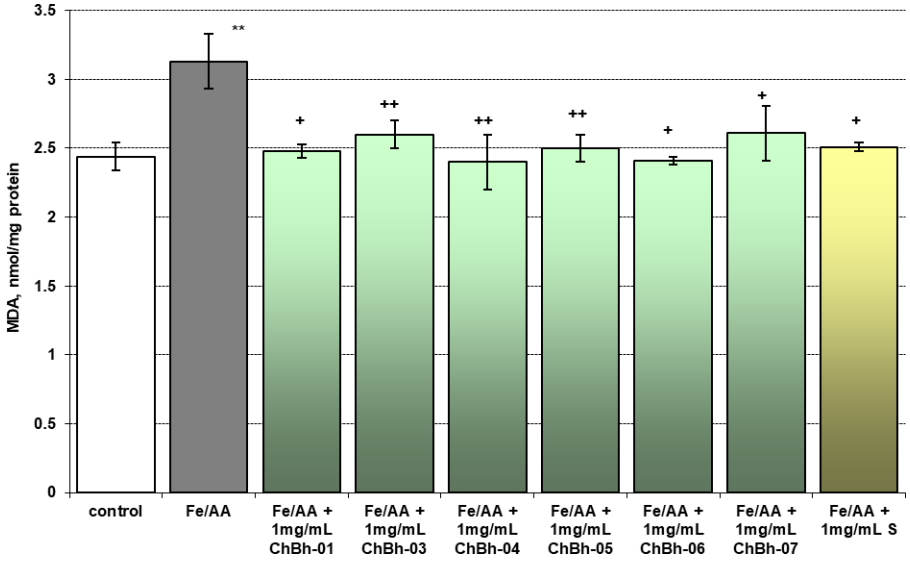


Figure 4. Effects of saponins (**ChBh-01**, **ChBh-03**–**ChBh-07**), isolated from the roots of *C. bonus-henricus*, and silymarin (S) (at a concentration of 1 mg/mL) under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation on MDA production in isolated rat liver microsomes. ** $p < 0.01$ vs. control (untreated microsomes); + $p < 0.05$, ++ $p < 0.01$ vs. Fe^{2+}/AA.

Compound **ChBh-01** statistically significantly reduced malondialdehyde production by 21%, **ChBh-03** and **ChBh-07** by 17%, **ChBh-04** and **ChBh-06** by 23%, and **ChBh-05** by 20%, compared to the iron–ascorbic acid combination. Silymarin (S) reduced malondialdehyde production by 20%.

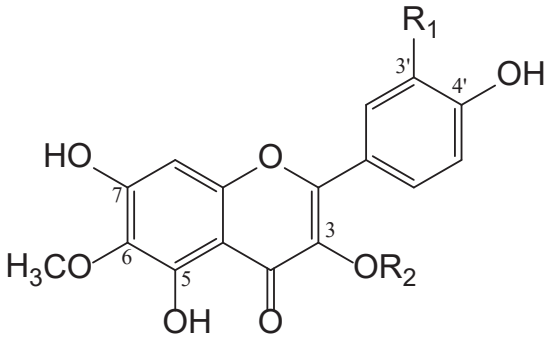
Among the investigated compounds, the saponins of bayogenin (**ChBh-04**) and 2β-hydroxygypsogenin (**ChBh-06**) exhibited the highest antioxidant activity, reducing MDA production by 23%, slightly surpassing the effect of silymarin (20%). Additionally, the saponins of medicagenic acid (**ChBh-01**) and bayogenin (**ChBh-05**) demonstrated substantial protective effects, reducing MDA levels by 21% and 20%, respectively, values comparable to those of the reference standard. The main difference between the two bayogenin saponins (**ChBh-04** and **ChBh-05**) lies in the sugar at the C-3 position: glucuronic acid in **ChBh-04** and arabinose in **ChBh-05**. This small structural difference makes **ChBh-04** slightly more active, although both are comparable to the reference standard, silymarin.

