Polyphenolic profile and anti-inflammatory activity of two extracts from *Tussilago farfara* leaves and *Cichorium intybus* roots

S. CONEA (1)*, O. VOSTINARU (2), C. MOGOSAN (2), C. C. TOMA (1), I. CUC HEPCAL (1), C. MORGOVAN (1), L. VLASE (3)

1Department of Pharmaceutical Sciences, Faculty of Pharmacy, Vasile Goldis Western University of Arad, 86 L. Rebreanu, 310048 Arad, Romania
2Department of Pharmacology, Physiology and Physiopathology, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, 6A, L. Pasteur, 400012, Cluj-Napoca, Romania
3Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, 41 Victor Babes, RO-400012, Cluj-Napoca, Romania

*Corresponding author
Simona Conea PhD.
Department of Pharmaceutical Sciences, Faculty of Pharmacy, Vasile Goldis Western University of Arad, 86 L. Rebreanu, 310048 Arad, Romania, e-mail: suciu_simona@yahoo.com

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**Summary**

This study evaluated the polyphenolic profile and anti-inflammatory activity of *Tussilago farfara* leaves and *Cichorium intybus* roots, species traditionally used in the treatment of asthma, bronchitis, gastrointestinal disorders, topical application is recommended for furunculosis, or for its analgesic effects other inflammatory disorders. Polyphenolic profile of the hydro-glycero-ethanolic extracts from *Tussilago farfara* leaves and *Cichorium intybus* roots was evaluated by a HPLC-MS method, while the anti-inflammatory effect of both extracts (500 mg/kg b.w.) administered orally was determined by carrageenan-induced rat paw oedema test. HPLC-MS analysis showed in *Tussilago farfara* leaves the presence of chlorogenic acid (171.168 μg/mL), isoquercitrin (73.706 μg/mL), rutin, quercitrin (75.896 μg/mL), quercetol and kaempferol, while in *Cichorium intybus* roots small amounts of quercitrin, quercetol, luteolin and apigenin were detected.

The results of the carrageenan-induced rat paw oedema test showed modest anti-inflammatory effects of the *Tussilago farfara* leaves and *Cichorium intybus* root extracts probably mediated by peripheral mechanisms in the early stage of inflammation.

**Introduction**

Several medicinal plants are used worldwide in traditional medicine as remedies for inflammatory skin disorders. Also in Romanian folk medicine species like *Eryngium planum*, *Eryngium campestre*, *Eryngium maritimum*, *Onopordon acanthium*, *Cichorium intybus*, *Tussilago farfara* and *Arctium lappa* are well known as cures for skin diseases.

*Tussilago farfara* L. (Asteraceae), commonly known as coltsfoot, is traditionally used to treat respiratory illnesses such as asthma, bronchitis, flu, for the expectorant, antimicrobial and sedative effects (Shikov et. al., 2014). Other uses of this plant include the treatment of gastrointestinal disorders, diarrhea, while topical application is recommended for furunculosis, or for its analgesic effects (Vereschagin cited by Shikov et al., 2014).

The medicinal product is represented by the leaves which are present in many pectoral and diaphoretic teas. Faradiol, tussilagin, angelic acid, hyperin, arniolid, seneconine, rutin and quercetin-glycosides were identified from this plant (Song et al., 2010). Some pharmacological studies demonstrated a significant antioxidant
capacity which can be mainly attributed to the phenolic compounds present in \textit{T. farfara} \cite{Kim et al., 2006}.

Tussilagone, a sesquiterpenoid isolated from the flower buds of \textit{Tussilago farfara}, inhibited the production of NO, TNF-\(\alpha\), and PGE2 as well as iNOS and COX-2 expression in LPS-stimulated RAW 264.7 cells and murine peritoneal macrophages \cite{Vogl et al., 2013}.

However, the safety of \textit{T. farfara} is controversial due to the presence of pyrroloidine alkaloids (low level of senkirkine and traces of senecionine) which are known to be hepatotoxic. However, in order to avoid any possible risk, the German public health authorities have restricted the daily intake of toxic pyrroloidine alkaloids to 1 \(\mu\)g \cite{Bundesgesundheitsamt, 1992}.

\textit{Cichorium intybus} \(L._{.}\), commonly known as chicory, belongs to Asteraceae family and is widely distributed in Asia and Europe. It contains many valuable compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins \cite{Molan et al., 2003, Muthusamy et al., 2008}. Chicory roots are also rich in glycosides, sterols and polyphenols which have been reported to possess anti-inflammatory activity by decreasing various mediators of inflammation such as prostaglandins, NO, TNF-\(\alpha\) IL-6, and IL-1 \cite{Lee et al., 2009}.

