Pharmacogn J. 2025; 17(5): 620-631
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Antioxidant and Neuroprotective Effects of *Chuquiraga spinosa* Less. and *Baccharis genistelloides* (Pers.) Lam. in a Rat Model of Transient Cerebral Ischemia-reperfusion

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History

- Submission Date: 29-08-2025;
- Review completed: 18-09-2025;
- Accepted Date: 03-10-2025.

DOI : 10.5530/pj.2025.17.77

Article Available online

http://www.phcogj.com/v17/i5

ABSTRACT

Background: Cerebral ischemia, a leading cause of disability and mortality, is strongly related to oxidative stress and inflammation, highlighting the need for neuroprotective antioxidant and cytokine-modulating agents. **Objective:** To characterize the phytochemical profile and evaluate the antioxidant and neuroprotective effects of hydroalcoholic extracts of *Chuquiraga spinosa* (ChS) and *Baccharis genistelloides* (BaG), individually and in combination, in a rat model of cerebral ischemia–reperfusion. **Methodology:** Phytochemical screening and GC-MS were performed with antioxidant assays (ABTS•+, DPPH•, FRAP). Neurological deficit was assessed (Bederson scale), while histopathology, oxidative stress markers (MDA, GSH, SOD, CAT, NOx), and cytokines (IL-6, TNF-α, IL-1β) were measured. Groups included Normal (no ischemia), Ischemia (oral placebo), Citicoline 300 mg/kg, ChS 500 mg/kg, BaG 500 mg/kg, and the oral combination ChS 500 + BaG 500 mg/kg, all administered for seven days prior to ischemia induction. **Results:** ChS had higher total phenolic content than BaG ($p = 0.0079$). GC-MS identified 23 compounds in ChS and 17 in BaG. The combination displayed greater antioxidant activity than either extract. At 24 h, ChS 500 mg/Kg and the combination reduced severe neurological deficit to 17% (vs. 83% in ischemia). Histopathology revealed less neuronal damage with the combination, comparable to ChS 500 mg/Kg. All treatments decreased MDA levels; the combination also enhanced GSH and CAT and significantly reduced TNF-α and IL-1β. **Conclusion:** *ChS* and *BaG* extracts exert neuroprotective effects against cerebral ischemia. Their combination shows synergistic antioxidant activity against free radicals and enhances the modulation of inflammatory cytokines, supporting a greater neuroprotective potential.

Keywords: Cerebral ischemia; neuroprotection; antioxidants; *Chuquiraga spinosa* Less.; *Baccharis genistelloides* (Pers.) Lam.

INTRODUCTION

Stroke remains one of the leading global causes of death and disability; although age-adjusted mortality has declined in several regions, the absolute burden continues to increase due to the ageing of the population and the epidemiological transition[1,2]. The World Health Organization has maintained it among the leading causes of global mortality since its most recent update[3].

In ischemic stroke, the interruption of blood flow triggers a complex cascade of events: glutamatergic excitotoxicity, mitochondrial dysfunction, overproduction of reactive oxygen and nitrogen species, disruption of the blood-brain barrier, and sustained neuroinflammation involving microglia, astrocytes, and infiltrating cells[4]. Among the central nodes of this inflammatory response is the NLRP3 inflammasome, whose activation correlates with increased tissue damage and neurological deficits in preclinical models[5].

Reperfusion therapies, including intravenous thrombolysis and mechanical thrombectomy, have transformed acute stroke management. However, their narrow therapeutic window, limited patient eligibility, and unequal availability prevent many patients from accessing them. Moreover, no neuroprotective drugs have yet been approved as adjuvants with proven benefits in clinical outcomes[6,7]. These limitations have renewed interest in multimodal strategies that reduce ischemia–reperfusion injury and enhance tissue protection beyond reperfusion[6].

Polyphenols and other plant-derived secondary metabolites are emerging as plausible candidates due to their ability to reduce oxidative stress, modulate Nrf2/ARE and NF-κB pathways, and dampen activation of NLRP3, showing consistent signals of neuroprotection in models of cerebral ischemia Polyphenols and other plant-derived secondary metabolites are emerging as plausible candidates due to their ability to reduce oxidative stress, modulate Nrf2/ARE and NF-κB pathways, and dampen NLRP3 activation, showing consistent signals of neuroprotection in cerebral ischemia models[8,9]. This mechanistic pleiotropy is particularly attractive for a multifactorial condition such as stroke[1,10].

In the Peruvian Andes, *Chuquiraga spinosa* Less. and *Baccharis genistelloides* (Lam.) Pers. are traditionally used and are rich in polyphenols

Cite this article: Justil-Guerrero HJ, Arroyo-Acevedo JL, Rojas-Armas JP, Palomino-Pacheco M, García-Bustamante CO, Cieza-Macedo EC, Huarcaya-Rojas JY, Torres-Lévano JD, Peña-Galindo JJ, Franco-Soto EO, Chávez-Flores JE, Cárdenas-Orihuela RA. Antioxidant and Neuroprotective Effects of *Chuquiraga spinosa* Less. and *Baccharis genistelloides* (Pers.) Lam. in a Rat Model of Transient Cerebral Ischemia-reperfusion. Pharmacogn J. 2025;17(5): 620-631.

(flavonoids, caffeoylquinic acids, terpenoids, among others) with documented antioxidant and anti-inflammatory effects[11-13]. *C. spinosa* has shown antioxidant properties and a reduction of inflammatory mediators in vitro and in vivo[11,14]. For *B. genistelloides*, more than one hundred compounds have been identified, including flavonoids such as quercetin and rutin, as well as clerodane-type diterpenes with relevant biological activities[12,15]. In vivo studies have reported that hydroalcoholic extracts of both species, at a dose of 500 mg/kg, reduce markers of oxidative stress and inflammation[14,16]. However, evidence of their neuroprotective potential in experimental models of cerebral ischemia remains scarce, and further exploration of their chemical constituents is needed.

Within this context, we aimed to describe the phytochemical characterization and to evaluate the **antioxidant activity** and **neuroprotective effect** of hydroalcoholic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides*, alone and in combination, in a rat model of transient middle cerebral artery occlusion (MCAO) followed by reperfusion. This evaluation included **in vitro assays** and **in vivo endpoints** related to oxidative stress, neuroinflammation, and histological/functional recovery.

MATERIALS AND METHODS

Handling of experimental animals

The experimental study was conducted in accordance with the guidelines of the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Research Council[17]. Housing conditions complied with the five animal welfare freedoms. Environmental temperature was maintained at 22 ± 2 °C, relative humidity at 55 ± 10%, and noise levels below 20 kHz. Animals were kept under a 12 h light/dark cycle with free access to purified water and a balanced rodent diet provided by the Universidad Nacional Agraria La Molina

The experimental protocol was reviewed and approved by the Ethics Committee of the Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos (Approval Certificate N° 007-CE-UDI-FFB-2020).

Design

This study was conducted using an experimental design with control groups. The independent variable corresponded to the hydroalcoholic extracts obtained from the plant species. The dependent variable was the neuroprotective effect. The extracts were previously characterized through phytochemical screening and evaluation of their antioxidant activity.

Reagents

Aluminum chloride hexahydrate ($AlCl_3 \cdot H_2O$), Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ascorbic acid (AA), quercetin (Q), gallic acid (GA), thiobarbituric acid (TBA), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), thiobarbituric acid, nitroblue tetrazolium (NBT), and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Species

The plant species were collected in Peru, in the department of Huancavelica, province of Tayacaja, district of Acostambo, annex of Parco Villanueva. The samples were subsequently deposited in the Herbarium of the Natural History Museum of the Universidad Nacional Mayor de San Marcos, where they were identified as *Chuquiraga spinosa* Less. (Certificate No. 242-USM-2019) and *Baccharis genistelloides* (Lam.) Pers. (Certificate No. 060-USM-2018).

