Vegetal extracts with gastroprotective activity. Part. I. Extracts obtained from *Centaurea cyanus* L. raw material

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Abstract

The aim of this study was the obtaining and characterization of two selective vegetal extracts based on polysaccharides and, respectively, polyphenols fraction isolated from *Centaurea cyanus* L. raw material in order to design one new, natural gastroprotective product.

Qualitative studies (HPTLC method) performed on the tinctures and some crude aqueous, ethanol and acetone extracts obtained from *Centaurea cyanus* raw material (flower head and aerial part) revealed the superiority of crude extracts and, respectively, of acetone solution as concerning polyphenols extraction; have been revealed the occurrence of quercetin, apigenin and caffeic acid derivates, known for the beneficial effect on different type of gastric injuries. More, HPTLC studies made on polysaccharides fractions isolated by the processing of crude aqueous extract revealed elevated levels of polysaccharides and minerals (over 60%, w) aside modest quantities of caffeic and quercetin derivates (less than 1%, w). Differently, polyphenols fraction isolated by the processing of crude acetone extract shown high contents of all gastroprotective compounds (over 10%, w). Accordingly, one final vegetal product based on these two selective extracts, polysaccharides and, respectively, polyphenols fraction, was designed.

Further, due to the fact that one of the most important features of a gastroprotective product is the capacity to protect gastric mucous tissue against acidic attack, pharmacological studies made on rats with stress-induced ulcer (this method assure the elevation of gastric acid synthesis) pointed out strong protective effect of this vegetal product based on *Centaurea cyanus* selective extracts.

**Keywords:** gastroprotective activity, *Centaurea cyanus* L. extracts

Introduction

*Centaurea cyanus* L. (*Asteraceae*), also called blue cornflower or bachelor’s button, is a vegetal species mainly found around the corn fields. As interest compounds, literature reports various flavonoids derivates, often cited being apigenin-4’-O-(6-O-malonil-glucoside)-7-O-glucuronide, apigenin-4-O-glucoside, apigenin-7-O-glucoside/cosmosiin, apigenin-7-O-apio-glucoside/apin, methyl-apigenin and methyl-vitexin, cyanidin-3-O-succinyl-glucoside-5-O-glucoside/centaurocyanin (the marker compound), cyanidin-3,5-diglucoside/cyanidin, quercetin-3-O-glucoside/rutoside, isorhamnetin, isorhamnetin-7-O-glucoside, naringenin and naringenin-7-O-gluco-rhamnose (Litvinenko V. I. et al., *Phytochemical study of Centaurea cyanus* - Chemistry of Natural Compounds, 24(6), p.672-674, 2007 [1]).

As to aromatic acids occurrence, were reveled phenyl carboxylic acid derivates such as: *cis* and *trans*-caffeic acids, protocatechic and chlorogenic acids, *p*-hidroxibenzoic, *p*-coumaric, vanilic, syringic, ferulic, salicilic and benzoic acids, as well as *cis/trans*-sinapic acids or *o/p*-hidroxiphenylacetic acids (Murav'eva D. A. et. al., Phenolcarboxylic acids of the flowers of *Centaurea cyanus* - *Chemistry of Natural Compounds*, 22(1), p.102, 2007 [2]).
Literature data also indicates protocatechic and caffeic acids as being the main aromatic acids, but, similar to our studies, studies made on indigenous Cyani flores (Hodisan Viorica et al., Clujul Medical, 58(4): 378-381, 1985 [3]) indicates, chlorogenic and isochlorogenic acids as being the major species. More, is mentioned the occurrence of amino acids, of sugars and of coumarins, umbelliferone and scopoletin derivates.

So, due to this valuable chemical content, Centaurea cyanus extracts, especially those of flower head part, are some of the most commonly raw materials in the cosmetics industry. Contrarily, only few human use Centaurea cyanus extracts/products have been evidenced.

