Research Article

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Centaurea cyanus L. Polysaccharides and Polyphenols Cooperation in Achieving Strong Rat Gastric Ulcer Protection

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Abstract: This work was aimed at testing gastroprotective effects of Centaurea cyanus L. (herba) polysaccharides (P) and polyphenols (A) fractions on stress-induced rat ulcer model. Studies evaluating acute toxicity in rats and antioxidant (chemiluminescence method) and antimicrobial (on Staphylococcus aureus ATCC6538 and Escherichia coli ATCC8739 strains) activities of Centaurea cyanus L. (herba) product, which combined polysaccharides and polyphenols fractions (PA), have also been done. Accordingly, in vivo pharmacological studies revealed high influence of PA product (500 mg kg⁻¹ of body weight) on deep, moderate and superficial gastric mucosal lesions, greater than that of chemical reference, Ranitidine. P product was proven more effective than Ranitidine in opposing the emergence of deep necrotic lesions only, suggesting the ability of polysaccharides compounds to consolidate gastric mucous layer as well as their certain tendency for cooperation with polyphenols fractions. The acute toxicity study indicated the lack of toxicity of PA product and maximum tolerated doses greater than 1875 mg kg⁻¹ of body weight. PA product provided augmented scavenger activity and week antimicrobial activity on Staphylococcus aureus ATCC6538 and Escherichia coli ATCC8739 strains, resulting in better opportunities for valorisation of the aerial part of Centaurea cyanus L. species in order to obtain new and effective natural medicines.

Keywords: cornflower, gastroprotective, antimicrobial, acute toxicity

1 Introduction

It is well known that a large amounts of currently active medicines are based on herbal products, isolated compounds, whole or selective vegetal extracts and powders from various plant sections. Thus, although weaker than allopath drugs when given the phytocompounds synergistic activity, plant-derived medicines are seldom more effective in treating human disorders. Moreover, literature data reveals that vegetal and chemical compounds synergism [1] leads to final effects more significantly than either of the compounds alone.

With regards to a gastric protection issue, recent statistical studies [2] indicated that 10% of worldwide population is affected by gastric diseases mainly due to peptic ulcer and gastroesophageal reflux diseases (GERD). The increasing prevalence of chronic inflammatory diseases (such as rheumatoid arthritis, inflammatory bowel disease, etc.) as well as obesity, which both require long term gastroprotective treatment, explains gastric protection issue subject actuality as well as the opportunity for finding new, natural herbal-based alternative or complementary medicines.

Our previous studies [3] described significant gastroprotective activity of a vegetal product obtained through processing Centaurea cyanus L. (herba) plant material. Briefly, pharmacological studies on rats with stress-induced gastric lesions treated with Centaurea cyanus L. (herba) derived product encompassing polysaccharides and polyphenols selective extracts indicated gastroprotective percentages of 100% (p < 0.02), 89% (p < 0.001) and, respectively, 83% (p < 0.001), Student’s t-test, in the specific case of deep, medium and, respectively, superficial mucosal lesions (measured as total length, mm), superior to that offered.
by chemical reference product, Ranitidine, a well-known gastroprotective medicine acting as histamine H₂-receptor inhibitor.

*Centaurea cyanus* L. (*Asteraceae* family) plant species, a common cornflower, bachelor’s button or *Cyanis flores* are rare in the wild. Being a common weed of the cornfields, the over-use of the herbicides leads to the destruction of its habitat: in United Kingdom it was reported that it has declined from 264 sites to just 3 sites in the last 50 years [4]. In Romania, Transylvania region, very recent doctoral thesis [5] reported a number of 22 species and 11 subspecies of *Centaurea* as follows: *C. stoebae* ssp. *stoebae* and *micranthos*, *C. reichenbachii*, *C. diffusa*, *C. jacea* ssp. *angustifolia* and *bannatica*, *C. nigrescens*, *C. pycnogonata*, *C. pycnochlaena*, *C. phrygia* ssp. *phrygia*, *erdneri*, *pseudophrygia*, *indurate*, *carpatica*, *ratezatensis* and *rarauensis*, *C. stenolepis* ssp. *zragradensis* and *stenolepis*, *C. uniflora* ssp. *nervosa*, *C. macroptilon* ssp. *oxylepis*, *C. calcitrapa*, *C. iberica*, *C. solstitialis*, *C. benedicta*, *C. cyanus*, *C. triumfetti* ssp. *axillaries*, *triumfetti*, *adscendens*, *stricta* and *pinnatifida*, *C. mollis* ssp. *mollis*, *C. atropurpurea*, *C. orientalis*, *C. kotschyanus* and *C. scabiosa* ssp. *spinulosa*, three of subspecies, *Centaurea phrygia* L. ssp. *rarauensis* *Phrygia* (Prod.) Dostál and ssp. *ratezatensis* (Prod.) Dostál, and *Centaurea pinnatifida* Schur, respectively being threatened with extinction across Europe.

