



Infusion of aerial parts of *Salvia chudaei* Batt. & Trab. from Algeria: Chemical, toxicological and bioactivities characterization

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A B S T R A C T

Ethnopharmacological relevance: *Salvia chudaei* Batt. & Trab. from Algeria is traditionally used to relieve several dysfunctions, including inflammatory and pain-related situations.

Aim of the study: This work aimed to confirm scientifically the referred properties. For that, the phenolic composition and antioxidant activity were evaluated as well as acute toxicity, anti-inflammatory and analgesic effects of different doses of the infusion of *S. chudaei* aerial parts.

Materials and methods: Infusion of aerial parts of *S. chudaei* was prepared and screened for phenolic composition by generalized methods TPC and TFC then by LC-DAD-ESI/MSn. DPPH and FRAP were used to evaluate antioxidant activity. Using mice, acute toxicity, anti-inflammatory by carrageenan-induced paw edema, and analgesic by acetic acid-induced writhing and formalin-induced pain activities were tested.

Results: The infusion showed 2018 mg GAE/100g DW of phenolics and 1956 mg ECE/100g DW of flavonoids. Phenolic profile by LC-DAD-ESI/MSn revealed the presence of ten compounds: syringic acid hexoside derivative, kaempferol-O-diglucuronide, kaempferol-O-deoxyhexoside-hexoside, kaempferol-O-glucuronide, apigenin-O-diglucuronide, caffeic acid, 4-O-caffeoylquinic acid, eriodictyol-O-glucuronide, rosmarinic acid hexoside, and rosmarinic acid. This acid was the major compound representing 54% of the total content of the identified compounds and an absolute content of 18 mg/g of extract. Additionally, the infusion exhibited a good antioxidant activity (DPPH: 81 $\mu\text{mol TE/g DW}$, FRAP: 438 $\mu\text{mol FSE/g DW}$). By oral administration to mice, the infusion showed a significant ($p < 0.05$) dose-dependent reduction of carrageenan-induced inflammation and inhibition of formalin-induced pain (late and early phase) and acetic acid-induced writhing compared with the control. On the other hand, infusion up to 8 g/kg b.w. showed no signs of toxicity or mortality.

Conclusion: This study reveals, for the first time, that the infusion of the aerial parts of *S. chudaei* is not toxic in a single dose and has remarkable antioxidant, anti-inflammatory, and analgesic activities, supporting the use of this species in folk medicine.

1. Introduction

The use of traditional medicines, particularly herbal medicines, has been widely increasing during the recent decades (Popović et al., 2016). Since the old times, people used medicinal plants for healing and other health care, probably due to their wide availability, as well as the high cost and difficult access to drugs in some regions of the world. Their use represents also the preservation of the cultural heritage, considering the traditional medicine practice as a part of peoples culture (Van Wyk and Wink, 2018).

Lamiaceae (Labiatae) family that comprises 236 genera and about 6900–7200 species (Stevens, 2016; Tamokou et al., 2017) contains many species with medicinal virtues (Ruiz-Vargas et al., 2019; Saad

et al., 2016; Sreeja et al., 2018). *Salvia* L. is the largest genus of this family (900–1000 species) (Walker et al., 2004); several *Salvia* species have very interesting pharmacological properties, namely antioxidant (Esmaili and Sonboli, 2010), anti-diabetic (Flores-Bocanegra et al., 2017), analgesic (Simon-Arceo et al., 2017), *in vitro/in vivo* anti-inflammatory activity (Akram et al., 2015), and anti-Alzheimer effects (Senol et al., 2017), which in several cases are attributed to secondary metabolites, especially phenolic compounds.

Phenolic compounds include a multitude of molecules (e.g. flavonoids, phenolic acids, stilbenes, tannins, and lignans), responsible for the main organoleptic properties of plant derived-foods and beverages, and contributing also to the vegetables and fruits nutritional properties (Tapas et al., 2008). Simultaneously, phenolic compounds are getting a

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large interest thanks to their association with many bioactivities, probably related to their antioxidant power (Shahidi and Yeo, 2018).