In view of these dates, this paper aimed at obtaining and characterization of two selective extracts based on polysaccharides and, respectively, polyphenols fraction isolated from C. cyanus raw material in order to design a final product with gastroprotective potential. The selection of these two types of selective extracts is based on the following points of view:

- Polyphenols species found in bachelor’s button are very effective as gastroprotective compounds; pharmacologically studies proved both, the capacity of quercetin derivates to inhibit inflammatory processes and to reduce gastric acid synthesis (Kelly Samara Lira Mota et al., Flavonoids with gastroprotective activity – Molecules, 14(3), p.979-1012, 2009 [5]), as well as the ability of caffeic acid derivates to inhibit oxidative processes and to control gall bladder function, one of the main gastric damaging factors (Viorica Istudor – Farmacognozie Fitochimie Fitoterapie, Editura Medicală, București, vol. 1; p.84-89, 113-114, 179, 1998 [6]).

Materials and methods

**Raw materials:** Centaurea cyanus L. of Fundulea Research Station origin.

**Extracts preparations:** Have been proposed the obtaining of two tinctures and, respectively, three aqueous, ethanol and acetone crude extracts, as well as of two selective extracts based on polysaccharides and, respectively, polyphenols fraction and the correspondingly final vegetal product based on these two selective extracts.

- Thus, the two tinctures were obtained from Centaurea cyanus–herba and, respectively, Centaurea cyanus-flores raw materials as following: compounds of 100g dried and minced herba and, respectively, flores were separately extracted in 1000ml of 70% ethanol (w), 10 days, at room temperature and dark conditions, with occasionally agitation. The obtained tinctures were separated by low pressure filtration and stored into dark bottles.
- Aqueous, ethanol and acetone crude extracts were obtained from Centaurea cyanus–herba raw material as follows: compounds of 100g dried and minced herba were separately extracted in 1000ml of distilled water, of 70% ethanol and, respectively, of 60% acetone solution at boiling temperature, 1 hour, with continuously agitation (w). Extractive solutions were filtered at low pressure and the resulted crude extracts stored into dark bottles.
- Selective extracts were obtained from crude aqueous and, respectively crude acetone extracts. Thus, polysaccharides fraction was isolated by the processing of the aqueous solution, while polyphenols fraction was obtained by the processing of acetone solution. Lastly, two selective extracts were obtained.
- Final vegetal product was obtained by the combination of these two selective extracts, polysaccharides and, respectively, polyphenols fraction (Lucia Pirvu et al. - Gastropotective vegetal product and the obtainment procedure - Patent no.121764B1 Int.Cl7, A61K36/00; A61P 1/04, 2008 [7]).
All these vegetal products (tinctures, crude extracts, selective extracts and the correspondingly final vegetal product) have been analyzed as concerning chemical content. Final vegetal product was tested as concerning pharmacological potential, too.

**Qualitative analytical determination** - Studies were performed according to Plant Drug Analysis (Hildebert Wagner & al., Second Edition, Springer, 1996 [8]) and High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plant (Eike Reikh & al., Thieme, N.Y.-Stuttgart, 2008 [9]) techniques. Studies projected the evaluation of key compounds presence; flavonoids and phenyl-carboxylic acid derivates known with gastroprotective potential. Thus, standard settings for polyphenols separation were selected:

- Adsorbent: Silica gel 60F254 – HPTLC plates 20x10, (Camag, Switzerland);
- Solvent system: ethyl acetate-acetic acid-formic acid-water / 100:12:12:26;
- Sigma/Aldrich reference compounds: solutions 10⁻⁳M solved into ethanol 75% (v/v);
- Identification: - exposure at 254/366 nm of origin dried chromatogram plate and, respectively, treatment with NP/PEG reactive followed by 366nm exposure.

**Quantitative analytical determination** - Quantitative measurements were realized by standard colorimetric/gravimetrical methods (FR.X, [10]); thus, total phenols content was measured by Folin – Ciocalteau’s method, total flavones content via AlCl₃ in base medium treatment, while polysaccharides content was measured using one gravimetrical method.