\textit{C. intybus} has been traditionally used for the treatment of fever, diarrhoea, jaundice and gallstones. Previous studies on animals have shown that \textit{C. intybus} possesses anti-hepatotoxic, anti-diabetic activities \cite{Saggu et al., 2014}, anti-bacterial \cite{Nandagopal et al., 2007}, anti-inflammatory \cite{Cavin et al., 2005}, anti-hyperglycaemic and anti-ulcerogenic activities \cite{Rifat-uz-Zaman et al., 2006}.

Although \textit{C. intybus} root is used in the treatment of inflammatory conditions, only a few in vitro studies investigated the anti-inflammatory activity of the aerial part \cite{Waseem et al., 2014}.

Aim. Our goal was to provide a scientific basis of the use of these plants in folk medicine as remedies for several inflammatory skin diseases.

Therefore we aimed to evaluate the anti-inflammatory activities of two hydro-glycerol-ethanol extracts prepared from the leaves of \textit{Tussilago farfara} \(L._{.}\) and \textit{Cichorium intybus} \(L._{.}\) aerial part.

Materials and methods

Collection and authentication of plant material

The aerial part of \textit{Cichorium intybus} \(L._{.}\) was collected from Baia Mare, Maramures County, Romania, in July 2015, while the leaves of \textit{Tussilago farfara} \(L._{.}\) were collected from Cavnic, Maramures, in May 2015. After identification and authentication, a voucher specimen was deposited for each species at the Herbarium of the Department of Pharmacognosy and Phytotherapy, Vasile Goldis Western University of Arad, Romania.

Preparation of the extracts

Dried aerial part of \textit{Cichorium intybus} and leaves from \textit{Tussilago farfara}, respectively, were powdered with a mechanical grinder. The powdered plant material (20 g) was extracted by maceration for 10 days at room temperature with a solvent mixture of water: glycerol: ethanol 1:1:1 in a 1:5 ratio (plant material/solvent). After filtration, solvent mixture was added to 100 mL final extract. For the pharmacological studies, extracts were spray-dried and suspended in a mixture of Tween 80 and normal saline solution (1:100 v/v).

Polyphenolic profile

The hydro-glycero-ethanolic extracts from both species were analyzed by a standardized HPLC-MS method in order to assess the polyphenolic profile \cite{Conea et al., 2014}.

Chemicals

Polyphenolic standards: caffeic acid, chlorogenic acid, p-coumaric acid, kaempferol, apigenin, rutin, quercitin, quercitrin, isoquercitrin, fisetin, hyperoside, myricetin were purchased from Sigma (St. Louis, USA), ferulic acid, gentisic acid, sinapic acid, patuletin, luteolin from Roth (Karlsruhe, Germany) and caftaric acid from...
Dalton (Canada). HPLC grade methanol and acetic acid were purchased from Merck (Darmstadt, Germany).

Chromatographic conditions

The experiment was carried out using an Agilent 1100 HPLC Series system (Agilent, USA) equipped with degasser, binary gradient pump, column thermostat, autosampler and UV detector. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL). For the separation, a reverse-phase analytical column was employed (Zorbax SB-C18 100 x 3.0 mm i.d., 3.5 μm particle); the temperature was 48 °C.

The detection of the compounds was performed on both UV and MS mode. The UV detector was set at 330 nm until 17.5 min, then at 370 nm. The MS system operated using an electrospray ion source in negative mode. The chromatographic data were processed using ChemStation and DataAnalysis software from Agilent, USA.

The mobile phase was a binary gradient prepared from methanol and solution of acetic acid 0.1% (v/v). The elution started with a linear gradient, beginning with 5% methanol and ending at 42% methanol, for 35 minutes; isocratic elution followed for the next 3 minutes with 42% methanol. The flow rate was 1 mL min−1 and the injection volume was 5 μL.

Identification and quantitative determinations of polyphenols

The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS traces/spectra of the analyzed samples were compared to spectra from a library obtained from a standard solution of polyphenols. Thus positive identification of compounds was based on spectral match. The UV trace was used for quantification of identified compounds from MS detection. The detection limits were calculated as minimal concentration producing a reproducible peak with a signal-to-noise ratio greater than three. Quantitative determinations were performed using an external standard method. Calibration curves in the 0.5–50 μg mL−1 range with good linearity (R2 > 0.999) for a five point plot were used to determine the concentration of polyphenols in plant samples.

Pharmacological studies

Animals

For the pharmacological experiments, four groups of male Crl:WI rats (n=6) were obtained from the Practical Skills and Experimental Medicine Centre of Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca (Romania). The animals were housed in polycarbonate type IV-S cages (Tecniplast, Italy) and maintained under standard conditions (22 ± 2 °C, a relative humidity of 45 ± 10%, 12:12-light:dark cycle). The animals had access to a standard pelleted feed (Cantacuzino Institute, Bucharest, Romania) and filtered water ad libitum throughout the experiment, except for the day when the test substances were administered. All experimental protocols were approved by the Ethics Committee of the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania and were conducted in accordance with the EEC Directive 63/2010 which regulates the use of laboratory animals for scientific purposes.

