https://doi.org/10.31925/farmacia.2022.2.12 **ORIGINAL ARTICLE**

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

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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. Antioxidant 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 efectele 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-caragenan, iar cea analgezică prin testul Randall-Selitto. Activitatea antioxidantă a extractelor etanolice a fost evaluată spectrofotometric prin testul de *scavenger* a 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 anti-oxidant 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* Afz.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 anti-bacterial 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, nephro-protective 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-Ciocâlteu method. Each ethanolic extract (1 mL) diluted 25 times was mixed with Folin-Ciocâlteu 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 DPPH solution was added. After 30 min of incubation at 40°C in a thermostatic bath, the decrease in the absorbance (n = 3) was measured at 517 nm, compared to ethanol.

The percent DPPH scavenging ability was calculated as:

$$\text{DPPH scavenging ability (I\%)} = (A_{control} - A_{sample})/ A_{control} * 100,$$

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 Haţieganu" 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:

$$\text{Oedema volume} = Vt - Vo \text{ (mL)},$$

$$\text{Oedema inhibition} = (1 - Et/Ec) * 100,$$

where Vo is the mean volume of oedema before the injection of λ-carrageenan; Vt is the mean volume of oedema at time t after the injection of λ-carrageenan; Et is the mean volume of oedema of treated animals; Ec 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

Total content of polyphenols (mg/g dry plant material) and antioxidant activity (I%)

Samples	TPC (mg GAE/g)		I% (DPPH)	
	H	E	H	E
T. alnifolia	30.87 ± 1.47	25.23 ± 1.02	93.744 ± 2.25	85.52 ± 1.75
H. crinita	6.95 ± 0.16	5.81 ± 0.15	34.94 ± 0.06	30.92 ± 0.06

Each value is the mean ± SD of three independent measurements; GAE = gallic acid equivalents. I% was obtained for the concentration of 1 mg/mL extract

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 poly-phenolic 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, proto-catechuic acid and isoquercitrin) 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

Polyphenols identified by LC-MS/MS in the studied extracts (µg/mL extract and µg/g dry vegetable product)

Polyphenolic compounds	m/z value	Rt ± SD (min)	*T. alnifolia*		*H. crinita*	
			µg/mL	µg/g	µg/mL	µg/g
Gentisic acid	153	3.69 ± 0.04	< 0.02		-	
Caffeic acid	179.1	5.6 ± 0.04	< 0.02		-	
Chlorogenic acid	353	6.43 ± 0.05	< 0.02		8.32 ± 0.67	83.2 ± 0.67
p-Coumaric acid	163	9.48 ± 0.08	3.99 ± 0.02	39.9 ± 0.02	1.52 ± 0.07	15.2 ± 0.07
Ferulic acid	193.2	12.8 ± 0.10	1.41 ± 0.05	14.1 ± 0.05	-	-
Gallic acid	169	1.50 ± 0.01	33.31 ± 1.69	333.1 ± 1.69	-	-
Protocatechuic acid	153	2.80 ± 0.01	7.47 ± 0.04	74.7 ± 0.04	0.26 ± 0.03	2.6 ± 0.03
Vanillic acid	167.1	6.7 ± 0.01	0.60 ± 0.08	6.0 ± 0.08	-	-
Epicatechin	289	9.00 ± 0.01	3.20 ± 0.15	32.0 ± 0.15	-	-
Isoquercitrin	463	19.60 ± 0.10	7.44 ± 0.46	74.4 ± 0.46	3.58 ± 0.01	35.8 ± 0.01
Rutin	609	20.20 ± 0.15	0.56 ± 0.03	05.6 ± 0.03	34.70 ± 0.79	347.0 ± 0.79
Quercitrin	447	23.64 ± 0.13	7.65 ± 8.34	76.5 ± 8.34	13.45 ± 0.54	134.5 ± 0.54
Quercetin	301	26.80 ± 0.15	0.28 ± 0.01	2.8 ± 0.01	0.22 ± 0.02	2.2 ± 0.02
Kaempferol	285.2	32.5 ± 0.17	1.61 ± 0.08	16.1 ± 0.08	4.92 ± 0.77	49.2 ± 0.77

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.

Table III

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

Extracts	Dose (mg/kg)	Volume of oedema in mL (percentage of inhibition)			
		1 h	2 h	3 h	4 h
Negative control		1.274 ± 0.046	1.772 ± 0.032	2.766 ± 0.043	2.758 ± 0.018
HHC	500	0.926 ± 0.062*** (27.32)	1.626 ± 0.055 ns (8.24)	2.034 ± 0.021*** (26.46)	2.398 ± 0.080*** (13.05)
EHC	500	1.002 ± 0.050** (21.35)	1.112 ± 0.063*** (37.25)	1.144 ± 0.041*** (58.64)	1.318 ± 0.025*** (52.21)
HTA	500	1.99 ± 0.071 (-)	2.494 ± 0.056 (-)	2.692 ± 0.074 ns (2.68)	2.48 ± 0.064** (10.08)
ETA	500	0.558 ± 0.025*** (56.20)	1.014 ± 0.035*** (42.78)	1.318 ± 0.019*** (52.35)	1.872 ± 0.050*** (32.12)
Diclofenac	20	1.106 ± 0.023* (13.19)	1.38 ± 0.057*** (22.12)	1.37 ± 0.089*** (50.47)	1.544 ± 0.035*** (44.02)

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

Extracts	Dose (mg/kg)	Threshold intensity (g)			
		1 h	2 h	3 h	4 h
Negative control		96 ± 2.92	59 ± 4.85	54 ± 2.92	61 ± 2.92
HHC	500	97 ± 3.00 ns	81 ± 4.00**	86 ± 3.32***	73 ± 5.39 ns
EHC	500	120 ± 6.32**	83 ± 4.36**	80 ± 4.18***	71 ± 4.30 ns
HTA	500	106.5 ± 4.58 ns	112.5 ± 3.35***	90 ± 6.32***	81.5 ± 5.68*
ETA	500	130 ± 7.58***	126 ± 7.97***	93 ± 5.15***	93 ± 5.15***
Diclofenac	20	94 ± 1.87 ns	106 ± 4.00***	95 ± 2.74***	89 ± 4.85***

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

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 iso-enzymes (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

of key molecules of the JAK/STAT and NK-κB 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]. Quercitrin, (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 inhibitory properties on the activity of certain pro-inflammatory cytokines (TNF-α, IL-1β, IL-8), cyclo-oxygenase 2, nuclear factor-κB (NF-κB), 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-κB 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 anti-oxidant 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.

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