**Pharmacological studies** - Have been proposed gastroprotective potential evaluation of the vegetal product based on *Centaurea cyanus* selective extracts. Studies fulfilled on rats with stress-induced ulcer; this model consists in the immersion and immobilization of rats into cold water, on dorsal position. Thus, stressful conditions results in the increasing of rats gastric acid synthesis and, accordingly, gastroprotective activity can be evaluated by the comparing of total length of each one superficial, medium or deep gastric lesions of the exposed-treated groups face to the same type of lesions of the exposed untreated group.

In this respect, animals groups treated as follows:

- **Group 1** (control group) – After the stress experiment (immobilization and immersion 4 hours into cold water) these animals were killed and the length of each one gastric lesion (superficial, medium or deep lesion) was measured;

- **Group 2** – One hour before stress experiment, these animals received (p.o.) human correspondingly doses of reference drug, Ranitidine. After this, animals were immobilized 4 hours into cold water and, once finished the stress experiment, animals were killed and the length of each one gastric lesion was measured (deep, medium and superficial lesions);

- **Group 3** – Similar, one hour before stress experiment, animals received (p.o.) vegetal product in doses of 500mg/kg body (previously tests made on doses of 250, 500 and, respectively, 1250mg vegetal product/kg body established this dose as being the effective dose). After this, animals were immobilized 4 hours into cold water and, once finished the stress experiment, rats were killed and the length of each one gastric lesion was measured.

Results are expressed as gastroprotective percents (GP/P%).

**Apparatus** - Extraction system (Jena, Germany), Concentrator (Büchi, Switzerland), HPTLC system - Camag Linomat Visualiser (Switzerland); Spectrophotometer UV-VIS - Hélios γ (Thermo Electron Corporation); Chemiluminometer - TurnerBioSystem (USA).

**Results and discussion**

Figure 1 presents HPTLC aspects of the two series of *Cyani flores/herba* tinctures, face to the three series of *Cyani herba* aqueous, ethanol and acetone crude extracts, respectively, comparatively to six series of reference compounds mixtures (ref.).

Tested samples disposed as follows:
T1: ref. - cyanidin;
T2: ref. - rutin, chlorogenic acid, apigenin-7-O-glucoside/cosmosiin and kaempferol;
T3: ref. - rutin, quercetin-3-O-galactoside/hyperoside and quercetin;
T4: ref. - rutin, chlorogenic acid, hyperoside, luteolin-7-O-glucoside/cinaroside, apigenin-8-C-glucoside/vitexin, umbelliferone and caffeic acid;
T5-T6: tincture of *Centaurea cyanus* L.-*flores*;
T7-T8: crude aqueous extract of *Centaurea cyanus* L.-*herba*;
T9-T10: crude ethanol extract of *Centaurea cyanus* L.-*herba*;
T11-T12: crude acetone extract of *Centaurea cyanus* L.-*herba*;
T13-T14: tincture of *Centaurea cyanus* L.-*herba*;
T15: ref. – apigenin-7-O-apiosylglucoside/apiin, vitexin-2”O-rhamnoside, hyperoside, cosmosiin, caffeic acid and quercetin;
T16: ref. - umbelliferone.

Figure 1 shows *Cyan* *flor*/*herba* tinctures as containing different polyphenols compounds. Thus, if *herba* tincture samples (T13-T14) reveal only two main polyphenols derivates, one yellow-orange fluorescent spots attributed to a quercetin diglycoside (rutin-like) and one blue fl. spots attributed to chlorogenic acid, *flores* tincture samples (T5-T6) emphasize at least eight compounds: two quercetin derivates (yellow-orange fl. spots attributed to quercetin-3/7-glycoside), two caffeic acid derivates (blue fl. spots attributed to chlorogenic and isochlorogenic acids, respectively), two apigenin derivates (green fl. spots attributed to apigenin-7-glucoside or cosmosiin and apigenin aglicone, respectively) and two coumarins derivates (dark-blue fl. spots disposed at the bottom of the chromatogram plate).

As concerning aqueous, ethanol and acetone crude extracts (T7-T12 tracks), excepting protocatechic and neochlorogenic acids presence, these are showing relatively similar polyphenols contents; have been revealed chlorogenic and neochlorogenic acid spots (Rf=0.49/0.54) aside rutin-like and, respectively, quercetin-7-glycoside spots (Rf=0.41/0.68), as well as isochlorogenic and, respectively, protocatechic acid spots (Rf=0.86/0.96).