Carrageenan-induced rat paw oedema test

The acute anti-inflammatory activity of extracts from Tussilago farfara leaves and Cichorium intybus aerial part was assessed by the carrageenan-induced rat paw oedema method followed by plethysmometric evaluation (Griesbacher et al, 1994; Adeyemi et. al., 2002). Initially, each extract was administered orally, by gastric intubation, 500 mg/kg bw, 1 hour before the induction of the inflammation. The rats in the negative control group (n = 6) were treated orally with normal saline solution. The animals from the positive control group (n = 6) were treated orally with a reference anti-inflammatory drug, diclofenac 20 mg/kg b.w. (Gerot Pharma Gmbh, Germany). Oedema was induced by a subplantar injection of 0.1 mL 1 % (w/v) λ-carrageenan into the left hind paw of each rat. The paw volume of each animal was
determined before carrageenan injection and then at 1, 2, 3 and 4h after the induction of inflammation, with a plethysmometer (model 7140, Ugo Basile, Varese, Italy). The anti-inflammatory effect of the standardized extracts from *Tussilago farfara* leaves and *Cichorium intybus* aerial part was determined at each time range with the formula:

Inhibition of oedema (%) = \[1 - \frac{(Ot}{Oc})\] x 100,

where Ot is the oedema in the treated group and Oc is the oedema in the negative control group.

**Statistical analysis**

Data were expressed as mean values ± SEM and were statistically analyzed by one way ANOVA method. The differences between the treated groups and the control group were evaluated by Dunnett’s ‘t’ test, p values<0.05 being considered statistically significant.

**Results and discussions**

**Polyphenolic profile**

Using the chromatographic conditions described above, the polyphenols eluted in less than 35 minutes (Figure 1, Figure 2).

<table>
<thead>
<tr>
<th>No.</th>
<th>Identified Compound</th>
<th>No.</th>
<th>Conc. (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>caftaric acid</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>chlorogenic acid</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>p-coumaric acid</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>isoquercitrin</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>quercitrin</td>
<td>13</td>
<td>4.291</td>
</tr>
<tr>
<td>6</td>
<td>luteolin</td>
<td>16</td>
<td>9.456</td>
</tr>
<tr>
<td>7</td>
<td>quercetol</td>
<td>14</td>
<td>0.944</td>
</tr>
<tr>
<td>8</td>
<td>apigenin</td>
<td>18</td>
<td>3.030</td>
</tr>
</tbody>
</table>

Legend: No.¹ - Number of detected compound on chromatogram; No.² – Number of standard; conc. – concentration.
Table 2. Polyphenolic compounds identified in Tussilago farfara leaves extract.

<table>
<thead>
<tr>
<th>No.</th>
<th>Identified Compound</th>
<th>No.</th>
<th>Conc. (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>chlorogenic acid</td>
<td>4</td>
<td>171.168</td>
</tr>
<tr>
<td>2</td>
<td>p-coumaric acid</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>isoquercitrin</td>
<td>9</td>
<td>73.706</td>
</tr>
<tr>
<td>4</td>
<td>rutin</td>
<td>10</td>
<td>1.599</td>
</tr>
<tr>
<td>5</td>
<td>quercitrin</td>
<td>13</td>
<td>1.599</td>
</tr>
<tr>
<td>6</td>
<td>quercetin</td>
<td>14</td>
<td>2.266</td>
</tr>
<tr>
<td>7</td>
<td>quercetol</td>
<td>17</td>
<td>20.448</td>
</tr>
<tr>
<td>8</td>
<td>kaempferol</td>
<td>17</td>
<td>20.448</td>
</tr>
</tbody>
</table>

Legend: No.1 – Number of detected compound on chromatogram; No.2 – Number of standard; conc. – concentration.

Chlorogenic acid was identified as the major polyphenolic compound of the Tussilago farfara leaves extract. Previous data have demonstrated the role of chlorogenic acid as an anti-oxidant, anti-inflammatory and analgesic agent. Chlorogenic acid significantly inhibited NO production and also the expression of COX-2 and iNOS in lipopolysaccharide (LPS)-stimulated murine cells (Hwang et al., 2014) and also demonstrated anti-inflammatory and antinociceptive effects in several in vivo experimental models (dos Santos et al., 2006).

Carrageenan-induced rat paw oedema test

The results of the carrageenan-induced rat paw oedema test are presented in Table 3.