*Centaurea cyanus* L. phytotherapy has been used as a traditional remedy for a long time and it has been used for tired eyes [6] as well as for its antipruritic, antitussive, astringent, diuretic, emmenagogic, mildly purgative and bitter tonic properties [7,8], also reducing inflammatory process through specific complement components inhibition [9]. The main active compounds are polyphenols, flavonoids and phenylcarboxylic acids such as: apigenin-4’-O-(6-O-malonilglucoside)-7-O-glucuronic, apigenin-4-O-glucoside, apigenin-7-O-glucoside/cosmosin, apigenin-7-O-apio-glucoside/ apiin, methyl-apigenin and methyl-vitexin, cyanidin-3-O-succinylglucoside-5-O-glucoside/centaurocyanin (marker compound), cyanidin-3,5-diglucoside/cyanidin, quercetin-3-O-gluc-ramnoside/rutin, isorhamnetin and isorhamnetin-7-O-glucoside, naringenin and naringenin-7-O-glucorhamnoside [10] aside cis/trans-cafeic acids, orto/para-hydroxiphenylacetic acids, *para*-hydroxibenoic and *para*-coumaric acids as well as sinapic, protocatechuic, chlorogenic, vanillic, syringic, ferulic, salicilic and benzoic [11] acids. Our previous studies [3] on *Centaurea cyanus* L. grown in Romania (studies carried out at different plant locations with use of three extraction solvents; water, ethanol and acetone) indicated five main phenolic derives as follows: quercetin-3-O-glucorhamnoside, quercetin-7-O-glucoside, apigenin-7-O-glucoside, chlorogenic and isochlorogenic acids added to less augmented, or present in specific plant parts only, quercetin-3-O-glucoside, apigenin, protocatechuic acid and two umbelliferone derivates.

This work was aimed at testing and studying correlation between two *Centaurea cyanus* L. (*herba*) plant material extracts, polysaccharides (P) and polyphenols (A) fractions respectively, to achieve gastroprotective activity on *Wistar* rats with gastric lesions produced via stress-induced ulcer model. Additionally, antioxidant activity of P, A and PA products (chemiluminescence method), as well as acute toxicity on rats and antimicrobial potency of PA on *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 microbial strains has also been investigated.

## 2 Experimental Procedure

### 2.1 Materials

#### 2.1.1 Plant material description

*Centaurea cyanus* L. *herba* plant material was purchased from Research Station for Medicinal and Aromatic Plants Fundulea, Ilfov, Romania. The plant material was shade dried and minced into medium-sized powder (3-5 mm). Taxonomic identification was done by the botanist’s team of National Institute of Chemical-Pharmaceutical R&D (ICCF), Bucharest, Romania. Voucher specimens were deposited in ICCF Plant Material Storing Room.

#### 2.1.2 Vegetal extracts preparation

The main purpose of technological studies is to obtain two *selective vegetal extracts*, such as polysaccharides and polyphenols fractions. Another goal is the preparation of a final product through a combination of these two selective extracts to assure exactly 4% (w/w) of flavones content expressed as hyperoside equivalents. The two *selective vegetal extracts* were obtained as follows: fifty (50) grams powder of *Centaurea cyanus* L. *herba* was (heat-assisted) extracted with 750 mL of distilled water using a glass extraction apparatus connected to a stirrer. The resulting aqueous extract was concentrated at low pressure and the concentrate was treated with four volumes of (98%, v/v) methanol. The resulting suspension was kept at cold (4°C) overnight and then filtrated at low pressure. The precipitate was dried in desiccator glass and grinded in
a porcelain herbal medicine grinder, thus resulting in a grey fine powder with 53–56% of polysaccharides content (gravimetrical estimation, w/w), 0.98–1.10% of flavones content (expressed as hyperoside equivalents, w/w) and 1.28–1.32% of phenols content (expressed as caffeic acid equivalents, w/w), further called polysaccharides selective fraction/extract (codified P). Plant material remaining after the first (hot water) extraction was further extracted with (40%, v/v) acetone solvent (it must be noted that acetone solvent usage has been chosen based on previous studies [3] on different Centaurea cyanus L. plant parts and extraction solvents) and the resulting polar extract was concentrated at low pressure. The residue was re-dissolved into 100 mL distilled water and then delipidized with chloroform (3 × 200 mL each operation). The aqueous fraction was concentrated at low pressure and then atomized, thus resulting in a grey fine powder measuring 10–12% total flavones content (hyperoside equivalents, w/w) and 12.2–14.5% total phenols content (caffeic acid equivalents, w/w), further called polyphenols selective fraction/extract (codified A). At the end of the process, the two selective extracts (P and A) where combined to exactly 4% (w/w) of flavones content (expressed as hyperoside equivalents), thus resulting in Centaurea cyanus L. final product (codified PA) made of polysaccharides and polyphenols compounds.

2.1.3 Chemicals, reagents and references

Chemicals (AlCl₃, CH₃COONa, H₂O₂, luminol, 0.2M TRIS-HCl pH 8.5, DMSO), reagents (Folin-Ciocalteau, Natural Product) and solvents (methanol, ethanol, ethyl acetate, formic acid, acetic acid and chloroform) as reference products* quercitrin (> 90%), hyperoside (> 97%), rutin (min. 95%), quercetin (95%), apigenin (> 97%), cosmol (97%), vitexin (> 96%), chlorogenic acid (> 95%), gentisic (95%) and rosmarinic acid (97%) were purchased of Fluka and Sigma-Aldrich Co (Bucharest, Romania).