More, the exposure of the origin chromatogram plate at 366nm (see Figure 2) revealed the occurrence of apigenin-7-glycoside/cosmosiin, too; green non-fluorescent spots disposed at the same zone with quercetin-7-glycoside (Rf=0.69).

* Spectral studies made on spots methanol extracts treated with AlCl₃ in base medium revealed for spot of Rf=0.68 a maximum absorption peak of 435nm, possibly only in the case of -(C’)-O- glycosides.
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Figure 2. HPTLC aspects of the origin chromatogram plate exposed at 366nm showing *Centaurea cyanus* tinctures (T5-T6/T13-T14), crude extracts (T7-T8/T9-T10/T11-T12) and reference (T1-T4/T15-T16) samples

Also, Figure 2 shows the lack of the two coumarins derivates in any of the three crude aqueous, ethanol or acetone extracts.

Table 1 present qualitative and quantitative aspect of *Centaurea cyanus* extracts.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Rf~</th>
<th>Spot coloration</th>
<th>Attributed compound</th>
<th>Quantitative appraisal of <em>Centaurea cyanus</em> extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tinctures</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T5-T6</td>
</tr>
<tr>
<td>1</td>
<td>0.17</td>
<td>Dark-blue, fl.</td>
<td>Coumarin derivate</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>0.22</td>
<td>Dark-blue, fl.</td>
<td>Coumarin derivate</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>Yellow-orange fl.</td>
<td>Rutine-like compound</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>0.49</td>
<td>Blue, fl.</td>
<td>Chlorogenic acid</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>0.54</td>
<td>Blue, fl.</td>
<td>Neochlorogenic acid</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0.66</td>
<td>Yellow-orange fl.</td>
<td>Quercetin-3-glycoside</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>0.68</td>
<td>Yellow-orange, fl.</td>
<td>Quercetin-7-glycoside</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>0.69</td>
<td>Green, fl.</td>
<td>Apigenin-7-glycoside</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>0.86</td>
<td>Blue, fl.</td>
<td>Isochlorogenic acid</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>0.96</td>
<td>Dark blue, fl.</td>
<td>Protocatechic acid</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0.97</td>
<td>Green, fl.</td>
<td>Apigenine</td>
<td>++</td>
</tr>
</tbody>
</table>

+++/: very abundant; ++/abundant; +/little; 0/not detected;

Thus, besides qualitative appraisals, these studies also revealed polyphenols extraction effectiveness, heat assisted extracts proving superior to room temperature extracts (tinctures).

As regards selective extracts qualitative composition, HPTLC studies (see Figure 3) indicate polysaccharides fraction (T5-T6 tracks) as containing only some of origin crude aqueous polyphenols derivates; chromatogram plate reveals chlorogenic acid occurrence aside small quantities of quercetin derivates and protocatechic acid traces. Differently, polyphenols fraction (T7-T8 tracks) revealed the same qualitative content as origin crude acetone extract, more augmented in the particular case of isochlorogenic acid occurrence (big blue fl. spot at \(Rf\sim0.86\), the most probably due to the heat chlorogenic acid isomerization.

In order to obtain these quantitative appraisals, HPLC studies fulfilled on identical volumes samples.

Concluding, analytical studies fulfilled on the two selective extracts obtained from *Centaurea cyanus* L.-*herba* raw material clearly revealed the occurrence of quercetin, apigenin and caffeic acid derivates, known with beneficial effect on different gastric injuries.

Further, based on the fact that one of the most important features of a gastroprotective product is the capacity to protect gastric mucous tissue against acidic attack, pharmacological studies fulfilled on *Wistar* rats with stress-induced ulcer.

