POLYPHENOLIC PROFILE AND ANTI-INFLAMMATORY, ANALGESIC AND ANTIOXIDANT EFFECTS OF ETHANOLIC AND HYDRO-ETHANOLIC EXTRACTS OF HEINSIA CRINITA AFZ.G. TAYLOR AND TETRACERA ALNIFOLIA WILLD

LIUCE SARRAH BOUMBA 1, ANAMARIA CRISTINA 2*, FREDY GELASE NSONDE NTANDOU 3, ILOIOARA ONIGA 4, DANIELA BENEDEC 4, ANA-MARIA VLASE 5, OLIVIU VOSTINARU 2, ANGE ANTOINE ABENA 6, CRISTINA MOGOSAN 2

1Laboratory of Chemistry of Organic Biomolecules and Pharmacodynamics, Department of Pharmacopoeia and Traditional Medicine, National Institute for Research in Health Sciences (IRSSA), Cité Scientifique, Brazzaville, Congo
2Department of Pharmacognosy, Faculty of Pharmacy, “Iuliu Hațieganu” University of Medicine and Pharmacy, 8 Victor Babeș Street, 400012, Cluj-Napoca, Romania
3Animal Physiology and Physiopathology Laboratory, Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, BP 69, Congo
4Department of Pharmaceutical Botany, Faculty of Pharmacy, “Iuliu Hațieganu” University of Medicine and Pharmacy, 8 Victor Babeș Street, 400012, Cluj-Napoca, Romania
5Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Iuliu Hațieganu” University of Medicine and Pharmacy, 8 Victor Babeș Street, 400012, Cluj-Napoca, Romania
6Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, B.P. 69, Congo

*corresponding author: anamaria.cristina@umfcluj.ro

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Abstract

The aim of this study was to determine the anti-inflammatory, analgesic and antioxidant effects and the polyphenols composition of Tetracera alnifolia Willd and Heinsia crinita Afz. G. Taylor extracts. The anti-inflammatory effect of the hydro-ethanolic and ethanolic extracts was evaluated by the carrageenan-induced rat paw oedema test and the analgesic effect by the Randall-Selitto test. Anti-inflammatory activity of the extracts was evaluated spectrophotometrically using the DPPH radical scavenging assay and the polyphenolic profile, by an HPLC-MS method. All extracts of H. crinita and T. alnifolia (500 mg/kg) showed significant anti-inflammatory and analgesic effects. The extract of T. alnifolia has shown an antioxidant effect greater than that of H. crinita. Gallic acid (33.31 µg/mL) and rutin (34.70 µg/mL) are the major polyphenolic compounds of T. alnifolia and H. crinita, respectively. This study justifies the use in the traditional medicine of T. alnifolia and H. crinita. The obtained results are promising for the use of these extract in the management of inflammatory diseases.

Rezumat

Scopul acestui studiu a fost de a evidenția compoziția chimică în polifenoli și efecțele antiinflamatoare, analgezice și antioxidante ale extractelor de Tetracera alnifolia Willd și Heinsia crinita Afz. G. Taylor. Acțiunea antiinflamatoare a extractelor hidro-etanolice și etanolice a fost evaluată prin testul edemului labei de șobolan indus de λ-carageenan-inducit rat paw edema test și analgezică prin testul Randall-Selitto test. Activitatea antiinflamatoare a extractelor a fost evaluată spectrophotometrically prin testul de scavenger radicalului DPPH, iar profilul polifenolic printr-o metodă HPLC-MS. Toate extractele de H. crinita și T. alnifolia (500 mg/kg) au prezentat efecte antiinflamatoare și analgezice semnificative. Extractul etanolic de T. alnifolia a prezentat un efect antioxidant mai bun decât cel al H. crinita. Acidul galic (33,31 µg/mL) și rutinul (34,70 µg/mL) sunt principalii compuși polifenolici din T. alnifolia, respectiv H. crinita. Studiul efectuat justifică utilizarea în medicina tradițională a speciilor T. alnifolia și H. crinita. Rezultatele obținute sunt promițătoare pentru utilizarea acestor extracte în managementul bolilor inflamatorii.