2.2 Experimental Design

2.2.1 Qualitative Analysis

Studies were performed according to Wagner et al. [12] and Reich et al. [13] with standard setting for vegetal polyphenols assessment: Silica gel 60F254 – HPTLC plates 10 × 10, (Merck); ethyl acetate - acetic acid - formic acid - water (100:12:12:26) solvent system; Sigma-Aldrich polyphenols references (prepared as 10⁻³ M solution in ethanol 70%, v/v); Natural Product (NP) and PEG4000 identification reagents; exposure at UV 366 nm. Briefly, volumes of 1–5 μL test vegetal extracts were loaded at 8 mm band length in 10 × 10 cm Silica gel 60F HPTLC plate; similarly, the reference compounds mixtures prepared at 10⁻¹ M solutions in 70% (v) ethanol. The loaded plate was then kept in TLC twin developing chamber at 18–19°C with respective mobile phase (solvent system) up to 90 mm. The developed plate was dried using a hair dryer and then immersed into identification reagents (Natural Product followed by PEG4000). The dried plate was next disposed into Photo-documentation chamber (Linomat 5) and the images captured at UV 366 nm. Polyphenols spots were assigned by comparison with references and Centaurea cyanus L. plant product literature data.

2.2.2 Estimation of Total Flavonoids Content

Total flavonoids were measured according to FRX (Romanian Pharmacopoeias, Xth edn. [14]), standard method. Briefly, 5.000 g of each, P, A and PA vegetal products were (heat assisted) extracted two times, separately, with 50 ml 50% (v/v) ethanol. The resulting ethanolic extracts were mixed together and finalized at 100 mL final volume with 50% (v/v) ethanol solution, thus resulting test extractive solutions (solution E). Subsequently, three aliquots of 25–100 μL solution E were treated with 600 μL of (2.5% w/v) AlCl₃ and 1000 μL of 10% (w/v) CH₃COONa and then finalized at 5000 μL with (50%, v/v) ethanol. Mixtures were incubated at room temperature for 30 minutes and absorbance of reactions at maximum wavelength (λ) 418 and 428 nm were measured. Total flavonoids content was estimated using hyperoside (ref.) standard calibration curve (r² = 0.9998) and the results were expressed in grams of total flavones (hyperoside equivalents) per 100 g vegetal product.

2.2.3 Estimation of Total Phenolics Content

Total phenolics were measured according to FRX [14], Folin-Ciocalteu method. Briefly, three aliquots of 50–500 μL solution E were treated with 200 μL of Folin-Ciocalteu reagent and completed at 5000 μL flasks with (5% w/v) sodium carbonate. Flasks were mixed and left in the dark place at room temperature for exactly 5 minutes, then absorbance at λ = 750 nm was measured. Total phenols content was estimated using caffeic acid
standard calibration curve (r² = 0.9997) and the results were expressed in grams total phenols (caffeic acid equivalents) per 100 g vegetal product.

### 2.2.4 Estimation of the Content of Minerals and Trace Elements

Minerals and trace elements (microelements) content was estimated using Atomic Absorption Spectrometry (AAS) method. Briefly, 1 g of vegetal raw material (*Centaurea cyanus* L. *herba*) was treated with 7 mL (65%) HNO₃, and 2 mL (30%) H₂O₂ then digested using the appropriate program (supplier’ instructions). The digested samples (triplicates) were then carefully transferred to 50 mL volumetric flasks using double distilled water solvent. The resulting samples were analyzed to determine the content of minerals and trace elements as follows; Fe, Mn, Zn, Cr, Cd and Pb elements were measured by directly reporting at standard calibration curve (dilution series 5, 25, 50, 100, and 250 × 10⁻³ mg mL⁻¹) while Na, K, Ca and Mg elements were measured after the treatment with lanthanum chloride as intermediary ionization step (dilution series 0, 0.4, 1, 2, 4, 6, 8 and 10 mg mL⁻¹).

### 2.2.5 In vitro Antioxidant Activity Study (Luminol-enhanced Chemiluminescence/CL Assay)

Antioxidant activity studies were performed according to N. Iftimie et. al method [15]. Briefly, aliquots (triplicates) of 50 μL test sample (prepared by dissolving 1 g vegetal product in 100 mL distilled water and 100 mL DMSO solvent) were mixed with 200 μL 10⁻³ M luminol (prepared in DMSO), 700 μL 0.2 M – TRIS-HCl pH 8.6 and 50 μL 10⁻¹ M H₂O₂ (prepared in bi-distilled water). In parallel, a reference sample (triplicate) consisting of 50 μL of the sample solvent (distilled water and DMSO) was mixed with 200 μL 10⁻³ M luminol, 700 μL 0.2 M – TRIS-HCl pH 8.6 and 50 μL 10⁻¹ M H₂O₂. Five seconds after reaction initiation, chemiluminescence reaction intensity (quantified in activity units/a.u.) of each vegetal sample and reference sample have been measured. Antioxidant activity (AA%) calculated (see formula). Antioxidant activities (AA%) of the three vegetal samples (P, A and PA) were measured in five dilution series (∗1, ∗2, ∗10, ∗50, ∗100, n = 3). IC₅₀ values (defined as concentration of total phenols content (in μg) expressed as caffeic acid equivalents per 1 mL sample, that inhibits 50% of luminal radicals) were calculated.

\[
AA\% = \frac{\text{CL intensity r.s.} - \text{CL intensity t.s.}}{\text{CL reaction intensity of the reference sample (a.u.)}} \times 100
\]

Where:
- CL intensity r.s. - CL reaction intensity of the reference sample [a.u.];
- CL intensity t.s. - CL reaction intensity of the tested sample [a.u.]