S. chudaei is an endemic plant of the central Sahara massif (Ozenda, 1991), known as Aouit in Tamahaq (indigenous language of the Touareg population in the South of Algeria) and as Tagrouft in Arabic. This plant is used to relieve numerous diseases and disorders such as ulcer, diarrhea, rheumatism, kidney diseases, dysmenorrhea, abdominal pain, spasms, urinary retention, urinary tract infection, and prostate pain, consumed as infusion, decoction, or powder (Hammiche and Maiza, 2006; Ramdane et al., 2015; Sekkoum et al., 2011). However, besides the use of this plant to relieve dysfunctions, no studies were carried out about its acute toxicity, anti-inflammatory activity, analgesic effect as well as its phenolic compound profile. Yet, some studies reported the composition of essential oil from aerial parts along with the antimicrobial and antioxidant activities of some organic extracts of *S. chudaei* (Hammoudi et al., 2017a; Krimat et al., 2015a, 2015b).

In order to confirm some of the reported healing virtues of *S. chudaei* infusion obtained from aerial parts, this research aimed to identify and quantify the molecules responsible for those medicinal benefits. To do that, different doses of infusion were tested in what concerns its toxicity, *in vivo* analgesic and anti-inflammatory effects, as well as its antioxidant activity, total phenolic and flavonoid contents, and phenolic profile by Liquid Chromatography coupled to Diode Array Detector and Electrospray Ionization tandem Mass Spectrometry (LC-DAD-ESI/MSn).

2. Materials and methods

2.1. Plant material

S. chudaei was harvested in January 2017 in the Ilamen station (longitude: 005° 29 30.6 E, latitude: 23° 14 26.9 N, altitude: 2055 m), located in the region of Tamanrasset in Southern Algeria. The authentication of the plant species was certified by the Higher National Agronomic School, El Harrach, Algiers, Algeria, according to Quezel and Santa (1963). A Standard specimen is conserved in the herbarium of Montpellier (voucher N°: MPU 010317). The plant aerial parts were air shade dried, at room temperature (22 ± 2 °C), then ground in a mill to small particles for further use.

2.2. Preparation of the infusion

The infusion (5% w/v) was prepared by adding boiling deionized water to the sample powder and left to stand for 5 min. Three independent extractions were prepared, the content filtered via Whatman paper N°3. The prepared infusion was thereafter used in the assays. For the LC-DAD-ESI/MSn analysis, the infusion extract was firstly lyophilized then dissolved in methanol/water (80:20, v/v).

2.3. Experimental animals

20–30g Swiss albino mice (NMRI) purchased from the Pasteur Institute of Algiers (Algeria) were acclimatized for 2 weeks before experiments, housed at 24 ± 1 °C, 50 ± 5% humidity, and 12/12 h light-dark cycle. They were fed with rodent chow from the Animals Food National Office (ONAB) Bejaia, Algeria, and had free access to water. The animals were handled following the care and use of the laboratory animals guide (Council, 2010). All procedures involving animals were approved by the University Animal Experiment Ethics Committee (approval ref no. 162/2011/8).

2.4. Acute oral toxicity

The toxicity was evaluated in animals of both sexes, according to the Organization for Economic Co-operation and Development (OECD) guideline 423 with slight modifications (OECD, 2001). 3 groups of NMRI mice, consisting of 3 males and 3 females each, were used for this

evaluation. Each group received 0.5 mL of *S. chudaei* infusion, in different doses as follows: group 1–2000 mg/kg b.w.; group 2–3000 mg/kg b.w.; and group 3–5000 mg/kg body weight (b.w.). After administration, the animals were carefully observed in the first 4 h for any abnormal behavior or toxicity sign and after, daily during 14 days to observe mortality.

2.5. Analgesic activity

To determine the analgesic activity two tests were evaluated: the Writhing and Formalin tests.

2.5.1. Writhing test

The writhing test using acetic acid (Liu et al., 2015) proceeded as follows. 5 groups, of 6 mice each, were used in this evaluation, the different groups receive orally as follows: group 1–0.5 mL saline solution; group 2–0.5 mL of paracetamol® at 100 mg/kg b.w.; group 3, 4, and 5–0.5 mL of *S. chudaei* infusion at 250, 500 and 1000 mg/kg b.w., respectively. After 30 min they were intraperitoneally injected with 200 µl of 0.6% acetic acid. 5 min after, the abdominal constrictions number was counted for 15 min. The results were expressed as protection percentage using the following formula:

$$\text{Protection\%} = [(NCc - Nct) / NCc] \times 100$$

where NCc represents constrictions number in control (saline solution) and Nct the constrictions number in tested drug (paracetamol® and the infusion at different doses).