Keywords: Tetracera alnifolia, Heinsia crinita, anti-inflammatory, antioxidant

Introduction

Anti-inflammatory drugs play an important role in therapy, especially in a series of inflammatory diseases affecting various organs or tissues [5,35]. Capable of treating inflammation, pain or fever, anti-inflammatory drugs are one of the most widely used pharmacological classes in the world and are easily accessible over the counter drugs [28]. However, it is known that due to their mechanism of action, anti-inflammatory drugs may present a risk of toxicity associated with prolonged use, including gastrointestinal, cardiovascular, renal and musculoskeletal complications [5, 28, 35]. This limitation has stimulated the interest in focusing on natural resources, in particular medicinal plants, which,
moreover, have always been the source of inspiration for several pharmacological molecules marketed to date [40]. In this context, medicinal plants have become an important alternative for discovering pharmacological active substances with reduced side effects [15]. *T. alnifolia* Willd. (Dilleniaceae) and *H. crinita* Aaf.G. Taylor (Rubiaceae) are two species of medicinal plants from tropical Africa and are used in traditional herbal medicine by the populations of this region. In traditional African medicine, the leaves or stems of the species *T. alnifolia* are used to treat toothache, stomachache, headache and also wounds [1, 8, 18, 26]. In addition to these, *T. alnifolia* is also used to treat people with rheumatism or arthritis [8, 20]. Pharmacological studies carried out on aqueous and alcoholic extracts of the leaves of *T. alnifolia* have shown anti-inflammatory and analgesic, but also antibacterial effects [2, 25, 30, 33]. These effects could be explained by the presence of the various secondary metabolites (flavonoids, saponins, anthraquinones etc.) it contains [30]. It was reported that the genus *Tetracera* contains mainly flavonoids and terpenoids [27]. Concerning *H. crinita*, it is used in traditional medicine to treat cough, pains or wounds [23, 24]. Previous pharmacological studies carried out on extracts from leaves or stems of *H. crinita* revealed antimicrobial activities, hypoglycaemic, hepatoprotective, nephroprotective and antihypertensive effects [16, 17, 31]. Anti-inflammatory and analgesic activities have also been proven for aqueous extracts [7, 44]. A detailed study on the chemical composition of polyphenols showed the presence of gallic acid, chlorogenic acid, rutin etc. [32]. Previous anti-inflammatory or analgesic studies of both species have focused on aqueous extracts and have been justified for all the secondary metabolites they contain, but other types of extracts were not previously studied. Thus, the objective of the present study is to determine the anti-inflammatory, analgesic and antioxidant effects and the polyphenols chemical composition of the hydro-ethanolic and ethanolic extracts of *T. alnifolia* and *H. crinita*.

Materials and Methods

**Plant material**
The leaves of *T. alnifolia* and *H. crinita* were harvested from the southeast of Brazzaville, Congo, in December 2018. The harvest was carried out in the presence of a botanist who identified each species. They were then authenticated at the national herbarium of the National Institute of Research in Exact and Natural Sciences under no. 1636/1967 and no. 2461/1968, respectively.

**Preparation of extracts**
The dried leaves of *T. alnifolia* and *H. crinita* were ground to a fine powder using an electric grinder. To carry out the analysis, the powder of each vegetal product was extracted by maceration for 72 hours at room temperature in two solvents: ethanol 96° (E) and ethanol/water 1:1 (H), in a ratio of 1:10 (plant material/solvent). The obtained extracts of *T. alnifolia* and *H. crinita* were kept in a cool place (4°C) [7, 43]. Prior to rats’ administration, the solvent was evaporated using a rotary evaporator to get a semi-solid mass, and orally administered as 0.5% Tween 80 suspension.