### 2.2.6 Antimicrobial Activity Assay

**Test organisms:** The tests were carried out using cylinder method in plates and two microorganisms: a Gram-negative bacterium (*Escherichia coli* ATCC 8739), and a Gram-positive bacterium (*Staphylococcus aureus* ATCC 6538). The test organisms were purchased from Meconti s.à r.l. through their distributor in Romania (Merck Romania S.R.L.).

**Test extracts:** Studies carried out on *Centaurea cyanus* L. product made of polysaccharides and polyphenols fractions (PA) passed in (20%, v/v) propylene glycol solution to assure 4 mg phenols content (expressed as caffeic acid equivalents) per 1 mL sample.

**Inoculum preparation:** The bacterial strains were activated by passaging the cells on Casein soya bean digest agar medium (CaSoA) and incubating for 18‒24 hours at 30‒35°C. After the incubation period, 1‒2 colonies were collected with a sterile loop and added in sterile distilled water in order to obtain microbial suspensions. The turbidity of these suspensions was adjusted to match the turbidity standard of 0.5 Mc Farland units corresponding to 1×10⁸ colony-forming units per millilitre (CFU mL⁻¹).

**Antimicrobial assay:** The tests were performed according to FRX [14] in sterile Petri dishes, each containing 15‒20 mL of CaSoA previously inoculated with 10⁻¹⁰ CFU mL⁻¹. Accordingly, 4 stainless steel cylinders of 8 mm diameter were placed on a solidified surface of the medium, in each dish. Afterwards, 0.2 mL of the sample was added to each cylinder as follows: one cylinder contained 20% propylene glycol reagent (the solvent in which the plant extracts were passed) and the other 3 contained triplicates of the vegetal sample (PA). The Petri dishes were then incubated for 18‒24 hours at 30‒35°C. After the incubation period, the growth inhibition zones were measured and the results were expressed as the arithmetic mean of three measurements for the same sample (triplicates). Antimicrobial activity was calculated on basis of the diameter of the growth inhibition in the following manner: (< 10 mm) – no antimicrobial activity; (10–15 mm) – weak antimicrobial activity; (16–20 mm)
moderate antimicrobial activity; (> 20mm) – certain antimicrobial activity.

2.2.7 In vivo Pharmacological Studies

Stress-induced rat ulcer model experiment: In vivo pharmacological studies were carried out on Wistar albino rats, male, of 180–200 g purchased from an authorized supplier, in accordance with the Romanian National Agency of Medicines and Medical Devices Informative Bulletins and FELASA guidelines and recommendations. Tests were done on two vegetal products, *Centurea cyanus* L. polysaccharides fraction (P) and *Centurea cyanus* L. final product combining polysaccharides and polyphenols fractions (PA) comparatively to one chemical reference, Ranitidine (R), an inhibitor of histamine h2 receptor known as controlling the main pathway of (human) gastric acid synthesis.

Briefly, animals were first accommodated in a controlled environment at (22 ± 2)°C and (55 ± 10)% humidity with 12h light-dark cycle and fed with standard pellet food; water *ad libitum*. Stress-induced rat ulcer model experiment was developed as follows: animals were fasted overnight and the next morning immobilized and immersed into cold water on dorsal position for four hours (the preliminary studies indicated four hours to be the required time necessary to achieve moderate to augmented rat gastric lesions and no mortality cases). In the specific case of animals treated with vegetal products (P and PA) or chemical reference (R), one hour before the stress experiment the animals received *per oral* (p.o.) the respective dose of test/reference product and then were stressed as described earlier. At the end of the four hours stress experiment, animals were anesthetised and killed. The stomachs were excised and washed with distilled water. The four PA doses (250, 500, 1250 and 1875 mg PA kg⁻¹) were solved into a minimum quantity of distilled water. The four PA doses were administered as single dose (p.o.); it must be noted that the dose, 1875 mg PA kg⁻¹, was selected on the basis of PA's physical properties and maximal administrable dose in rats. The animals were observed periodically for toxicity signs and death within 24 h and daily for next 14 days, finally estimating PA potential toxicity.

Statistical analysis: Chemical quantitative analytical and microbiological results were expressed as means along standard deviation (SD) of three measurements. *In vivo* pharmacological gastroprotective activity was considered on basis of One-Way ANOVA analysis.

The four animals’ groups were as follows:

*Group 1* (control group/C) – Fasted animals were four hours stressed by immobilization. After that they were anesthetised and killed and the total lengths of each deep, medium and superficial gastric lesions were measured.

*Group 2* (group treated with chemical reference/R) – One hour before stress experiment, fasted animals received (p.o.) the human corresponding dose of the chemical reference drug, *Ranitidine*, 27 mg kg⁻¹ of body weight, respectively. Animals were then stressed for four hours and, at the end of the stress experiment, animals were anesthetised and killed and the total lengths each, deep, medium and superficial gastric lesions were measured.

*Group 3* (group treated with *Centurea cyanus* L. polysaccharides fraction/P) – Similarly, one hour before the stress experiment, fasted animals received (p.o.) the vegetal product (P), 500 mg kg⁻¹ of body weight. After that, animals were 4 hours stressed and then anesthetised and killed and the total lengths of each gastric lesion have been measured.