In vitro evaluation of flavonoids from the aerial parts of *C. bonus-henricus* on isolated liver microsomes from young and old rats under conditions of non-enzyme-induced lipid peroxidation

The effects of nine flavonol glycosides of patuletin, 6-methoxykaempferol, and spinacetin, previously isolated from the aerial parts of *C. bonus-henricus* L. (Kokanova-Nedialkova et al. 2017) (Fig. 5), were evaluated on non-enzyme-induced lipid peroxidation in isolated rat liver microsomes.

Microsomes were isolated by repeated differential centrifugation and incubated with flavonoids at a concentration of 100 μM. Malondialdehyde (MDA) production was measured as a biomarker of lipid peroxidation to evaluate the antioxidant potential of the flavonoids. Initially, the effect of age on MDA production was investigated in microsomes derived from young and old rats.

The results demonstrate that MDA production was statistically significantly increased in microsomes isolated from old rats compared to those from young rats by 28%.



A = Glu(1→6)–Glu
B = [Api(1→2)]–Glu(1→6)–Glu
C = (5‴-O-E-FA) Api(1→2)[Glu(1→6)]–Glu

Patuletin glycosides

ChBhnf-1 $R_1 = OH, R_2 = B$
ChBhnf-2 $R_1 = OH, R_2 = A$
ChBhnf-7 $R_1 = OH, R_2 = C$

Spinacetin glycosides

ChBhnf-4 $R_1 = OCH_3, R_2 = B$
ChBhnf-6 $R_1 = OCH_3, R_2 = A$
ChBhnf-8 $R_1 = OCH_3, R_2 = C$

6-Methoxykaempferol glycosides

ChBhnf-3 $R_1 = H, R_2 = B$
ChBhnf-5 $R_1 = H, R_2 = A$
ChBhnf-9 $R_1 = H, R_2 = C$

Figure 5. Flavonoids from the aerial parts of *C. bonus-henricus* L.

Iron/ascorbate (Fe^{2+}/AA)-induced lipid peroxidation in microsomes from young rats resulted in a statistically significant increase in MDA production by 150% relative to the control (untreated microsomes). In microsomes isolated from old rats, this process was more pronounced, with MDA levels increasing by 260% compared to the control (untreated microsomes) (Fig. 6).

In microsomes obtained from young rats under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation, the flavonoids **ChBhnf-01** to **ChBhnf-09**, isolated from the aerial parts of *C. bonus-henricus* L., at a concentration of 100 μM, exhibited a statistically significant antioxidant effect comparable to that of silibinin.

The results indicate that flavonoid **ChBhnf-01** reduced MDA production by 30%, **ChBhnf-02** by 42%, **ChBhnf-03** by 27%, **ChBhnf-04** by 39%, **ChBhnf-05** by 18%, **ChBhnf-06** by 23%, **ChBhnf-07** by 22%, **ChBhnf-08** by 28%, and **ChBhnf-09** by 50% relative to Fe^{2+}/AA-induced levels. Silibinin (at a concentration of 100 μM) significantly reduced MDA production by 48% under the same conditions. Among the tested flavonoids, **ChBhnf-09** (a glycoside of 6-methoxykaempferol) showed the most pronounced effect, exceeding that of the positive control silibinin, which is likely due to the presence of ferulic acid in its sugar moiety. Other flavonoids demonstrated moderate activity, with reductions in MDA production ranging from 18% (**ChBhnf-05**) to 42% (**ChBhnf-02**) (Fig. 7).

On liver microsomes isolated from old rats, under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation, the tested flavonoids **ChBhnf-01**–**ChBhnf-09** (100 μM) exhibited a more pronounced statistically significant antioxidant effect compared to their activity on microsomes obtained from young rats. The effects are comparable to those of silibinin.