Extraction of Chemical Components

The extraction of bioactive compounds was carried out following the protocol described by Zucolotto et al.[18]. The leaves were washed with potable water, dried in the shade for 24 hours, and subsequently oven-dried at 40 °C ± 2 for 48 hours. They were then ground in an electric mill until particles of 3 mm were obtained. The extracts were obtained by maceration with 70° ethanol, in a ratio of 1:10 (w/v) for 7 days. At the end of this process, the solvent was recovered using a rotary evaporator, and the extracts were oven-dried at 40 °C. Moisture content was determined gravimetrically: 1 g of each extract was weighed, dried in an oven at 100–105 °C for 2 hours, cooled, and reweighed. The percentage of moisture was calculated based on the weight difference. Hydroalcoholic extracts were obtained from *Chuquiraga spinosa* Less. (ChS), with 1.5% moisture, and from *Baccharis genistelloides* (Pers.) Lam. (BaG), with 2.1%.

Phytochemical Analysis

Preliminary Qualitative Analysis.

The identification of bioactive compounds was performed through phytochemical screening, following the protocol described by Lock[19]. After preparation of the extracts with the corresponding solvents, they were fractionated into aliquots and subjected to specific tests for different groups of compounds. Ferric chloride ($FeCl_3$) was used for phenolic compounds; Shinoda and aluminum chloride ($AlCl_3$) tests for flavonoids; gelatin tests for tannins; Dragendorff and Mayer tests for alkaloids; Bornträger's test for quinones; sulfuric vanillin test for terpenes; Liebermann–Burchard test for terpenoids and steroids; Baljet A and B reagents for sesquiterpene lactones; and Frothing test for saponins. The presence of metabolites was determined through color changes or precipitate formation, which enabled a preliminary identification of the bioactive compounds in the analyzed simples.

Total Phenolic Content

The quantification of phenolic compounds was performed using the Folin–Ciocalteu reduction method, following the procedure previously described by Singleton et al.[20]. A total of 25 mg of each extract was weighed and dissolved in methanol to obtain a concentration of 100 µg/mL. From this solution, 500 µL were taken and mixed with 250 µL of the Folin–Ciocalteu reagent. After 5 minutes, 1250 µL of a 20% sodium carbonate solution were added to alkalinize the mixture. Following a 2-hour incubation in darkness, absorbance was measured at 760 nm using a GENESYS® 10S UV-VIS spectrophotometer (Waltham, MA, USA). Each type of extract was analyzed in triplicate. The total phenolic concentration was determined using a calibration curve prepared with standard gallic acid solutions, and the results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Total Flavonoid Content

The quantification of total flavonoids was carried out using a colorimetric method based on the formation of flavonoid–$AlCl_3$ complexes, according to the procedure described by Dewanto et al.[21]. A total of 25 mg of each extract was weighed and diluted in methanol to obtain a concentration of 100 µg/mL. To 0.5 mL of this solution, 2.25 mL of H_2O and 0.15 mL of 5% $NaNO_2$ were added. After 6 minutes, 10% $AlCl_3 \cdot H_2O$ was incorporated, and the mixture was incubated for 5 minutes at room temperature. Subsequently, 1 mL of 1 M NaOH was added, vortexed, and the absorbance was measured at 510 nm using a UV-VIS spectrophotometer. Each extract was analyzed in triplicate.

The total flavonoid content was calculated using a calibration curve prepared with standard quercetin solutions, and the results were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

Gas Chromatography Analysis

The identification of chemical components was performed by gas chromatography coupled with mass spectrometry (GC-MS) using a TRACETM 1310 gas chromatograph (Waltham, MA, USA) coupled to an ISQTM 7000 mass spectrometer (Bartlesville, OK, USA). Solutions were prepared by diluting 10 mg of the extracts in 1 mL of methanol. From each diluted solution, 1 µL was injected for the analysis of volatile compounds. The GC-MS system employed a DB-5MS column (30 m × 250 µm × 0.25 µm) with a temperature program starting at 50 °C (5 min), followed by an increase of 3 °C/min up to 155 °C, and then 15 °C/min up to 250 °C, maintaining this temperature for 2 min. The injection was carried out in split mode (40:1), using helium as the carrier gas at a flow rate of 1 mL/min[22]. The identification of bioactive compounds was based on comparison of retention time (RT) and mass spectra with the NIST20 database and specialized literature[23]. The relative area of each compound was expressed as a percentage, calculated from the ratio of the corresponding peak area to the total area of all identified peaks, without applying correction factors.

Antioxidant Capacity

DPPH• Radical Scavenging Capacity

Antioxidant activity by DPPH• radical reduction was evaluated by measuring the decrease in violet absorbance, according to the method described by Prieto[24]. A 0.4 mmol DPPH• solution was prepared in HPLC-grade methanol. Extracts were diluted into five concentrations and adjusted to a final volume of 100 µL in a microplate, to which 100 µL of the DPPH• solution were added. A control containing only the DPPH• solution and a blank with solvent were included. Samples were incubated at 25 °C for 30 min, and absorbance was measured at 517 nm using an EMPEROR microplate reader (Shenzhen, China). The radical scavenging capacity of the extracts was compared by calculating the 50% inhibitory concentration (IC_{50}), expressed in µg/mL.

ABTS•$^+$ Radical Scavenging Capacity

Antioxidant activity was determined by the ABTS•$^+$ radical decolorization assay, according to the method described by Re et al.[25]. The ABTS•$^+$ radical was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) in darkness for 16 h at room temperature. The resulting solution was diluted to an absorbance of 0.700 ± 0.020 at 734 nm. Extracts were tested at five concentrations by mixing 100 µL of each sample with 1 mL of the ABTS•$^+$ solution and incubating in darkness for 30 min. Absorbance was measured at 734 nm using a UV-VIS spectrophotometer. Results were expressed as the 50% inhibitory concentration (IC_{50}), in µg/mL.

Ferric Reducing Antioxidant Power (FRAP)

Antioxidant capacity by the FRAP method was determined according to the procedure described by Belyagoubi et al.[26], which is based on the reduction of ferric iron (Fe^{3+}) to ferrous ion (Fe^{2+}), producing a color change from light blue to intense blue. The freshly prepared FRAP reagent was made using sodium acetate–acetic acid buffer (pH 3.6), TPTZ, and $FeCl_3$ in a ratio of 25:2.5:2.5 mL, and the final volume was adjusted to 80 mL with distilled water. The calibration curve was prepared with ascorbic acid (AA) at concentrations of 1, 2, 3, 4, 5, and 10 ppm, yielding a correlation coefficient between 0.8 and 1. For the analysis, three replicates of each extract at 100 ppm were prepared; from this solution, 0.5 mL was mixed with 1.5 mL of the FRAP reagent, resulting in a final concentration of 10 ppm. Samples were incubated in darkness for 60 min, and absorbance was measured at 593 nm using a UV-VIS spectrophotometer. Results were expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg AAE/g).

Protective effect of central carotid artery occlusion–induced cerebral ischemia in rats

Experimental design

Thirty-six male Holtzman rats (weighing 200–250 g) were distributed into six groups of six animals each:

1. Normal group (surgery without occlusion) with vehicle p.o. (Normal)
2. Ischemia group with vehicle p.o. (Ischemia)
3. Ischemia group with 300 mg/kg Citicoline p.o. (Citicoline 300)
4. Ischemia group with 500 mg/kg ChS p.o. (ChS 500)
5. Ischemia group with 500 mg/kg BaG p.o. (BaG 500)
6. Ischemia group with combined treatment p.o. (ChS 500 + BaG 500)

The extracts, the drug, or the vehicle were administered from seven days before until one hour prior to ischemia induction.