*Group 4* (group treated with *Centurea cyanus* L. product combining polysaccharides and polyphenols fractions/PA) – One hour before the stress experiment, fasted animals received (p.o.) the vegetal product (PA) at identical dose, 500 mg kg⁻¹ of body weight. Further, animals were stressed for four hours and later they were anesthetised and killed and the total lengths of each, deep, medium and superficial gastric lesions were measured.

It must be noted that the dose of 500 mg vegetal product *per* 1 kg of body weight resulted from prior, dose-effect studies on doses of 200, 500, 1250 and 1875 mg PA kg⁻¹ body weight.

Acute toxicity assessment: These studies were aimed at testing *Centurea cyanus* L. *herba* derived product PA acute toxicity on rats. Briefly, tests were done on five groups of *Wistar* rats (200 ± 15 g), male and female, and four PA doses solved into a minimum quantity of distilled water. The four PA doses (250, 500, 1250 and 1875 mg PA per kg body weight) were administered as single dose and p.o.; it must be noted that the end dose, 1875 mg kg⁻¹, was selected on the basis of PA's physical properties and maximal administrable dose in rats. The animals were observed periodically for toxicity signs and death within 24 h and daily for next 14 days, finally estimating PA potential toxicity.

2.3 Instruments

The instruments used in studies were: CAMAG TLC Visualizer Linomat 5 instrument (Switzerland) used for qualitative (HP)TLC studies; UV/vis Hélios γ Thermo Electron Corporation spectrophotometer (UK) used for quantitative analytical studies; AAS-Vario γ, Analytik Jena (Germany) instrument used for atomic absorption assay and Turner BioSystems (SUA) 20/20® Luminometer
equipment used to estimate antioxidant activity potency of the test samples.

3 Results and Discussion

3.1 Analytical results

Fig. 1 shows chemical qualitative composition (TLC method) in polyphenols of *Centaurea cyanus* L. (herba) polysaccharides fraction (P) and *C. cyanus* L. (herba) polyphenols fraction (A), comparable to references (ref.) [3]. As shown in Fig. 1, *Centaurea cyanus* L. polyphenols fraction (T7-T8 tracks) has five main polyphenols species, likely rutin (s3), chlorogenic acid (s4), quercetin-7-O-glucoside (s7), apigenin-7-O-glucoside (S8) and isochlorogenic acid (s9), quercetin derivate being the major spot. *Centaurea cyanus* L. polysaccharides fraction (T5-T6 tracks) indicated a more decreased number and quantities of polyphenols species, chlorogenic acid (s4) – major spot added to only traces of rutin (s3), quercetin-3-O-glucoside (s6) and protocatechuic acid (s10).

It must be noted that previous analytical (HP)TLC studies [3] on different *Centaurea cyanus* L. plant parts (flores and herba) and extraction solvents (tinctures versus crude aqueous, ethanolic and acetonic extracts) also revealed the occurrence of neo- and isochlorogenic acids, apigenine and two coumarins compounds (s1, s2), likely umbelliferone derivate, some of the fewest coumarins species ascribed with beneficial, antioxidant and anti-inflammatory properties [16-18].

Quantitative AAS study of *Centaurea cyanus* L. (herba) was done on three charges of 1 g plant material each and the results (Table 1) represent the mean values of three replicates ± standard deviation (S.D.).

The results indicate the presence of K, Ca, Mg, Na, Fe, Mn and Zn (micro)elements in an equilibrate ratio (more K and Ca and less Na) and no heavy metals (ex., Cr, Cd, Pb) thus suggesting the opportunity for valorisation of the aerial part (herba) of *Centaurea cyanus* L. species as a natural source of (micro)elements.

3.2 Antioxidant activity results

Table 2 presents comparative IC50 values (μg total phenols content expressed as caffeic acid equivalents per 1 mL sample) of the three *Centaurea cyanus* L. herba derived products (P, A and PA); tests were done using two solvents, dimethyl sulfoxide (DMSO) and distilled water.

![Figure 1](image)

**Figure 1:** (HP)TLC aspects [3] of *Centaurea cyanus* L. (herba) polysaccharides and polyphenols fractions comparable to reference compounds (ref.). Track T1, quercetin-3-O-glucorhamnoside/rutin, apigenin-8-C-glucoside/vitexin, apigenin-7-O-glucoside/cosmosiin and gentisic acid (ref.); Track T2, rutin, quercetin-3-O-galactoside/hyperoside and quercetin (ref.); Track T3, rutin, chlorogenic acid, cosmosiin and apigenin (ref.); Track T4, rutin, chlorogenic acid, hyperoside, cosmosiin, quercetin-3-O-rhamnoside/quercitrin, rosmarinic acid and quercetin (ref.); Tracks T5-T6, *Centaurea cyanus* L. (herba) polysaccharides fraction (P); Tracks T7-T8, *Centaurea cyanus* L. (herba) polyphenols fraction (A).