Accordingly, gastroprotective activity was evaluated by separately counting of the total length of each one deep, medium or superficial gastric lesions of the exposed-treated groups (group 2 and group 3), face to the same type of lesions presented of exposed untreated group (group 1/control group). Results are expressed as percents (±GP%). More, by comparing each type of gastric injuries of the group 3 with each type of gastric injuries of the group 2 have been evaluated vegetal product effectiveness face to reference product, too. Results also expressed as gastroprotective percents (±P%) – see Table 2.

**Table 2.** Gastro protective activity of the vegetal product based on *Centaurea cyanus* selective extracts

<table>
<thead>
<tr>
<th>Tested group</th>
<th>Total length (mm) of the gastric lesions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>deep</td>
<td>medium</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± ES</td>
<td>4.13 ± 1.25</td>
<td>18.25 ± 2.08</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± ES</td>
<td>0.46 ± 0.16</td>
<td>7.40 ± 2.15</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>t, p&lt;</td>
<td>2.96, p&lt;0.02</td>
<td>3.54, p&lt;0.01</td>
</tr>
<tr>
<td>± GP%</td>
<td>-88.86%</td>
<td>-59.45%</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± ES</td>
<td>0</td>
<td>2.04 ± 0.55</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>t, p&lt;</td>
<td>2.88, p&lt;0.02</td>
<td>8.27, p&lt;0.001</td>
</tr>
<tr>
<td>± GP%</td>
<td>-100.00%</td>
<td>-98.82%</td>
</tr>
<tr>
<td>± R%</td>
<td>-100.00%</td>
<td>-72.43%</td>
</tr>
</tbody>
</table>

X ± ES = the total length of lesions ± standard deviation; n = the number of animals; t; p< Student's *t* test; ±GP% = protection percent shown of the two exposed-treated groups face to exposed-untreated group; ± R% = protection percent of group treated with vegetal product face to group treated with reference product.
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Therefore, pharmacological screening fulfilled on vegetal product based on Centaurea cyanus selective extracts shows gastroprotective percents (±GP%) of 100% (p<0.02), 89% (p<0.001) and, respectively, 83% (p<0.001) in the specific case of deep, medium and, respectively, superficial gastric lesions, even superior to that offered of reference product; in this case revealed percents of 89% (p<0.02), 59% (p<0.01) and, respectively, 54% (p<0.01) on the same type of lesions. As concerns vegetal product - reference product comparison (±P%), results had statisticyal significance in all tested situations; deep (100%, p<0.02), medium (72%, p<0.05) and, respectively, superficial (63%, p<0.01) lesions, too.

These good results aded to other in vivo and in vitro studies emphasizing both, the lack of the toxicity and high antioxidant potential of this vegetal product based on Centaurea cyanus L.-herba extracts (Lucia Pirvu et al. - Scavenger properties of some natural products with application in the therapy of gastro-intestinal diseases - Scientific Bulletin of UPB, Serie B, Chemistry and Materials Science, 67(3), 23-27, 2005 [11]). Consequently, the obtaining procedure of these selective extracts as well as of some other selective extracts of Plantago lanceolata L.-folium origin (results in course of publication) was patented.

Conclusions

Qualitative studies (HPTLC method) performed on tinctures and crude aqueous, ethanol and acetone extracts obtained from Centaurea cyanus herba and flores raw materials revealed the attendance of some very effective gastroprotective compounds: quercetin, apigenin and caffeic acid derivates. Also, analytical studies fulfilled on polysaccharides and, respectively, polyphenols fraction, isolated from crude aqueous and, respectively, crude acetone Centaurea cyanus L.-herba extracts emphasized polysaccharides fraction as containing only some of origin crude aqueous polyphenols derivates; were revealed chlorogenic acid occurrence aside small quantities of quercetin derivates and protocatechic acid traces (less than 1%, w). Differently, polyphenols fraction revealed high contents of all gastroprotective species; quercetin, apigenin and caffeic acid derivates (over 10%, w).

Further, based on the fact that one of the most important attribute of a gastroprotective drug is the capacity to protect gastric mucous tissue against acidic attack, pharmacological studies made on rats with stress-induced ulcer proved high effectiveness of the vegetal product based on Centaurea cyanus selective extracts, even greater than of reference drug.

References

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