Induction of transient focal cerebral ischemia

Focal cerebral ischemia was induced by intraluminal middle cerebral artery occlusion (MCAO), following the protocol described by Uluç et al.[27] with minor modifications. The procedure was performed under sterile conditions and in compliance with ethical standards for the use of laboratory animals. Rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), i.p., and placed in the supine position on a heating surface to maintain body temperature at 37 ± 0.5 °C. A midline cervical incision was made to expose the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA).

The ECA was ligated and a small incision was made in it to insert a silicone-coated monofilament nylon suture (diameter 0.37–0.40 mm, rounded tip) into the arterial lumen. The filament was carefully advanced through the ICA for about 18–20 mm from the bifurcation point to occlude the middle cerebral artery. Proper occlusion was confirmed by resistance to advancement and absence of hemorrhage.

After 60 minutes of occlusion, the filament was withdrawn to allow blood reperfusion, and the surgical wound was closed. Animals were placed in individual cages with free access to food and water and monitored during the postoperative period. In the normal group, the same arterial exposure was performed without filament insertion.

Evaluation of functional effects

Neurological functions in rats were assessed 24 hours after induction of focal cerebral ischemia using the neurological scale described by Bederson et al.[10]. This behavioral test allowed the identification of motor and postural deficits associated with ischemic injury by observing simple and reproducible responses. For the assessment, each animal was gently held by the tail, and the position of the forelimbs, resistance to lateral push, and presence of spontaneous circling movements were observed. Neurological scores were assigned according to the following criteria:

0: No observable neurological deficit; symmetric extension of forelimbs.

1: Flexion of the forelimb contralateral to the lesion side (Mild).

2: Contralateral flexion accompanied by reduced resistance to lateral push (Moderate).

3: In addition to the above signs, the animal exhibits circling movements toward the affected side (Severe).

This scale provided a rapid and semiquantitative assessment of neurological damage, useful for comparing the neuroprotective effects of the treatments. All evaluations were performed by an observer blinded to the assigned treatment.

Collection of biological samples

After the evaluation of neurological functions, the animals were anesthetized, and blood was obtained by cardiac puncture followed by exsanguination, as described by Tobar et al.[28]. Serum was separated by centrifugation at 2000 rpm for 10 minutes. The brain was extracted, washed with cold saline solution, and sagittally divided into hemispheres. A portion of the right hemisphere was homogenized in RIPA buffer at a concentration of 0.1 g/mL. After centrifugation at 10,000 rpm, the supernatant was stored at −20 °C for biochemical analyses.

Evaluation of tissue damage

The left hemisphere of the brain was fixed in 10% formalin for 48 hours, then dehydrated in ascending alcohol series, cleared with xylene, and embedded in paraffin. Coronal sections of 5 µm were obtained at the cortex and hippocampus, corresponding to the infarct region, mounted on slides, and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

The stained sections were examined under a LEICA DM 750 light microscope with a LEICA ICC50 W camera (Heerbrugg, Switzerland) at 400× magnification. Histopathological parameters assessed included pyknosis, neuropil vacuolization, loss of neuronal architecture, and vascular congestion. Each parameter was scored semi-quantitatively on a scale from 0 to 3, where: 0 = no alterations; 1 = mild damage; 2 = moderate damage; and 3 = severe damage

Biochemical parameters of oxidative stress and neuroinflammation

Malondialdehyde

The concentration of malondialdehyde (MDA) was determined in brain tissue supernatant using thiobarbituric acid, following the method described by Esterbauer and Cheeseman[29]. A volume of 0.5 mL of sample was mixed with 1 mL of 20% trichloroacetic acid. After shaking, the mixture was centrifuged at 3000 rpm for 10 minutes under refrigeration. Then, 1 mL of the supernatant was combined with 1.5 mL of 0.67% thiobarbituric acid dissolved in 0.25 N HCl. The mixture was shaken and incubated in a boiling water bath for 30 minutes, followed by cooling in an ice-water bath. Absorbance was measured at 535 nm using a UV-VIS spectrophotometer. MDA concentration was calculated using a molar extinction coefficient of 156 $mM^{-1} \cdot cm^{-1}$. Results were expressed as micromoles per gram of tissue (µmol/g).

Glutathione

The concentration of glutathione (GSH) was determined in brain tissue supernatant by its reaction with DTNB, following the method described by Sedlak and Lindsay[30]. A volume of 0.5 mL of sample was mixed with 1.5 mL of 0.02 M TRIS buffer (pH 8.2) and 0.1 mL of DTNB at a concentration of 0.01 M. The volume was then adjusted to 10 mL with absolute methanol. The tubes were sealed with rubber stoppers and left to stand for 15 minutes. They were then centrifuged at 3000 rpm for 15 minutes. Absorbance was measured at 412 nm using a UV-VIS spectrophotometer. Six samples were analyzed in total. GSH concentration was calculated using a molar extinction coefficient of 13.1 $mM^{-1} \cdot cm^{-1}$. Results were expressed as micromoles per gram of tissue (µmol/g).

Superoxide dismutase

Superoxide dismutase (SOD) activity was determined in brain tissue supernatant by the reduction of NBT to blue formazan, following the method of Beauchamp and Fridovich[31]. Solutions of 50 mM potassium phosphate buffer (pH 7.8), 0.3 mM NBT, 0.04 mM riboflavin, 0.1 mM EDTA, and 13 mM methionine were prepared. In test tubes, 2.6 mL of buffer, 0.3 mL of each solution, and 0.1 mL of sample were mixed. The tubes were then incubated for 15 minutes at 25 °C, covered with aluminum foil, and exposed to fluorescent light for 10 minutes. Absorbance was measured at 560 nm using a UV-VIS spectrophotometer. SOD activity was calculated by dividing the difference in absorbance between samples with and without SOD by the control absorbance, and multiplying by the dilution factor. Results were expressed as units per milligram of protein (U/mg protein)

Catalase

Catalase (CAT) activity was assessed in brain tissue supernatant by the decomposition of H_2O_2 into water and oxygen, following the methodology described by Chance and Maehly[32]. For this, 2.9 mL of 50 mM phosphate buffer containing 0.05% H_2O_2 solution was added to 100 µL of supernatant in a tube. Absorbance was measured immediately at 240 nm, recording values every 15 seconds for 1 minute. CAT activity was calculated by dividing the change in absorbance per minute by the milligrams of protein per milliliter and multiplying the result by a factor of 7500. Results were expressed as units per milligram of protein (U/mg protein).

Nitrates and nitrites

The concentration of total nitrites and nitrates (NO_3^-/NO_2^-) was determined in brain tissue supernatant by nitrate reduction using the Griess reagent, as described by Palmer et al.[33]. For this, 800 µL of sample was mixed with 700 µL of distilled water and 100 µL of 1 M NaOH. After 5 minutes, 100 µL of 30% $ZnSO_4$ was added, and the mixture was shaken until a whitish suspension formed. The mixture was centrifuged at 3000 rpm for 15 minutes. To 920 µL of the supernatant, a small amount of zinc powder was added, shaken, and left to stand for 45 minutes, followed by centrifugation under the same conditions. Then, 800 µL of the resulting supernatant was mixed with 800 µL of a solution containing 2% sulfanilamide and 0.1% N-1-naphthylethylenediamine. Absorbance was measured at 540 nm using a UV-VIS spectrophotometer. Nitric oxide quantification was performed using a calibration curve prepared with sodium nitrite standard solutions. Results were expressed as micromoles per gram of tissue (µmol/g).

Cytokines

The quantification of IL-6, IL-1β, and TNF-α cytokines in brain tissue supernatant was performed using a sandwich ELISA technique, with commercial rat-specific kits from ELK Biotechnology (Denver, CO, USA). Samples and standards were prepared and added to microplates pre-coated with specific capture antibodies. After an incubation step, successive washes were performed before adding the detection antibody conjugated to an enzyme. Following a second incubation and additional washes, the enzymatic substrate was added, and the reaction was stopped by adding a stop solution. Absorbance was measured at 450 nm using a microplate reader, and cytokine concentrations were determined from a standard curve and expressed as picograms per gram of tissue (pg/g).