The results showed that flavonoids **ChBhnf-01**, **ChBhnf-03**, and **ChBhnf-07** statistically significantly reduced MDA production by 50%; **ChBhnf-02** by 51%; **ChBhnf-04** by 47%; **ChBhnf-05** by 46%; **ChBhnf-06** by 49%; **ChBhnf-08** by 53%; and **ChBhnf-09** by 62% relative to Fe^{2+}/AA. Silibinin (100 μM) statistically significantly reduced MDA production by 61% under the same conditions. Among the studied flavonoids, **ChBhnf-09** again shows the most pronounced effect (62%), slightly higher than the positive control silibinin (61%). Other flavonoids

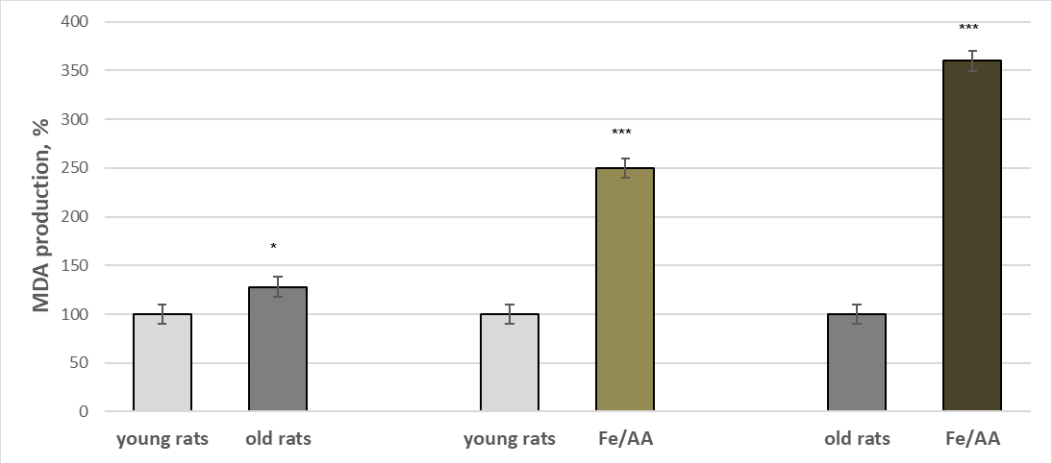


Figure 6. Comparison of microsomal MDA levels between young and old rats (* $p < 0.05$) and MDA levels after Fe^{2+}/AA-induced lipid peroxidation in microsomes from young and old rats relative to the control.*** $p < 0.001$ vs. control (untreated microsomes).

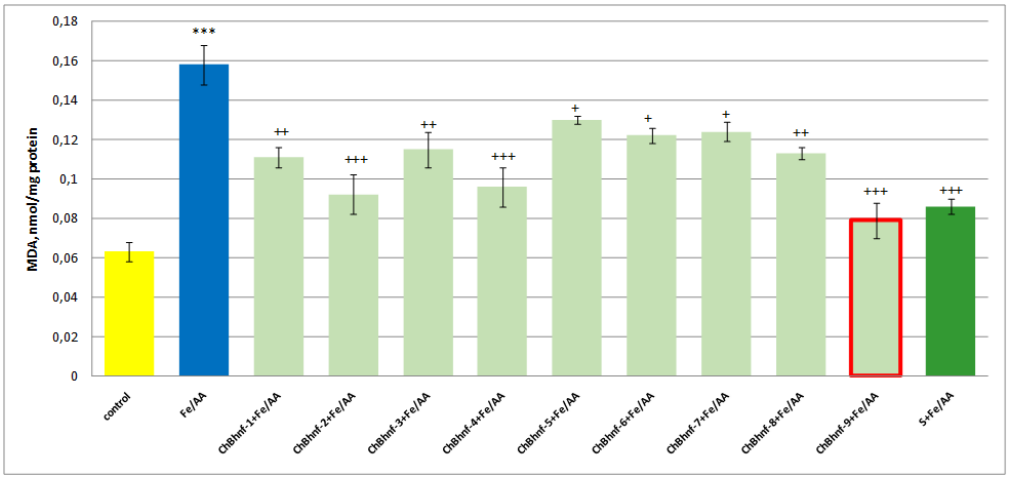


Figure 7. Effects of flavonoids (**ChBhnf-01**–**ChBhnf-09**), isolated from the aerial parts of *C. bonus-henricus*, and silibinin (S) at a concentration of 100 μM under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation on MDA production in isolated rat liver microsomes from young rats. *** $p < 0.001$ vs. control (untreated microsomes); + $p < 0.05$, ++ $p < 0.01$, and +++ $p < 0.001$ vs. Fe^{2+}/AA.