**Chemistry**

**Determination of total polyphenol content (TPC)**
The total phenolic content (TPC) of the extracts was determined by the slightly modified Folin-Ciocalteu method. Each ethanolic extract (1 mL) diluted 25 times was mixed with Folin-Ciocalteu reagent (1.0 mL) and distilled water (10.0 mL) and diluted to 25.0 mL with a 290 g/L solution of sodium carbonate. The samples were incubated in the dark for 30 min. The absorbance was measured at 760 nm. Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL, prepared in ethanol-water (50:50, v/v). TPC values were determined using an equation obtained from the calibration curve of gallic acid graph ($R^2 = 0.999$). The results were expressed as mg of gallic acid equivalent (GAE)/g dry plant material [12, 13].

**Antioxidant activity test by DPPH radical scavenging assay**
The antioxidant capacity of the extracts (H and E) was determined in vitro by the DPPH radical scavenging assay. The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. A DPPH solution (0.1 g/L) in ethanol was prepared. 100 μL of each extract was diluted to 10 mL with ethanol in a volumetric flask. Then, 4 mL of each diluted solution was taken, over which 4 mL of a 290 g/L solution of sodium carbonate. The total phenolic content (TPC) of the extracts was determined by the slightly modified Folin-Ciocalteu method. Each ethanolic extract (1 mL) diluted 25 times was mixed with Folin-Ciocalteu reagent (1.0 mL) and distilled water (10.0 mL) and diluted to 25.0 mL with a 290 g/L solution of sodium carbonate. The samples were incubated in the dark for 30 min. The absorbance was measured at 760 nm. Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL, prepared in ethanol-water (50:50, v/v). TPC values were determined using an equation obtained from the calibration curve of gallic acid graph ($R^2 = 0.999$). The results were expressed as mg of gallic acid equivalent (GAE)/g dry plant material [12, 13].

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The percent DPPH scavenging ability was calculated as:

$$DPPH\text{ scavenging ability (I\%) = } \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100,$$

where $A_{control}$ is the absorbance of DPPH radical + ethanol (prepared with 4.0 mL DPPH solution and 4.0 mL ethanol) and $A_{sample}$ is the absorbance of DPPH radical + sample extract [4, 12, 41].

**LC-MS/MS analysis**
The phytochemical analysis of the extracts was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). An Agilent 1100 HPLC Series system (Agilent, Santa Clara, CA, USA) equipped with degasser, auto sampler, binary gradient pump, column thermostat, and UV detector was used. The HPLC system was coupled with an Agilent Ion Trap 1100 SL mass spectrometer (LC/MSD Ion Trap VL). A reverse-phase analytical column (Zorbax SB-C18, 100 mm x 3.0 mm i.d., 3.5 μm) was
used for separation, while the mobile phase consisted in a mixture of methanol: 0.1% acetic acid (v/v) and a binary gradient (start 5% methanol, at 35 min 42% methanol; 3 min isocratic elution with 42% methanol, then 7 min with 5% methanol). The flow rate was 1 mL/min, the injection volume was 5 μL and the column temperature 48°C and a combined detection: UV (330 nm, 370 nm) and MS mode. ChemStation and Data Analysis software from Agilent, USA were used to process the chromatographic data. The same analytical conditions were used for the identification of catechin, epicatechin, gallic acid, syringic acid, vanillic acid and protocatechuic acid using a different binary gradient (start with 3% methanol; at 3 min 8% methanol, from 8.5 min until 10 min with 20% methanol, then 3% methanol to rebalance the column) and compounds detection in MS mode. In both cases the MS system operated in the following conditions: an electrospray ion source in negative mode (capillary +3000 V, nebulizer 60 psi (nitrogen), dry gas nitrogen at 12 L/min, dry gas temperature 360°C). The identified compounds were quantified on the base of their peak area and the calibration curve of their corresponding standards, the results being expressed as μg of phenolic compound per mL of liquid extract [37-39, 42].