Data Processing and Analysis

Neurological and histopathological scores were expressed as frequencies and percentages, presented as median and range. Median comparisons were performed using the Kruskal–Wallis test, while differences between groups were assessed with Dunn's test and Bonferroni correction. Oxidative stress and neuroinflammation parameters were expressed as mean ± 95% confidence interval (95% CI). These data were first analyzed by testing variance homogeneity (Levene's test) and distribution normality (Shapiro–Wilk test). Subsequently, a one-way ANOVA was conducted, followed by Tukey's multiple comparisons test to assess significant differences among group means. In all cases, a p value < 0.05 was considered statistically significant. All analyses were performed using R statistical software (version 4.3.3, R Foundation for Statistical Computing).

RESULTS

Table 1A shows the presence of various secondary metabolites in the BaG and ChS extracts, including alkaloids, flavonoids, phenolic compounds, saponins, tannins, terpenes/steroids, and sesquiterpene lactones. Table 2A presents the quantitative results of the total phenolic and flavonoid content in both extracts. The total phenolic content was significantly higher in ChS compared with BaG (p = 0.0079). In contrast, no significant differences were observed in the total flavonoid concentration between the extracts (p = 0.052).

GC-MS analysis of *Baccharis genistelloides* extract (BaG) identified a total of 17 compounds, classified into different chemical groups. Phenolic monoterpenes such as carvacrol were detected, along with simple phenolic compounds such as 4-vinylphenol. Sugars and glycosidic derivatives were also identified, including 3-O-methyl-D-glucose, ethyl α-D-glucopyranoside and glyceraldehyde. Quinones such as anthraquinone were also present. Among fatty acids and their esters, methyl palmitate (hexadecanoic acid, methyl ester), ethyl palmitate (hexadecanoic acid, ethyl ester), α-linolenic acid and its esters (9,12,15-octadecatrienoic acid, methyl/ethyl ester), linoleic acid and its esters (9,12-octadecadienoic acid, methyl/ethyl ester), and palmitic acid (hexadecanoic acid) were identified. Vitamin derivatives such as L-(+)-ascorbic acid 2,6-dihexadecanoate, as well as sterols like cholest-3-ene, were also recorded. In addition, compounds such as 1,2-bis(trimethylsilyl)benzene and short-chain alcohol esters like 1-butanol, 3-methyl-, acetate were detected.

The GC-MS analysis of *Chuquiraga spinosa* extract (ChS) identified 23 metabolites. Among the lactone compounds and oxygenated heterocyclic derivatives, 2-hydroxy-γ-butyrolactone and 3-n-butylthiolane were detected. Within the group of organic acids and their derivatives, phosphonoacetic acid, dodecanoic acid, 2-bromotetradecanoic acid, n-hexadecanoic acid, and octadecanoic acid were identified, along with their corresponding methyl esters: hexadecanoic acid, methyl ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester; trans-13-octadecenoic acid, methyl ester; 11-octadecenoic acid, methyl ester; and heptadecanoic acid, 16-methyl-, methyl ester. In the group of phenolic and related compounds, canolol and 9-octadecenamide (Z)- were found, whereas terpenoids and phytohormones included isogibberellin. The analysis also revealed the presence of the glucosinolate desulphosinigrin and the organosilicon compound isopropyl pentakis(trimethylsilyl) disilicate. Furthermore, glycosides and carbohydrates such as maltose, tuberonic acid glucoside, sweroside, and β-D-galactopyranoside were identified, along with the cardiotonic glycoside digitoxin. These findings highlight the remarkable chemical diversity of the extract, encompassing both primary and secondary metabolites with potential biological relevance.

As shown in Figure 1, in each antioxidant activity assay, the combination of extracts (ChS + BaG) produced a significant increase compared to the activity obtained with each extract alone. Likewise, when comparing the individual extracts, ChS exhibited significantly higher activity. In the DPPH• and ABTS•$^{+}$ assays, the controls (AA and Trolox, respectively) showed significantly greater antioxidant activity (p < 0.001) compared with the evaluated groups.

The percentage distribution of neurological scores shown in Figure 2 indicates that the untreated ischemia group exhibited the highest frequency of rats with severe functional impairment (83%). Moreover, when comparing the median of this group with that of the normal group, a significant difference was observed (p < 0.001), as detailed in Table S1. On the other hand, the groups treated with ChS 500 and with the combination ChS 500 + BaG 500 showed a lower proportion of severe impairment (17% in both cases). However, the combination group displayed a lower proportion of mild impairment (33%) compared to the ChS 500 group (67%). This finding is supported by Table S1, where the median score in the combination group was 1.5, versus 2 in the ChS 500 group, although this difference was not statistically significant. Finally, the citicoline-treated group did not present cases of severe impairment but showed 50% of cases with mild and moderate impairment, respectively.

Table 1: Phytochemical constituents of the ethanolic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides*.

	Bioactive compounds	Tests	ChS	BaG
A	Alkaloids	Mayer	+	+
		Dragendorff	+	+
	Flavonoids	Shinoda	+	+
		$AlCl_3$	+	+
	Quinones	Bornträguer	-	-
	Phenolic compounds	$FeCl_3$	+	+
	Saponins	Froting	+	+
	Tannins	Gelatina	+	+
	Terpenes and steroids	Lieberman - Burchard	+	+
	Terpenes	Vainillina sulfúrica	+	+
	Sesquiterpene lactones	Baljet A y B	+	+

(+) **Positive, (-) Negative**

	Group	Total phenolic (mg GAE/g) Mean ± SD	Total flavonoid (mg QE/g) Mean ± SD
B	ChS	153.07 ± 1.89	67.71 ± 1.89
	BaG	119.47 ± 6.47	44.80 ± 6.47
	p value	0.0079	0.052

A: Preliminary phytochemical screening. B: Total phenolic and flavonoid content (n = 3) in both extracts. p-values correspond to Student's t-test between the ChS and BaG groups.

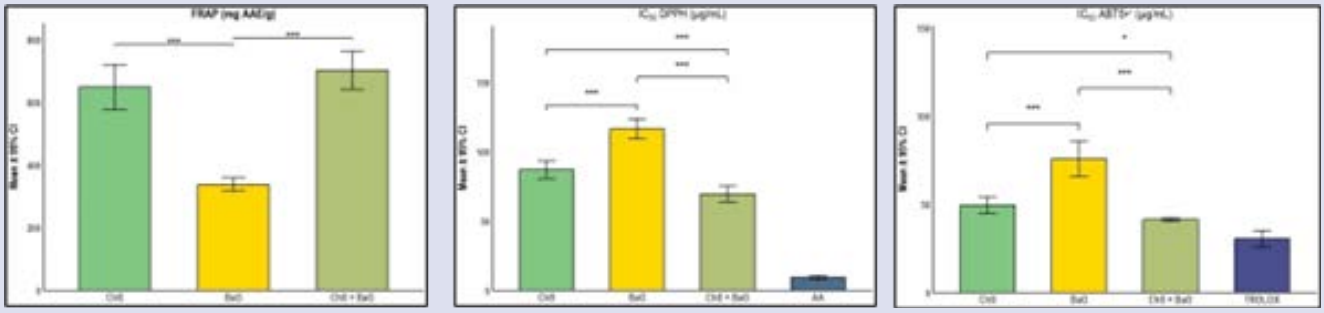

Figure 1. Evaluation of antioxidant capacity

The figures show mean values ± 95% confidence interval (95% CI) for FRAP, IC_{50} DPPH, and IC_{50} ABTS (n = 3 replicates per group). Asterisks indicate significant differences between groups according to Tukey's test (*$p < 0.05$; ***$p < 0.001$).