Table 3. Effect of the Cichorium intybus root extract (EC) and Tussilago farfara leaves extract (ET) on carrageenan-induced rat paw oedema

<table>
<thead>
<tr>
<th>Group</th>
<th>O. 1h (% I)</th>
<th>O. 2h (% I)</th>
<th>O. 3h (% I)</th>
<th>O. 4h (% I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.23 ± 0.21</td>
<td>2.68 ± 0.20</td>
<td>3.01 ± 0.14</td>
<td>3.47 ± 0.23</td>
</tr>
<tr>
<td>EC 500 mg/kg</td>
<td>1.67 ± 0.21*</td>
<td>2.52 ± 0.37 (25.56)</td>
<td>2.89 ± 0.49 (4,31)</td>
<td>3.05 ± 0.54 (12.0)</td>
</tr>
<tr>
<td>ET 500 mg/kg</td>
<td>1.18 ± 0.23**</td>
<td>3.04 ± 0.51 (47.08)</td>
<td>3.29 ± 0.27 (-)</td>
<td>3.36 ± 0.25 (3.17)</td>
</tr>
<tr>
<td>Diclof.</td>
<td>1.63 ± 0.10*</td>
<td>1.08 ± 0.09** (26.90)</td>
<td>1.13 ± 0.09** (59.70)</td>
<td>1.53 ± 0.10* (55.90)</td>
</tr>
</tbody>
</table>

Legend: O. – oedema, % I – inhibition of oedema expressed in %, EC – extract of Cichorium intybus root, ET – extract of Tussilago farfara leaves, Diclof. - diclofenac (positive control); *p≤0.05 vs. control, **p≤0.01 vs. control.

The inflammatory oedema developed soon after the subplantar injection of carrageenan, reaching its peak at 4 h (oedema of 3.47 ± 0.23 mL). The administration of the Cichorium intybus extract (500 mg/kg b.w.) reduced modestly the oedema formation, in the first phase of oedema formation (25.56%). The effect was statistically significant and inferior to the reference anti-inflammatory drug, diclofenac (26.90%) 1h after inflammation was induced. Later, at 2h, 3h, and 4h, C. intybus extract has shown no effect on induced inflammation (p>0.05 vs. control).

The administration of the Tussilago farfara extract (500 mg/kg b.w.) intensely reduced the inflammation in the first phase of oedema formation (47.08%). The effect was statistically significant comparing to control group and superior to diclofenac (26.90%) 1h after carrageenan-induced inflammation. Surprisingly, later at 2h and 3 h, Tussilago...
farfara extract at dose of 500 mg/kg b.w. raised inflammation while at 4h the effect on oedema formation was similar to that noticed in control group (p> 0.05). The reference drug, diclofenac has shown a maximum inhibition rate of 62.45%, 3 h after the induction of the inflammation.

The development of oedema in the rat hindpaw following an injection of carrageenan has been characterized as a biphasic event in which several mediators are released, producing an inflammatory response. The initial inflammatory reaction to carrageenan (0–1 h), has been attributed to the release of histamine, serotonin, bradykinin and also complement and reactive oxygen species. In contrast, the second accelerating phase of swelling (2–4 h), has been correlated with the elevated production of prostaglandins in the inflammatory area (Vinegar et. al., 1969).

Our experimental data suggests extract from Tussilago farfara leaves was able to influence only the first phase of carrageenan-induced oedema formation, probably by inhibiting the release of pro-inflammatory mediators.

Our results are in contradiction with a previous study in which an ethanolic extract from C. inthybus root (500mg/kg b.w.) reduced oedema in both the phases of inflammation, the maximum effect was maximum in the second phase of inflammation (62.36%) (Rizvi et al., 2014).

**Conclusions**

Our study evaluated the polyphenolic profile and anti-inflammatory activity of two extracts from Tussilago farfara leaves and Cichorium intybus aerial part. By HPLC-MS analysis several polyphenolic compound were detected in Tussilago farfara leaves extract such as chlorogenic acid (main polyphenolic compound), isoorcetin, quercitin and small amounts of rutin, quercetol and kaempferol. The administration of the Cichorium intybus extract (500 mg/kg b.w.) modestly reduced the oedema formation, only in the first phase of oedema formation (25.56%).

The administration of the Tussilago farfara extract (500 mg/kg b.w.) intensely reduced the oedema formation only in the first phase of oedema formation (47.08%), the effect being superior to diclofenac (26.90%) 1h after carrageenan-induced inflammation.

Probably the anti-inflammatory effects of both extracts can be related to the ability of polyphenols to inhibit the synthesis and release of some pro-inflammatory mediators (Hwang et al., 2014; Dos Santos et al., 2006).

Further research is required to assess the topical anti-inflammatory activity of both extracts in order to verify the traditional use in folk medicine in some skin diseases.

**References**