Table 1: (Micro)Element concentrations (mg kg⁻¹ ± S.D., n = 3) in *Centaurea cyanus* L. (herba) plant material

<table>
<thead>
<tr>
<th>(Micro)Elements</th>
<th>Mean ± S.D (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>57533 ± 2589</td>
</tr>
<tr>
<td>Ca</td>
<td>24640 ± 229</td>
</tr>
<tr>
<td>Mg</td>
<td>2700 ± 32.4</td>
</tr>
<tr>
<td>Na</td>
<td>190 ± 4.75</td>
</tr>
<tr>
<td>Fe</td>
<td>175 ± 5.42</td>
</tr>
<tr>
<td>Mn</td>
<td>61.334 ± 0.25</td>
</tr>
<tr>
<td>Zn</td>
<td>51.970 ± 0.08</td>
</tr>
</tbody>
</table>

Table 2: Comparative IC50 values of *Centaurea cyanus* L. herba derived products

<table>
<thead>
<tr>
<th>Tested extracts</th>
<th>IC50 (μg mL⁻¹)</th>
<th>DMSO</th>
<th>water</th>
<th>R²</th>
<th>DMSO</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Centaurea cyanus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polysaccharides fraction (P)</td>
<td>9.10</td>
<td>6.50</td>
<td>0.9866</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polyphenols fraction (A)</td>
<td>3.12</td>
<td>3.12</td>
<td>0.9993</td>
<td>0.9995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>combined product (PA)</td>
<td>4.20</td>
<td>4.20</td>
<td>0.9919</td>
<td>0.9960</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2 shows maximum antioxidant (AA%) activities for each P, A and, PA *Centaurea cyanus* L. herba derived products at respective dilution series: (× 10) P, (× 50) A and (× 2) PA.
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IC₅₀ assessment (Table 2) indicates that all tested extracts are potent antioxidant agents, with polyphenols fraction (A) showing the best scavenging activity (IC₅₀ = 3.12 μg mL⁻¹ in both, DMSO and distilled water). Correspondingly, scavenger potency of polysaccharides fraction (P) was weaker than that of polyphenols fraction (A) and different in the two studied solvents (IC₅₀ = 6.5 μg mL⁻¹ in distilled water and, IC₅₀ = 9.10 μg mL⁻¹ in DMSO solvent, respectively); the combined product (PA) emphasized an intermediary value, also identical in both solvents (IC₅₀ = 4.20 μg mL⁻¹).

Antioxidant activity comparison (Fig. 2) between the three vegetal products (P, A and PA), prepared in water and DMSO, confirmed their high scavenger potential by revealing maximum antioxidant activities (AA% values) ranging from 85% to 99%. Moreover, studies indicated that antioxidant potency of polysaccharides fraction (P) significantly amplified in water referring to that in DMSO versus the identical combined product (PA) antioxidant activity in water and DMSO clearly suggesting the major role of the polyphenols fraction (A) in achieving augmented antioxidant activity of the (PA) product.

### 3.3 Microbiological results

Studies were aimed at antimicrobial activity assessment of *Centaurea cyanus* L. (*herba*) polysaccharides (P) and polyphenols (A) fractions versus combined (PA) product in two solvent systems, water and DMSO (mean value, n = 3).

![Figure 2](image.png)

Figure 2: Comparative antioxidant activity of *Centaurea cyanus* L. (*herba*) polysaccharides (P) and polyphenols (A) fractions versus combined (PA) product in two solvent systems, water and DMSO (mean value, n = 3).

<table>
<thead>
<tr>
<th>No. Test Sample</th>
<th>Test bacteria</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Centaurea cyanus L. combined product (PA)</td>
<td>Staphylococcus aureus ATCC 6538</td>
<td>11.5 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli ATCC 8739</td>
<td>10 ± 1.15</td>
</tr>
<tr>
<td>2 Oxaciline</td>
<td>Staphylococcus aureus ATCC 6538</td>
<td>22 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli ATCC 8739</td>
<td>23 ± 0.32</td>
</tr>
</tbody>
</table>

Values are the inhibition zone (mm) ± S.D. of three replicates; Where: diam. < 10 mm means no activity, diam. 10–15 mm means weak activity, diam. 16–20 mm means moderate activity, and diam. > 20 mm means certain activity.

The results indicated that the PA product was providing week antimicrobial activity on both, *S. aureus* ATCC 6538 and *E. coli* ATCC 8739 confirming *Centaurea cyanus* L. derived products use in mild, eye and urinary infections.

### 3.4 Pharmacological results

*In vivo* pharmacological, gastroprotective activity studies were carried out on four groups of *Wistar* rats: group 1 – control group (C); group 2 – group treated with chemical reference product, *Ranitine* (R); group 3 – group treated with *Centaurea cyanus* L. (*herba*) polysaccharides fraction (P); group 4 – group treated with *Centaurea cyanus* L. *herba* derived product combining polysaccharides and polyphenols fractions (PA).

Studies (Figs. 3-5) indicated that immobilisation and immersion into cold water and dorsal position (stress-induced rat ulcer model) caused important rat gastric damages, as the control group (C) proved; there was a total...
measured 2.55 ± 0.09 mm of deep necrotic lesions (DNL),
11.57 ± 0.60 mm total length of moderate haemorrhagic
lesions (MHL) and 18.35 ± 0.29 mm total length of superficial
mucosal irritations (SMI). Comparatively, the group
treated with chemical reference product, (R), indicated
0.51 ± 0.32 mm total length of deep necrotic lesions (DNL),
2.20 ± 1.13 mm total length of moderate haemorrhagic
lesions (MHL) and 3.78 ± 0.60 mm total length of superficial
mucosal irritations (SMI), while the groups (P) and (PA),
treated with C. cyanus herba derived products, shown
0.12 ± 0.06 vs. 0.00 mm total length of deep necrotic
lesions (DNL), 4.48 ± 1.26 vs. 0.99 ± 0.13 mm total length
of moderate haemorrhagic lesions (MHL) and 4.8 ± 0.83
vs. 0.73 ± 0.46 mm total length of superficial mucosal
irritations (SMI), p < 0.001.