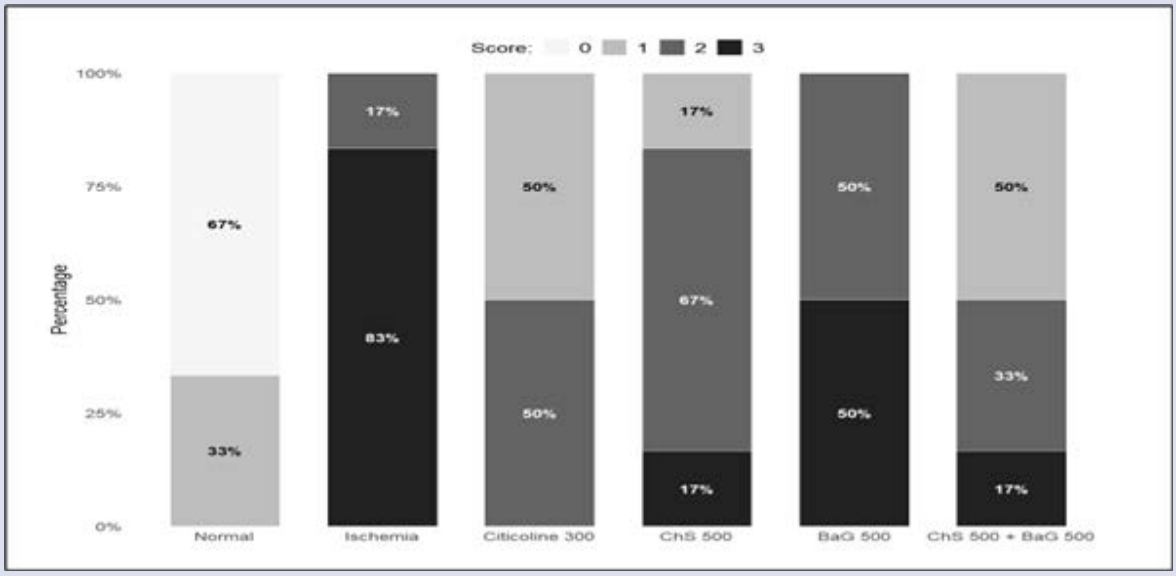

Figure 2. Percentage distribution of neurological scores according to the level of functional impairment.

Scores were assigned based on the degree of functional impairment observed in the behavioral test 24 hours after ischemia induction (range: 0 = No impairment, 1 = Mild, 2 = Moderate, 3 = Severe).

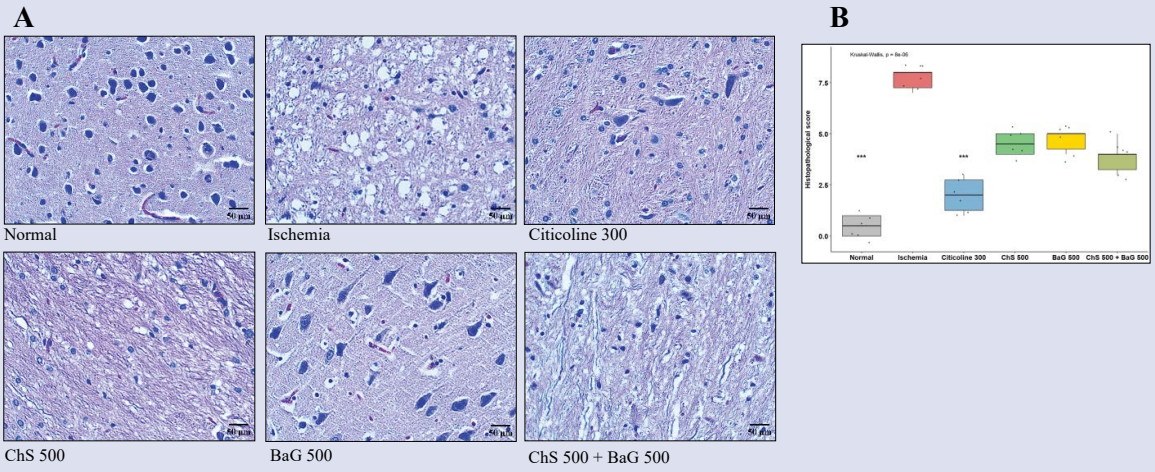

Figure 3. Microphotographs of brain tissue in the different experimental groups.

A: Sections were stained with H&E. Images were visualized at a total magnification of 400×. B: boxplot represents the median and range of total histopathological scores for each group. A p value < 0.001 (***) indicates a significant difference compared to the ischemia group, as determined by Dunn's test with Bonferroni correction.

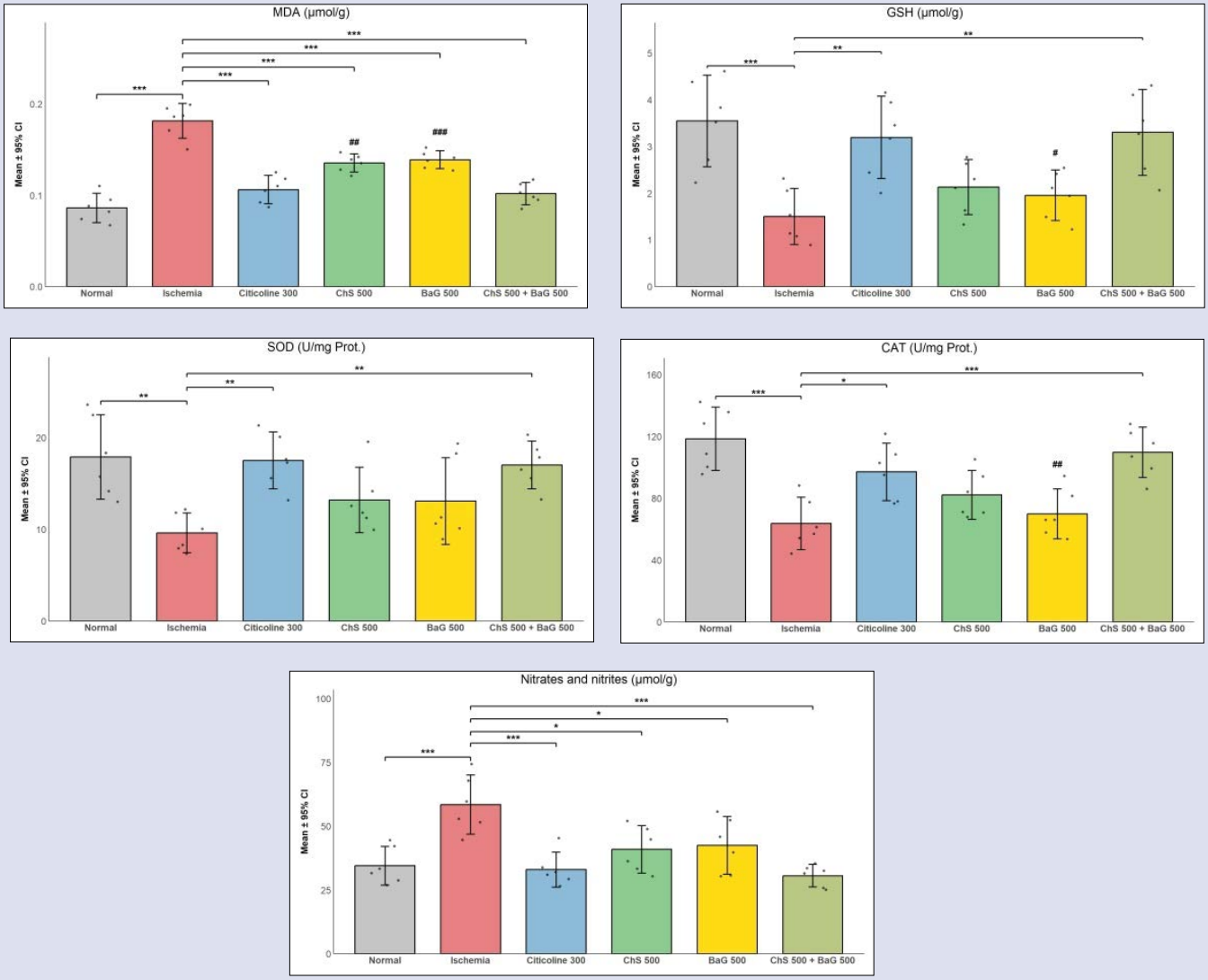

Figure 4. Biochemical parameters of oxidative stress

The figures represent mean values ± 95% confidence interval (95% CI) of MDA, GSH, SOD, CAT, and total nitrates/nitrites (NO_3^-/NO_2^-) in each experimental group (n = 6 rats per group). Asterisks indicate significant differences compared with the Ischemia group according to Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Hash symbols indicate significant differences compared with the group treated with ChS 500 + BaG 500 (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$).