**In vivo assays**

**Animals**

To assess the pharmacological activities, 6 groups of female Charles River Wistar rats (n = 5), weighting between 230 and 370 g (average 280.5 g) were used. These animals were provided by the Centre for Practical Skills and Experimental Medicine of the “Iuliu Hatieganu” University of Medicine and Pharmacy (Cluj-Napoca, Romania). The animals were housed in open-top type IV-S polycarbonate cages (Tecniplast, Italy) and kept at a controlled room temperature (22 ± 2°C; relative humidity 45 ± 10%) with a 12/12 h light/dark cycle. They were fed a standard diet of pellets and water ad libitum. Prior to any experimentation, the animals were fasted for 12 hours. The procedures on animals were carried out in accordance with the EEC Directive 63/2010, which regulates the care and use of laboratory animals for scientific purposes and approved by the Ethics Commission of the University and the Veterinary Health and Food Safety Department in Cluj. Two plant extracts were administered, the hydro-ethanol extract 1:1 (100 g, [1:10]) and the concentrated ethanol extract 96° (100 g, [1:10]). Each extract was tested at a dose of 500 mg/kg body weight. Rats in the negative control group were treated with normal saline solution. Animals in the positive control group received a reference anti-inflammatory/analgesic drug, diclofenac 20 mg/kg b.w. Oral administration of the extracts was made 1 h before inflammation induction. 

**Carrageenan-induced rat paw oedema test**

The anti-inflammatory effect of the extracts was determined using the λ-carrageenan induced rat paw oedema test [14, 45]. The acute inflammation was induced 1 h after oral administration of the extracts, by an intraplantar injection of 100 μL of λ-carrageenan 1% saline solution (Sigma Aldrich, St. Louis, MO, USA) into the left hind paw of the rats. The paw volume (mL) was measured with a plethysmometer (Ugo Basile 7140, Varese, Italy) at 0, 1, 2, 3 and 4 hours after injection of λ-carrageenan. The volume of oedema and percentage of oedema inhibition were expressed as follows:

\[
\text{Oedema volume} = V_t - V_0 \text{(mL),}
\]

\[
\text{Oedema inhibition} = \left(1 - \frac{E_t}{E_c}\right) \times 100, \quad \text{where} \quad V_0 \text{ is the mean volume of oedema before the injection of λ-carrageenan; } V_t \text{ is the mean volume of oedema at time } t \text{ after the injection of λ-carrageenan; } E_t \text{ is the mean volume of oedema of treated animals; } E_c \text{ is the mean volume of oedema of animals in the negative control group.}
\]

**Randall-Selitto test in rats**

The antinociceptive activity of the extracts was evaluated by the Randall and Selitto test [14, 36] under inflammatory conditions. This test was performed simultaneously with the λ-carrageenan-induced rat paw oedema test and evaluates the nociceptive withdrawal threshold. In this model of inflammatory pain, a linearly increased mechanical force is applied on the inflamed paw and the pain threshold is determined at 0, 1, 2, 3 and 4 hours after the intraplantar injection of λ-carrageenan, using the analgesimeter (Ugo Basile 37215, Varese, Italy). The analgesimeter applies a linearly increased force (in grams) until the animal produces a response characterized by paw retraction or vocalization interpreted as mechanical hypernociception. The maximum applied weight where a response was obtained, was noted at each time interval.

**Statistical analysis**

Data were expressed as mean values ± S.E. and were statistically analysed by two-way ANOVA method. The differences between the treated groups and the control group were evaluated by Dunnett’s test, p < 0.05 being considered statistically significant.

**Results and Discussion**

**Total polyphenol content and in vitro antioxidant activity**

The total content of polyphenols (TPC) expressed as gallic acid equivalents (GAE) and the antioxidant activity of the extracts (H and E) of *T. alnifolia* and *H. crinita* were determined spectrophotometrically, and the results are presented in Table I. The content of total polyphenols was considerably higher in *T. alnifolia* extracts (> 25 mg GAE/g dry plant material), compared to *H. crinita* extracts. The antioxidant activity determined by DPPH radical bleaching method was in accordance with the polyphenols content, being higher for *T. alnifolia* extracts. Previous studies on *T. alnifolia* reported a lower content...
of total poly-phenols (15.15 ± 0.54 mg GAE/100 g) and a good antioxidant activity (IC_{50} = 41 - 51.5 µg/mL)[2].