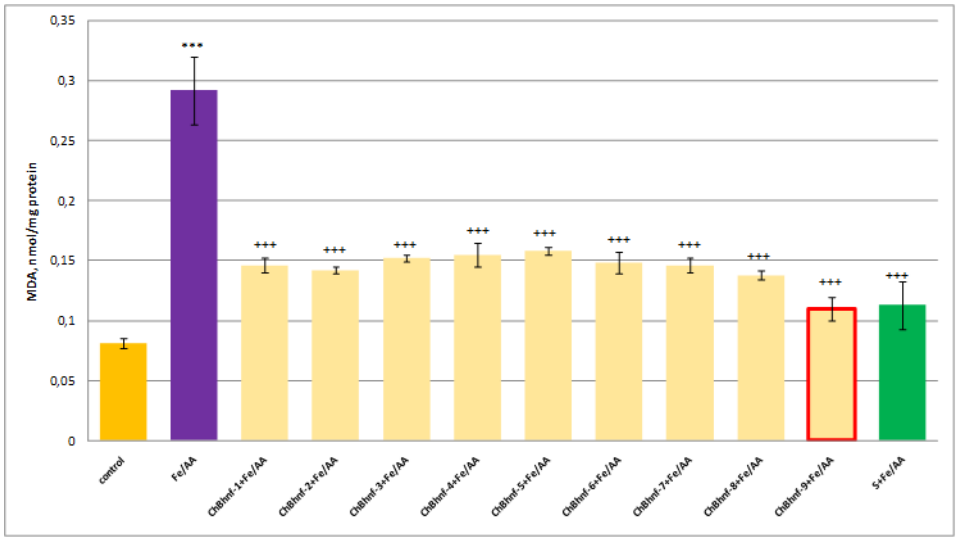


Figure 8. Effects of flavonoids (**ChBhnf-01**–**ChBhnf-09**), isolated from the aerial parts of *C. bonus-henricus*, and silibinin (S) at a concentration of 100 μM under conditions of non-enzyme (Fe^{2+}/AA)-induced lipid peroxidation on MDA production in isolated rat liver microsomes from old rats. *** $p < 0.001$ vs. control (untreated microsomes); +++ $p < 0.001$ vs. Fe^{2+}/AA.

also demonstrated high activity for the reduction of MDA production, ranging from 46% (**ChBhnf-05**) to 53% (**ChBhnf-08**) (Fig. 8).

The antioxidant activity observed for the compounds in this study further supports and confirms previous findings on saponins and flavonoids from *Astragalus* (Stambolov et al. 2025; Kondeva-Burdina et al. 2018) in models of non-enzymatic lipid peroxidation.

Conclusion

The present study demonstrates that under non-enzymatic (Fe^{2+}/AA)-induced lipid peroxidation conditions, saponins (**ChBh-01**, **ChBh-03**–**ChBh-07**) from the roots of *C. bonus-henricus* L. exhibited pronounced antioxidant activity comparable to that of silymarin, a well-established antioxidant and hepatoprotective agent. Notably, the bayogenin (**ChBh-04**) and 2β-hydroxygypsogenin (**ChBh-06**) saponins showed the strongest effects. The results also demonstrate that MDA production during non-enzymatic lipid peroxidation was higher in microsomes from old rats (by 28%) than in those from young rats. Under the same conditions, the flavonoids (**ChBhnf-01**–**ChBhnf-09**) from the aerial parts of the plant exhibited a significant antioxidant effect in microsomes from old rats compared to their effect in microsomes from young rats. The strongest activity was observed for **ChBhnf-09** (a 6-methoxykaempferol glycoside), likely due to the presence of ferulic acid in its sugar moiety.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: The experiments were carried out under Permission No. 304, valid until 28 June 2026, issued by the Bulgarian Food Safety Agency.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

Funding

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.