In the histopathological analysis (Figure 3), animals in the normal control group showed no alterations, with a histopathological score of 0.5. In contrast, the untreated ischemia group exhibited the highest degree of injury, characterized by marked pericellular edema, severe vacuolization, and significant reduction of neuronal basophilia, consistent with severe damage, with a score of 8.0. In the citicoline 300 group, only mild changes were observed, including slight vacuolization and minimal nuclear pyknosis, with a score of 2.0, showing a significant difference ($p < 0.001$) compared to the ischemia group. Animals treated individually with ChS 500 or BaG 500 displayed a similar pattern, characterized by moderate edema and vacuolization together with slight disruption of architecture, with scores of 4.5 and 5.0, respectively. The group treated with the combination of extracts exhibited mild to moderate damage, with discrete vacuolization and nuclear pyknosis and partial loss of neuronal architecture, with a score of 4.0, although no significant difference was found compared to the ischemia group.

In Figure 4, it can be observed that the ischemia group showed significant alterations in oxidative stress biochemical parameters compared with the normal group. In contrast, the groups treated with citicoline and extracts exhibited significant prevention of these alterations. For MDA, all treatments achieved a significant reduction ($p < 0.001$). Regarding GSH and the enzyme SOD, both increased significantly ($p < 0.01$) only with the administration of citicoline and the extract combination. As for CAT activity, a significant improvement was observed with citicoline ($p < 0.05$), and an even more pronounced effect with the extract combination ($p < 0.001$). Finally, NO_3^- and NO_2^- levels were significantly reduced ($p < 0.001$) by both citicoline and the extract combination, whereas treatment with BaG 500 and ChS 500 also resulted in a significant reduction ($p < 0.05$).

It was also assessed whether administration of the extract combination better regulated oxidative stress parameters than the individual extracts. Regarding MDA levels, the combination produced a significantly greater reduction compared with ChS 500 and BaG 500 ($p < 0.01$ and $p < 0.001$, respectively). For GSH levels and CAT activity, the combination was significantly superior only to BaG 500 ($p < 0.05$ and $p < 0.01$, respectively). Finally, when evaluating SOD activity and

Table 2: Chemical components of the extracts identified by GC-MS

	NO	Compound	Formula	Molecular Weight	CAS Number	Rt (min)	Ra (%)
Baccharis genistelloides extract (BaG)	1	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130.18	123-92-2	6.22	10.94
	2	Glyceraldehyde	$C_3H_6O_3$	90.08	56-82-6	9.52	1.09
	3	1,2-Bis(trimerhylsilyl)benzene	$C_{12}H_{22}Si_2$	222.48	17163-29-0	10.43	0.80
	4	Carvacrol	$C_{10}H_{14}O$	150.22	499-75-2	10.50	1.18
	5	4-Vinylphenol	C_8H_8O	120.15	2628-17-3	12.50	0.69
	6	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.18	644-99-3	18.50	37.84
	7	Anthraquinone	$C_{14}H_8O_2$	208.22	84-65-1	18.58	1.57
	8	Ethyl a-d-glucopyranoside	$C_8H_{16}O_6$	208.21	580-07-4	20.01	6.74
	9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	112-39-0	22.50	0.76
	10	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	628-97-7	23.50	8.16
	11	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.46	301-00-8	24.56	1.30
	12	Linolenic Acid	$C_{18}H_{30}O_2$	280.45	463-40-1	24.70	1.14
	13	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.49	1191-41-9	25.50	16.31
	14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	112-63-0	25.67	0.79
	15	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.5	544-35-4	26.11	8.45
	16	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.94	137-66-6	35.12	1.48
	17	Cholest-3-ene, (5B)	$C_{27}H_{46}$	370.66	481-21-0	36.13	0.76
		Total components					**100.00**
Chuquiraga spinosa extract (ChS)	1	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	19444-84-9	4.46	1.17
	2	3-n-Butylthiolane	$C_8H_{16}S$	144.28	28564-83-2	7.98	0.60
	3	Phosphonoacetic acid	$C_2H_5O_5P$	140.03	1551-24-2	11.26	1.08
	4	isopropyl pentakis(trimethylsilyl) disilicate	$C_{18}H_{52}O_7Si_7$	577.2	71579-69-6	15.76	0.76
	5	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32	0143-07-07	19.05	1.22
	6	Canolol	$C_{10}H_{12}O_3$	180.2	28343-22-8	19.37	0.92
	7	Isogibberellin	$C_{19}H_{22}O_6$	346.38	468-44-0	19.71	0.60
	8	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279.31	5115-81-1	21.30	0.90
	9	2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	307.27	10520-81-7	23.61	0.63
	10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	112-39-0	26.94	8.97
	11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	57-10-3	27.84	12.00
	12	Ocatadecanoic acid	$C_{18}H_{36}O_2$	284.48	57-11-4	28.24	4.74
	13	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.63	101230-69-7	28.64	0.32
	14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.48	112-63-0	30.21	5.62
	15	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	42199-38-2	30.30	15.29
	16	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	6198-58-9	30.39	5.81
	17	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.5	5129-61-3	30.75	1.93
	18	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.48	301-02-0	31.97	1.69
	19	Maltose	$C_{12}H_{22}O_{11}$	342.3	69-79-4	35.10	17.04
	20	Tuberonic acid glucoside	$C_{18}H_{28}O_9$	388.41	124649-25-8	35.31	7.35
	21	Sweroside	$C_{16}H_{22}O_9$	358.34	14215-86-2	35.67	8.69
	22	ß-D-Galactopyranoside	$C_{17}H_{37}BO_6Si_2$	404.22	56211-13-3	36.59	2.09
	23	Digitoxin	$C_{41}H_{64}O_{13}$	764.94	71-63-6	37.67	0.55
		Total components					**100**

Rt, retention time; Ra, relative area.

NO_3^-/NO_2^- levels, no significant differences were observed with the combination.

In Figure 5, the group of untreated rats with induced ischemia showed a significant increase in brain cytokine levels compared with the normal group. In contrast, the groups treated with citicoline and extracts showed a significant reduction compared with the ischemia group. For IL-6, citicoline, ChS 500, and the combination of extracts significantly decreased its levels ($p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively). For TNF-α, citicoline, ChS 500, and the combination also reduced levels significantly ($p < 0.001$, $p < 0.01$, and $p < 0.001$, respectively). For IL-1β, a significant decrease was observed only with the combination ($p < 0.05$).

Additionally, it was assessed whether the administration of the extract combination reduced cytokine levels more effectively than the individual extracts. In this case, the combination was significantly better ($p < 0.05$) than BaG 500 in reducing TNF-α.