The comparison with control group (C), One-Way
ANOVA unifactorial analysis applied to compare mean
depth necrotic lesion (DNL) values (Fig. 3) of the four rat
groups (C = 2.55 mm, R = 0.51 mm, P = 0.12 mm, and
PA = 0.00 mm; F = 977.883; p < 0.001 < α = 0.05) together with
the Post Hoc - Multiple Comparisons/Tamhane analysis
(Levene Statistic = 5.43, df1 = 3, df2 = 20, p = 0.007 < α = 0.05
for Test of Homogeneity of Variances) respectively,
demonstrated significant statistical differences among
values (p < 0.001) as well as high effectiveness of Centaurea
cyanus L. herba derived products, P and PA, more effective
than chemical reference, Ranitidine (R).

In the specific case of moderate haemorrhagic lesion
(MHL) (Fig. 4), One-Way ANOVA unifactorial analysis
applied to compare the mean values of the four rat groups
(C = 11.58 mm, R = 2.20 mm, P = 4.48 mm, and PA = 0.99 mm;
F = 1352.42; p < 0.001 < α = 0.05) together with the
Post Hoc – Multiple Comparisons/Tamhane analysis
(Levene Statistic = 7.75, df1 = 3, df2 = 20, p = 0.001 < α = 0.05
for Test of Homogeneity of Variances) also demonstrated
significant statistical differences among values (p < 0.001)
and the superiority of the vegetal product made
of Centaurea cyanus L. herba polysaccharides and
polyphenols fractions, PA; the product containing
polysaccharides fraction only, P, has been shown less
effective than both, chemical reference product, R and
vegetal combined product, PA.

One-Way ANOVA unifactorial analysis applied to
compare the mean values of the superficial mucosal
irritations (SMI) (Fig. 5) of the four rat groups (C = 18.36 mm,
R = 3.78 mm, P = 4.76 mm, and PA = 0.73 mm; F = 10245.10;
p < 0.001 < α = 0.05) together with the Post Hoc - Multiple
Comparisons/Bonferroni analysis (Levene Statistic = 1.081, df1 = 3, df2 = 20, p = 0.380 > α = 0.05 for Test of
Homogeneity of Variances) demonstrated significant
statistical differences among values (p < 0.001) and,
similarly, high effectiveness of Centaurea cyanus L. herba
derived product with polysaccharides and polyphenols
fractions, PA; the product containing polysaccharides
fraction, P, has also been proven less effective than both,
reference, R and vegetal PA product.

Summarizing all of the results, in vivo pharmacological
studies clearly revealed high effectiveness of Centaurea
cyanus L. (herba) derived product made of polysaccharides
and polyphenols fractions (PA) on stress induced deep,
moderate and superficial gastric mucosal lesions,
even greater than that of chemical reference product,
Ranitidine (R). Comparatively, Centaurea cyanus L.
polysaccharides product (P) has been proven less effective
than PA product in all studied cases of deep, moderate
and superficial gastric mucosal lesions, but more effective
than Ranitidine (R) in opposing the development of deep
necrotic lesions. Given that, it was concluded that the ability of *Centaurea cyanus* L. polysaccharides compounds to consolidate gastric mucous layer as well as their certain cooperation with polyphenols fraction in achieving strong rat gastric ulcer protection, was most probable through their antioxidant and cytoprotective combined effects.

Furthermore, the acute toxicity study indicated the lack of toxicity of *Centaurea cyanus* L. (*herba*) combined product (PA); no toxicity signs and death during the oral acute experimental study occurred, the maximum tolerated dose on rats of both sexes being evaluated at levels as greater than 1875 mg kg⁻¹ body weight.

Other example of plant-derived product with certain gastroprotective properties refers to *Sida corymbosa* R. E. Fries (*Malvaceae* family) water extract [19]; the water extract demonstrated a dose-dependent (250, 500 and 1000 mg kg⁻¹ body weight, p < 0.05) activity in reducing ulcer indices on both, ethanol and diclofenac rat ulcer models. Moreover, results indicated the reduction of the severity of haemorrhagic gastric lesions promoting wound healing and reducing inflammation, all attributed to synergistic effects of *Sida corymbosa* R. E. Fries compounds.

Also, the methanol extract of leaves of *Calamintha officinalis* Mill. (*Lamiaceae* family) [20], shown as containing numerous polyphenols compounds such as eriocitrin, eriodictyol, acacetin, linarin, benzoic, caffeic, chlorogenic and p-coumaric phenolic acids as well as catechins and terpenes, tested on rats with ethanol-induced ulcer indicated a gastroprotective activity comparable to that of the reference chemical product, sucralfate. It has also been concluded that the gastroprotective properties of *Calamintha officinalis* Mill. are on basis of phytochemicals synergistic activity added to antioxidant properties, known as playing an important role by removing damaging agents from gastric mucosa.