Table I

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC (mg GAE/g)</th>
<th>I% (DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>E</td>
</tr>
<tr>
<td>T. alnifolia</td>
<td>30.87 ± 1.47</td>
<td>25.23 ± 1.02</td>
</tr>
<tr>
<td>H. crinita</td>
<td>6.95 ± 0.16</td>
<td>5.81 ± 0.15</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of three independent measurements; GAE = gallic acid equivalents.

LC-MS/MS analysis

The HPLC analysis of the extracts revealed the presence of several phenolic acids as well as flavonoids (Table II). Identification of the compounds was based on their retention times, and UV and MS spectra, compared to standards. The concentrations of identified polyphenolic compounds in both analysed samples are presented in Table II. In the extract of T. alnifolia, 14 polyphenolic compounds were determined (both phenolic acids and flavonoids), while 8 compounds were quantified in H. crinita extract. The major compound of T. alnifolia extract was gallic acid (33.31 µg/mL), while in H. crinita extract rutin had the highest concentration (34.70 µg/mL).

Among the eight compounds detected in the extract of H. crinita, five (chlorogenic acid, kaempferol, quercetin, quercitrin and rutin) were also identified in the work of Oboh et al. [32]. The other three compounds identified by us (p-coumaric acid, protocatechuic acid and isoorcitrin) were not detected [32]. This difference could be explained by the possible variety of the raw material, the extraction solvent or the detection method.

Table II

<table>
<thead>
<tr>
<th>Polyphenolic compounds</th>
<th>m/z value</th>
<th>Rt ± SD (min)</th>
<th>T. alnifolia</th>
<th>H. crinita</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg/mL</td>
<td>µg/g</td>
</tr>
<tr>
<td>Genticisic acid</td>
<td>153</td>
<td>3.69 ± 0.04</td>
<td>&lt; 0.02</td>
<td>-</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>179.1</td>
<td>5.6 ± 0.04</td>
<td>&lt; 0.02</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>353</td>
<td>6.43 ± 0.05</td>
<td>&lt; 0.02</td>
<td>8.32 ± 0.67</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>163</td>
<td>9.48 ± 0.08</td>
<td>3.99 ± 0.02</td>
<td>39.9 ± 0.02</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>193.2</td>
<td>12.8 ± 0.10</td>
<td>1.41 ± 0.05</td>
<td>14.1 ± 0.05</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>169</td>
<td>1.50 ± 0.01</td>
<td>33.31 ± 1.69</td>
<td>333.1 ± 1.69</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>153</td>
<td>2.80 ± 0.01</td>
<td>7.47 ± 0.04</td>
<td>74.7 ± 0.04</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>167.1</td>
<td>6.7 ± 0.01</td>
<td>0.60 ± 0.08</td>
<td>6.0 ± 0.08</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>289</td>
<td>9.00 ± 0.01</td>
<td>3.20 ± 0.15</td>
<td>32.0 ± 0.15</td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>463</td>
<td>19.60 ± 0.10</td>
<td>7.44 ± 0.46</td>
<td>74.4 ± 0.46</td>
</tr>
<tr>
<td>Rutin</td>
<td>609</td>
<td>20.20 ± 0.15</td>
<td>0.56 ± 0.03</td>
<td>05.6 ± 0.03</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>447</td>
<td>23.64 ± 0.13</td>
<td>7.65 ± 8.34</td>
<td>76.5 ± 8.34</td>
</tr>
<tr>
<td>Quercetin</td>
<td>301</td>
<td>26.80 ± 0.15</td>
<td>0.28 ± 0.01</td>
<td>2.8 ± 0.01</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>285.2</td>
<td>32.5 ± 0.17</td>
<td>1.61 ± 0.08</td>
<td>16.1 ± 0.08</td>
</tr>
</tbody>
</table>

Each value is the mean of 3 independent measurements ± SD (n = 3); Rt = retention time.

Carrageenan-induced rat paw oedema test

The results of the anti-inflammatory activity by the rat paw oedema test are presented in Table III. The hydro-ethanolic extract of H. crinita inhibited the formation of oedema at 1, 3 and 4 hours after the inflammation induction. The hydro-ethanolic extract of T. alnifolia (HTA) only showed a significant effect at 4 hours. The ethanolic extracts of H. crinita (EHC) and T. alnifolia (ETA) inhibited the evolution of oedema from the first hour with an inhibition percentage of 21.35 and 56.20, respectively. Diclofenac, the reference drug, prevented the formation of oedema at every hour.