DISCUSSIÓN

The metabolites identified in ChS and BaG, described in Tables A1 and A2, are consistent with those reported in the literature, particularly phenolic compounds, flavonoids, and tannins[13,15]. In the n-hexane fraction of *Baccharis genistelloides*, terpenes and phenolic compounds have also been identified[34]. These metabolites have shown pharmacological properties, mainly antioxidant and anti-inflammatory

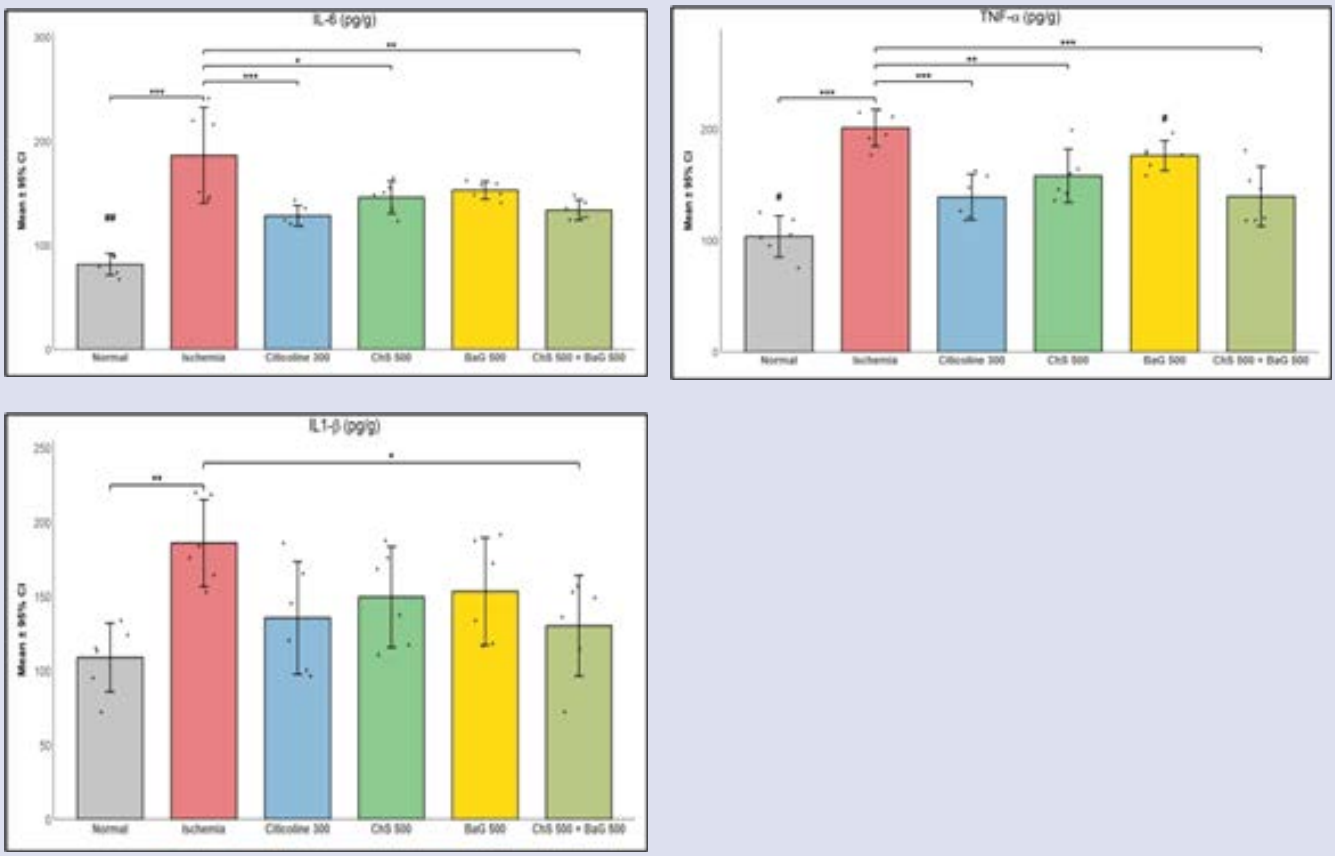

Figure 5: Evaluation of Neuroinflammation

The figures show mean values ± 95% confidence interval (95% CI) of IL-6, TNF-α, and IL-1β in each experimental group (n = 6 rats per group). Asterisks indicate significant differences compared with the Ischemia group according to Tukey's test (* $p < 0.05$; **$p < 0.01$; *** $p < 0.001$). Number signs indicate significant differences compared with the group treated with ChS 500 + BaG 500 (# $p < 0.05$; ## $p < 0.01$).

effects[11,15]. To further explore components of pharmacological interest, in this study the chemical constituents of both extracts were analyzed using GC-MS.

In BaG, 17 compounds were identified. Carvacrol, a phenolic monoterpene, was detected, known for its ability to inhibit the production of pro-inflammatory cytokines (IL-1β, TNF-α), reduce lipid peroxidation, and enhance antioxidant enzymes[35,36]. Hexadecanoic acid, methyl ester, and hexadecanoic acid, ethyl ester (methyl and ethyl palmitate) were also identified, described for their ability to reduce edema and leukocyte infiltration, as well as decrease pro-inflammatory cytokine levels in animal models[37]. L-(+)-ascorbic acid 2,6-dihexadecanoate (ascorbyl palmitate) was also detected, which has demonstrated anti-inflammatory effects by effectively inhibiting the NLRP3 inflammasome[38]. Other compounds, such as α-linolenic acid, have shown the ability to mitigate neuroinflammation by reducing reactive oxygen species (ROS) through activation of the NrF-2/HO-1/JnK signaling pathway, as well as decreasing pro-inflammatory cytokine production by inhibiting p-JnK and NF-κB activation[39]. Anthraquinone has also been associated with anti-inflammatory immunomodulatory effects via NF-κB inhibition and antioxidant effects through NrF2 regulation[40].

In ChS, pharmacologically relevant components were identified, such as canolol, which has demonstrated in vitro antioxidant capacity and cytoprotective action against oxidative stress by inhibiting the p38 MAPK pathway. It also showed anti-inflammatory effects by reducing markers such as iNOS, IL-1β, TNF-α, IFN-γ, and COX-2[41]. Another compound, sweroside, exhibited antioxidant properties in ABTS, CUPRAC, and FRAP assays, along with antidiabetic and neuroprotective effects demonstrated in both in vitro studies and molecular modeling[42].

Both extracts were evaluated for their antioxidant activity against ABTS•$^+$ and DPPH• radicals. The results shown in Figure 1 are consistent with previous reports on ChS[11] and *Baccharis* species[43]. A noteworthy finding was that the combination of both extracts significantly enhanced free radical scavenging capacity. This synergistic effect may be attributed to the interaction among the various phenolic compounds and other metabolites identified in the chemical profile. Scientific literature supports that combining hydroalcoholic extracts can potentiate antioxidant activity[44], a principle reinforced by the present work. Overall, these results provide a solid scientific basis for the development of low-cost, accessible antioxidant and anti-inflammatory formulations.

Following phytochemical characterization and demonstration of the synergistic antioxidant activity of the extract combination, the effect was experimentally evaluated in an integral system. For this purpose, a transient ischemia model in rats was selected, a pathology in which oxidative stress plays a central etiological role due to reperfusion. This transition from an in vitro model to a complex organism allows investigation not only of the ability of these extracts to regulate oxidative stress in an integrated biological environment, but also to assess their effects on functional, morphological, and neuroinflammatory parameters, thereby advancing the understanding of the neuroprotective potential of both extracts.

In the present study, the ischemia-induced untreated group showed a significant increase in neurological deficit compared to the normal group, with 83.3% of the animals reaching level 3 on the Bederson scale at 24 h (Figure 2), indicating severe motor impairment. This finding is consistent with reports from experimental models of transient middle cerebral artery occlusion, where blood flow interruption triggers the ischemic cascade characterized by energy failure, excitotoxicity, calcium overload, and neuronal death in cortical and striatal regions supplied by this artery[27]. This was corroborated histopathologically in Figure 3, showing vacuolization and a significant reduction in neuronal basophilia, together with marked pericellular edema and intense vacuolization, reflecting reperfusion injury after occlusion. Post-occlusion reperfusion exacerbates damage through the production of reactive oxygen species and activation of the inflammatory response mediated by microglia and pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6, amplifying neuronal dysfunction and behavioral deterioration[45,46].