Concerning plant compounds gastroprotective mechanism, studies indicated quercetin derivates [21-23] as well as naringenin [24-26], (+)-cyanidin [27] and myricetin [28] derivates as being effective gastric acid synthesis inhibitors through inhibiting histidine decarboxylase activity and histamine release. On the other hand, caffeoylquinic acid derivates have been associated, besides augmented antioxidant properties [29], with gall bladder regulatory properties [26], an important threat for gastric mucosa integrity in the case of non-compliance with digestion sequences. Furthermore, some specific plant polysaccharides are associated with anti-inflammatory properties, including *Cyani flores* polysaccharides [9], and also with mucous tissue bioadhesive and cytoprotective effects [30], all proving real gastroprotective benefits of some particular plant-derived products. Table 4 presents gastroprotective mechanism of some specific plant compounds.

### 4 Conclusions

This work was aimed at testing *Centaurea cyanus* L. (*herba*) polysaccharides (P), polyphenols (A), their combined (PA) gastroprotective effects on stress-induced rat ulcer and their impact on the overarching model. Additionally, acute toxicity study on rats and antioxidant and antimicrobial activity on standard *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 strains has been investigated.

Chemiluminescence’s studies indicated high scavenger potency of all tested vegetal products (P, A and PA) and the major role of the polyphenols fraction (A) in achieving augmented antioxidant activity of the (PA) product. In vivo pharmacological studies clearly revealed high effectiveness of (PA) product on stress-induced deep, moderate and superficial rat gastric lesions, greater than that of reference product, Ranitidine (R). By comparison, polysaccharides product (P) has been proved less effective than (PA) product on all tested lesions, but more effective than reference product (R) in opposing the emergency of deep gastric mucosal lesions; it must be reminded that while polysaccharides product (P) encompassed 1% (mean value, w/w) total flavones expressed as hyperoside equivalents, the combined product (PA) was made so as to assure 4% (w/w) total flavones content (hyperoside equiv.). Also, acute toxicity studies on (PA) product indicated maximum tolerated doses greater than 1875 mg kg⁻¹ body weight.
Table 4: Examples of plant compounds and their most probable gastroprotective mechanisms

<table>
<thead>
<tr>
<th>Gastroprotective mechanism</th>
<th>Biochemical pathway</th>
<th>Vegetal compounds acting through respective pathway</th>
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<tbody>
<tr>
<td>Gastric acid synthesis inhibition</td>
<td>Histidine decarboxylase inhibition</td>
<td>Naringenin [24,25,26], quercetin [21,22,23], (+)-3-cyanidol [27], myricetin [28].</td>
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<td></td>
<td>Proton pump (H+/K+ATPase) inhibition</td>
<td>7-hydroxycoumarin/umbelliferone [31], genistein [46].</td>
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<td></td>
<td>Stimulation of synthesis of mucosal hexosamine</td>
<td>Naringenin [25].</td>
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<tr>
<td>Gastric mucosa strengthening</td>
<td>Mucopolysaccharides layer (chemical) adhesion</td>
<td>Polysaccharides (mucilages and pectines) from Althaea officinalis, Plantago lanceolata, Malva moscata, Tilia cordata, Calendula officinalis [30].</td>
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<tr>
<td></td>
<td>Mucopolysaccharides layer (physical) protection</td>
<td>Polysaccharides (pectines and arabinogalactans) from Aloe barbadensis [32], Panax ginseng [33,34], Acmella oleracea [35,36], Bupleurum falcatum [37,38].</td>
</tr>
<tr>
<td></td>
<td>Stimulation of synthesis of antioxidant compounds</td>
<td>Genistein, daidzein, kaempferol and biochanin A increase metallothionein level [39].</td>
</tr>
<tr>
<td>Antioxidant, anti-inflammatory effects</td>
<td>Stimulation of activity of antioxidant enzymes</td>
<td>Naringenin and (+)-catechin increase cytosolic glutathione S-transferase activity [40].</td>
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<tr>
<td></td>
<td>Lipid peroxidation inhibition</td>
<td>Quercetin, rutin, hesperetin, naringenin [41,42,43].</td>
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<tr>
<td></td>
<td>Stimulation of PGE₂ synthesis</td>
<td>Generally, flavones and flavanones sub-classes [44].</td>
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<td></td>
<td></td>
<td>Ponciretin, hesperetin, naringenin and diosmetin [45], tectorigenin, genistein, irigenin [46] as well as gallic acid derivates [47], epigallocatechingallates and epicatechingallates from green tea [48-49] or magnolol, berberine and cinnamic acid [50].</td>
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<td>Polysaccharides from Alcea rosea and Alcea flavovirens enhance gastric tissue RNA synthesis;</td>
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<td></td>
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<td>Rutin and Quercetin flavonols have stimulatory effects on gastric tissue DNA, respectively, DNA and RNA synthesis [51].</td>
</tr>
<tr>
<td>Antimicrobial effect</td>
<td>Anti-Helicobacter pylori activity</td>
<td></td>
</tr>
<tr>
<td>Gastric tissue regeneration</td>
<td>Stimulation of DNA and RNA synthesis at the level of gastric tissue</td>
<td></td>
</tr>
<tr>
<td>Gastric muscle relaxing effect</td>
<td>Adrenaline relaxing effect prolongation</td>
<td>Luteolin aglycone [52]</td>
</tr>
</tbody>
</table>

References