References

Deby C, Goutier R (1990) New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. Biochemical Pharmacology 39(3): 399–405. https://doi.org/10.1016/0006-2952(90)90043-K

Fukunaga I, Takebe T (2025) *In vitro* liver models for toxicological research. Drug Metabolism and Pharmacokinetics 62: e101478. https://doi.org/10.1016/j.dmpk.2025.101478

Grozeva N (2011) *Chenopodium bonus-henricus* L. (Perennial Goosefoot) in Bulgaria: II. Morphology, Chorology and Ecology. Trakia Journal of Sciences 9(3): 8–12. http://www.uni-sz.bg/tsj/Vol9N3_2011/N.Grozeva_2.pdf

Guengerich FP, Dannan GA, Wright ST, Martin MV, Kaminsky LS (1982) Purification and characterization of liver microsomal cytochromes p-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or beta-naphthoflavone. Biochemistry 21(23): 6019–6030. https://doi.org/10.1021/bi00266a045

Kokanova-Nedialkova Z, Nedialkov P (2017) Antioxidant properties of 6-methoxyflavonol glycosides from the aerial parts of *Chenopodium bonus-henricus* L. Bulgarian Chemical Communications 49(D): 253–258. http://www.bcc.bas.bg/BCC_Volumes/Volume_49_Special_D_2017/BCC2017-49-SE-D-253-258.pdf

Kokanova-Nedialkova Z, Nedialkov P, Kondeva-Burdina M, Simeonova R, Tzankova V, Aluani D (2017) *Chenopodium bonus-henricus* L. – A source of hepatoprotective flavonoids. Fitoterapia 118: 13–20. https://doi.org/10.1016/j.fitote.2017.02.001

Kokanova-Nedialkova Z, Nedialkov P, Kondeva-Burdina M, Simeonova R (2019) Hepatoprotective activity of a purified methanol extract and saponins from the roots of *Chenopodium bonus-henricus* L. Zeitschrift für Naturforschung C 74(11–12): 329–337. https://doi.org/10.1515/znc-2019-0002

Kokanova-Nedialkova Z, Nedialkov PT, Momekov G (2019a) Saponins from the roots of *Chenopodium bonus-henricus* L. Natural Product Research 33(14): 2024–2031. https://doi.org/10.1080/14786419.2018.1483928

Kondeva-Burdina M, Shkondrov A, Simeonova R, Vitcheva V, Krasteva I, Ionkova I (2018) In *vitro/in vivo* antioxidant and hepatoprotective potential of defatted extract and flavonoids isolated from *Astragalus spruneri* Boiss. (Fabaceae). Food and Chemical Toxicology 111: 631–640. https://doi.org/10.1016/j.fct.2017.12.020

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mahajan P, Palkar M, Pingili RB (2024) Drug reactive metabolite-induced hepatotoxicity: a comprehensive review. Toxicology Mechanisms and Methods 34(6): 607–627. https://doi.org/10.1080/15376516.2024.2332613

Nnane IP, Tao X (2005) Encyclopedia of analytical science (2nd Edn.). In: Worsfold P, Townshend A, Poole C (Eds) Drug metabolism: Metabolite isolation and identification. Elsevier, Amsterdam, 305–311. https://doi.org/10.1016/B0-12-369397-7/00109-6

Soldatow VY, Lecluyse EL, Griffith LG, Rusyn I (2013) *In vitro* models for liver toxicity testing. Toxicology Research 2(1): 23–39. https://doi.org/10.1039/C2TX20051A

Stambolov I, Kondeva-Burdina M, Shkondrov A, Krasteva I (2025) *In vitro* activity of two cycloartane-type triterpenoid saponins from *Astragalus glycyphyllos* on liver microsomes, hepatocytes, and isoforms of cytochrome P450. Pharmacia 72: 1–7. https://doi.org/10.3897/pharmacia.72.e152827

Zaidi SI, Agarwal R, Eichler G, Rihter BD, Kenney ME, Mukhtar H (1993) Photodynamic effects of new silicon phthalocyanines: *in vitro* studies utilizing rat hepatic microsomes and human erythrocyte ghosts as model membrane sources. Photochemistry Photobiology 58(2): 204–210. https://doi.org/10.1111/j.1751-1097.1993.tb09550.x