In contrast, groups receiving citicoline and the extracts showed a reduction in neurological scores, although not statistically significant, with citicoline and the extract combination standing out. This suggests a neuroprotective effect likely linked to the antioxidant and anti-inflammatory capacity of the active compounds described above, which may attenuate oxidative stress and the acute inflammatory response after ischemia. Histopathological findings showed that citicoline exerted greater neuronal protection against ischemia, significantly reducing vacuolization and architectural loss compared to the untreated group. The combined treatment produced an intermediate effect, suggesting a possible synergistic benefit. Individual treatments with ChS 500 or BaG 500 provided only partial protection. These results highlight the need for future studies to explore the potential of therapeutic combinations in cerebral ischemia.

In the ischemic untreated group, the biochemical pattern observed—marked by increased MDA ($p<0.001$), decreased GSH ($p<0.001$), and reduced antioxidant enzymatic activities SOD ($p<0.01$) and CAT ($p<0.001$), together with elevated nitrites/nitrates ($p<0.001$)—is consistent with ischemia-reperfusion injury dominated by oxidative and nitrosative stress. Ischemia followed by reperfusion triggers a burst of ROS and NO production; excess ROS promotes lipid peroxidation of neuronal membranes, measured as MDA, while GSH is rapidly consumed in radical neutralization and detoxification pathways, increasing neuronal vulnerability as its reserve declines[47]. At the same time, SOD and CAT activities may decrease due to oxidative inactivation or overconsumption under sustained radical excess, lowering the capacity to dismutate superoxide and degrade H_2O_2, thus facilitating progression of peroxidation and damage to proteins and nucleic acids[48]. The rise in nitrites/nitrates reflects increased NO production and consequent peroxynitrite generation through reaction with superoxide; peroxynitrite is a strong oxidant that modifies lipids and enzymes, activates pro-apoptotic and energy-depleting pathways, and contributes to the functional loss observed in the ischemic untreated group[49].

In groups treated with citicoline, extracts, and their combination, a significant decrease in MDA was observed, consistent with the ability of several compounds identified in the extracts to act as direct radical scavengers and modulators of antioxidant enzymes. In ChS, total phenols and flavonoids, together with metabolites such as canolol and sweroside, explain the reduction in MDA. Canolol has been shown to inhibit the p38 MAPK pathway and consequently iNOS and COX-2 expression, decreasing both ROS and RNS, while sweroside exerts antioxidant and neuroprotective effects through Nrf2-dependent redox systems[41,42]. In BaG, compounds such as carvacrol, α-linolenic acid, and lipophilic derivatives like ascorbyl palmitate and hexadecanoic acid palmitates contribute to the reduction in nitrites/nitrates through their ability to inhibit iNOS overexpression, regulate NF-κB, and activate the Nrf2/HO-1 axis, thereby reducing peroxynitrite formation and nitrosative stress[35-38]. These compounds also promote recovery of endogenous antioxidant defenses, as reflected in the significant increase of GSH, SOD, and CAT observed in the extract combination, where synergy between metabolites of both extracts may potentiate the antioxidant response. Citicoline has been demonstrated to stabilize the antioxidant system and restore brain cell structures damaged by oxidative stress during transient ischemia[50]. In this study, the extract combination proved superior to individual extracts in reducing MDA and showed better improvement in GSH and CAT activity compared to BaG, supporting the idea of synergism between extract components. Moreover, the combination regulated oxidative stress similarly to citicoline, suggesting comparable potency that warrants further investigation. Overall, these results support that the observed biochemical neuroprotection arises from both direct antioxidant actions of extract molecules and activation of enzymatic systems with negative modulation of pro-inflammatory pathways.

Cytokine analysis revealed that all treated groups significantly decreased IL-6 and TNF-α levels, reflecting substantial modulation of the post-ischemic inflammatory response in the brain. Citicoline stabilizes membranes and restores redox balance, thereby attenuating NF-κB activation and transcription of pro-inflammatory cytokines[51]. Effects observed with the extracts and their combination may be linked to metabolites identified in ChS, such as canolol, which inhibits p38MAPK activation, thereby reducing pro-inflammatory cytokine production in brain tissue [41]. Likewise, sweroside present in ChS, through its antioxidant properties[41,42], may contribute to cytokine reduction. In BaG, compounds such as carvacrol and palmitic acid derivatives have been shown to lower TNF-α and IL-1β levels and to attenuate leukocyte infiltration and COX-2 activation[35-37]. Additionally, α-linolenic acid and anthraquinone exert immunomodulatory and antioxidant roles through regulation of NF-κB and Nrf2[39,40]. This also explains why the extract combination significantly decreased IL-1β, as well as showing superiority in reducing TNF-α compared to BaG, suggesting that ChS components mainly drive the anti-TNF-α effect, while BaG constituents provide anti-inflammatory support without additional advantage on IL-6 or IL-1β beyond that achieved by ChS.

This study identified pharmacologically relevant findings, including synergistic antioxidant activity of both extracts and regulation of oxidative stress and neuroinflammation in the ischemia model. However, due to the variety of compounds, it was not possible to determine which specific components interacted synergistically; thus, it is necessary to evaluate individual and specific combinations of the main bioactive metabolites previously identified, to establish which directly contribute to the observed activity. Another limitation is that the experimental model used was transient focal cerebral ischemia in rats, making direct extrapolation to human pathophysiology difficult. Finally, the study focused on an acute time point (24 h post-ischemia), so it remains to be established whether the effects observed persist in subacute or chronic phases of brain injury, which is relevant for proposing potential therapeutic use.

The search for agents that protect against lipid peroxidation and enhance the antioxidant enzyme defense system should be considered a rational strategy to prevent cerebrovascular diseases. Natural products with such properties represent an ideal choice with minimal risk of iatrogenic adverse effects.

CONCLUSION

The **synergistic antioxidant activity against free radicals** of hydroalcoholic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides*, demonstrated in vitro (ABTS•$^+$, DPPH•, FRAP assays), was reflected in their ability to attenuate oxidative stress, neuroinflammation, behavioral deficits, and histological alterations in

the transient focal cerebral ischemia model. The combined treatment consistently showed the strongest effect, in agreement with the phytochemical profile of the extracts. Overall, the findings support the potential of a combined extract formulation as a neuroprotective adjuvant comparable to citicoline, while underscoring that confirmation of synergy and translational applicability will require additional studies, including fractionation, dose–response analysis, therapeutic window evaluation, and subacute assessments of efficacy and safety.

ACKNOWLEDGMENTS

This study was approved by the Vicerrectorado de Investigación y Posgrado of the Universidad Nacional Mayor de San Marcos (protocol code: A17010062). The authors express their gratitude to the Faculty of Medicine, Universidad Nacional Mayor de San Marcos, for providing the necessary facilities.

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Cite this article: Justil-Guerrero HJ, Arroyo-Acevedo JL, Rojas-Armas JP, Palomino-Pacheco M, García-Bustamante CO, Cieza-Macedo EC, Huarcaya-Rojas JY, Torres-Lévano JD, Peña-Galindo JJ, Franco-Soto EO, Chávez-Flores JE, Cárdenas-Orihuela RA. Antioxidant and Neuroprotective Effects of *Chuquiraga spinosa* Less. and *Baccharis genistelloides* (Pers.) Lam. in a Rat Model of Transient Cerebral Ischemia-reperfusion. Pharmacogn J. 2025;17(5): 620-631.