Randall-Selitto test in rats

The results of the Randall-Selitto test in rats are presented in Table IV. Extracts of H. crinita and T. alnifolia produced a significant analgesic effect compared to negative control group. Extracts of H. crinita (HHC, EHC) showed a significant analgesic effect at 2 and 3 hours. The hydro-ethanolic extract of T. alnifolia (HTA) presented antinociceptive activity starting with the second hour. The ethanolic extracts of H. crinita (EHC) and T. alnifolia (ETA) significantly increased the threshold reaction pain, from the first hour at the threshold of 120 g and 130 g, respectively.
Carrageenan induced oedema is an excellent representative model of acute inflammation. This pharmacological model of inflammation is used to assess the influence of the substances on the inflammation, but also on the inflammatory pain evaluated with the Randall-Selitto test. Carrageenan is a polysaccharide with a biphasic mechanism of action. The first phase (0 - 1 h) is characterized by the release and action of serotonin, histamine, bradykinin, complement and reactive oxygen species. The second phase (1 hour later) is marked by prostaglandins which accelerate the progression of oedema and cause pain [11].

Extracts of *H. crinita* (HHC, EHC) and ethanol extract of *T. alnifolia* (ETA) act significantly from the first hour of the oedema evolution. These extracts could therefore have an inhibitory action on one of the inflammatory mediators released during the first phase or second phase. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) which inhibits the isoenzymes (COX-1 and COX-2) responsible for the synthesis of prostanoids. The main mechanism for the potent analgesic and anti-inflammatory properties of NSAIDs is the inhibition of the predominant prostanoid PGE2 synthesis [3]. The ethanolic extracts for both plants have the best effect, superior to diclofenac, in the first three hours after the induction of inflammation. Oedema inhibition rates were higher than diclofenac especially in the case ethanolic extract of *H. crinita* (EHC).

Extracts of *H. crinita* and *T. alnifolia* (500 mg/kg) increase the pain threshold significantly, compared to negative control group. We therefore suggest that the action of all these extracts involves the inhibition of PGE2 synthesis. This time, the best effect, superior to diclofenac, is for ethanolic extract from *T. alnifolia* (ETA). Our results on the ethanolic extract of *H. crinita* (EHC) corroborates the work of Ukeme *et al.* [44]. Indeed, they also demonstrated the anti-inflammatory and analgesic effect of the ethanolic extract of *H. crinita*. Similarly, Adeyemi *et al.* [2] showed the anti-inflammatory and analgesic effect of the hydro-ethanolic extract of *T. alnifolia*.

The effects observed in our study could be partly explained by the action of polyphenolic compounds identified in *H. crinita* and *T. alnifolia*. Indeed, polyphenols have important therapeutic properties including anti-inflammatory and antioxidant effects [46]. In our study, we identified chlorogenic acid (in *H. crinita*), *p*-coumaric acid, protocatechuic acid, quercitrin, isoquercitrin, rutin and gallic acid as well represented quantitatively polyphenolic compounds in the studied extracts. Chlorogenic acid is a phenolic acid which has an anti-inflammatory effect on the metabolism of arachidonic acid; it inhibits and regulates the expression

### Table III

Effects of hydro-ethanolic (H) and ethanol (E) extracts of *H. crinita* (HC) and *T. alnifolia* (TA) on oedema induced by λ-carrageenan

<table>
<thead>
<tr>
<th>Exacts</th>
<th>Dose (mg/kg)</th>
<th>Volume of oedema in mL (percentage of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td><strong>Negative control</strong></td>
<td>1.274 ± 0.046</td>
<td>1.772 ± 0.032</td>
</tr>
<tr>
<td><strong>HHC</strong></td>
<td>500</td>
<td>0.926 ± 0.062***</td>
</tr>
<tr>
<td><strong>EHC</strong></td>
<td>500</td>
<td>1.002 ± 0.050**</td>
</tr>
<tr>
<td><strong>HTA</strong></td>
<td>500</td>
<td>1.99 ± 0.071</td>
</tr>
<tr>
<td><strong>ETA</strong></td>
<td>500</td>
<td>0.558 ± 0.025***</td>
</tr>
<tr>
<td><strong>Diclofenac</strong></td>
<td>20</td>
<td>1.106 ± 0.023*</td>
</tr>
</tbody>
</table>

All data are presented as mean ± standard error of mean, n = 5 rats, *p < 0.05; **p < 0.01; ***p < 0.001; ns = non-significant compared to the control.

### Table IV

Effects of hydro-ethanolic (H) and ethanol (E) extracts of *H. crinita* (HC) and *T. alnifolia* (TA) by the Randall and Selitto test

<table>
<thead>
<tr>
<th>Exacts</th>
<th>Dose (mg/kg)</th>
<th>Threshold intensity (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td><strong>Negative control</strong></td>
<td>96 ± 2.92</td>
<td>59 ± 4.85</td>
</tr>
<tr>
<td><strong>HHC</strong></td>
<td>500</td>
<td>97 ± 3.00 ns</td>
</tr>
<tr>
<td><strong>EHC</strong></td>
<td>500</td>
<td>120 ± 6.32**</td>
</tr>
<tr>
<td><strong>HTA</strong></td>
<td>500</td>
<td>106.5 ± 4.58 ns</td>
</tr>
<tr>
<td><strong>ETA</strong></td>
<td>500</td>
<td>130 ± 7.58***</td>
</tr>
<tr>
<td><strong>Diclofenac</strong></td>
<td>20</td>
<td>94 ± 1.87 ns</td>
</tr>
</tbody>
</table>

All data are presented as mean ± standard error of mean, n = 5 rats, *p < 0.05; **p < 0.01; ***p < 0.001; ns = non-significant compared to the control.
of key molecules of the JAK/STAT and NK-xB signalling pathways [29]. Its antioxidant action involves reducing the production of oxygen free radicals, reducing the level of lipid peroxidation by regulating the activity of antioxidant enzymes [29]. Quercitin, (quercetin-3-rhamnoside), is a flavonoid with antioxidant, anti-inflammatory, anti-apoptotic, anti-platelet and anti-thrombotic effects [34]. Rutin (quercetin-3-O-rutinoside) is a flavonoid with anti-inflammatory properties on the activity of certain pro-inflammatory cytokines (TNF-α, IL-1β, IL-8), cyclo-oxygenase 2, nuclear factor-xB (NF-xB), and inducible nitric oxide synthase. It also has the capacity to give electrons to reactive free radicals by converting them into more stable species and stopping the reaction in the free radical chain [10, 19, 21]. Gallic acid is a phenolic acid involved in various signalling pathways (NF-xB and MAPK). It inhibits the activities of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), intracellular adhesion molecules (CCL-2, ICAM-1, TIMP-1, CCL5, CXCL8), cyclo-oxygenase, nitric oxide. It induces the release of endogenous antioxidant enzymes (superoxide dismutase, catalase) [6, 22]. It also has a high reducing power but a low chelating capacity [9]. Thus, these compounds, identified in H. crinita and T. alnifolia can act in synergy with other compounds or have a singular action in anti-inflammatory, antioxidant and analgesic mechanisms.

Conclusions

Our study evaluated the polyphenolic profile, anti-inflammatory, analgesic and antioxidant effects of H. crinita and T. alnifolia leaves extracts. The chemical analyses revealed a high content of total polyphenols in T. alnifolia, with gallic acid as major compound. The in vitro antioxidant activity was also better for T. alnifolia extract. The administration of H. crinita and T. alnifolia extracts reduced the formation of carrageenan-induced oedema in rats at a single dose of 500 mg/kg. These extracts also produced an interesting analgesic effect. However, additional studies are necessary in order to confirm the therapeutical importance of the studied species.

Conflict of interest

The authors declare no conflict of interest.

References

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