2.5.2. Formalin test

The antinociceptive activity of the sample extracts was assessed by the formalin test (Hunzka et al., 1985). For that, 5 groups containing 5 mice each were formed. The different groups received *per os*: 1–0.5 mL of saline; 2–50 mg/kg b.w. paracetamol®; 3–250 mg/kg b.w. of the infusion; 4–500 mg/kg b.w. of the infusion; 5–1000 mg/kg b.w. of the infusion. After 1h, 20 µl of formalin (2.5% formaldehyde in 0.9% NaCl saline) were subcutaneously injected into the right paw dorsal surface. Immediately, the licking and biting time of the injected paw was recorded for 5 min (early phase), and after 15–30 min (late phase). The protection percentage was calculated as follows:

$$\text{Protection\%} = [(Tc - Tt) / Tc] \times 100$$

in which, Tc represents the time of licking and biting in the control (saline solution), Tt the time of licking and biting in the tested drug (paracetamol® and the infusion at different doses).

2.6. Anti-inflammatory activity

The evaluation of the anti-inflammatory activity was carried out by paw edema provocation with carrageenan (Levy, 1969). 1% carrageenan in saline solution (0.9% NaCl), was injected into the mice right hind paw plantar surface. In this experiment 5 groups of 6 mice each were used; the control group received 0.5 mL of saline solution; the reference group received 50 mg/kg b.w. of diclofenac; and the other three groups received 250, 500, and 1000 mg/kg b.w. of *S. chudaei* infusion. The edema was induced after 30 min of the drug administration. 4 h later, mice were sacrificed by cervical dislocation and immediately both right and left hind paws were cut at the talocrural joint and weighted.

The results are expressed as inhibition edema percentage (IEP) using the formula below:

$$\text{IEP} = [(Epc - Ept) / Epc] \times 100$$

in which, EP represents edema percentage in control (c, saline solution) and in tested drug (t, diclofenac and infusion at different doses).

The edema percentage (EP) is calculated as follows:

$$EP = [(RPW - LPW) / LPW] \times 100$$

where RPW representing the right paw weight and LPW the left paw weight.

2.7. Total phenolic content (TPC)

Total phenolics were determined by spectrophotometry (BioTek Instruments, Inc.) (Alves et al., 2010) with some modifications. Briefly, 30 μ l of the sample were mixed with 150 μ l of Folin-Ciocalteu reagent (1:10, v/v) and 120 μ l of sodium carbonate (7.5%, m/v). The mixture was then incubated during 15 min at 45 °C, followed by 30 min at room temperature, protected from light. The absorbance was measured at 765 nm. The calibration curve was prepared with gallic acid (5–80 mg/L; $r = 0.9998$) and the results expressed as mg of gallic acid equivalents/100g of sample dry weight (mg GAE/100g DW).

2.8. Total flavonoid content (TFC)

TFC were determined according to Costa et al. (2014). In brief, 100 μ l of sample was mixed with 400 μ l distilled water and 30 μ l NaNO₂ 5%; after 5 min at room temperature 30 μ l AlCl₃ were added to the mixture; 1 min after, 200 μ l NaOH 1M and 250 μ l distilled water were added. Absorbance measurements were performed at 510 nm. A calibration curve was prepared with epicatechin (2.5–400 mg/L; $r = 0.9946$), and results expressed as mg of epicatechin equivalents/100g of sample dry weight (mg ECE/100g DW).

2.9. Antioxidant activity

2.9.1. DPPH[•] radical scavenging activity

This method is based on the reduction of the free radical DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) by the antioxidants present in the infusion. The evaluation of the scavenging power was performed according to Costa et al. (2014), with some modifications. Briefly, 30 μ l of infusion was mixed with 270 μ l of DPPH[•] (6.0×10^{-5} mol/L in ethanol). The absorption decrease of DPPH[•] was measured at 525 nm in intervals of 2 min, in order to observe the kinetics reaction. The reaction endpoint was reached in 20 min. The results were expressed as mg trolox equivalent/g of sample dry weight (mg TE/g DW) using a standard curve (5.62–75.87 mg/L, $r = 0.9978$).

2.9.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out according to Costa et al. (2014). In brief, 35 μ l of sample was mixed with 265 μ l of FRAP reagent (0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM of ferric chloride). The mixture was incubated for 30 min at 37 °C in dark. Absorbance was measured at 595 nm. A calibration curve was prepared with ferrous sulfate (25–500 μ mol/L; $r = 0.9997$) and the results expressed as mg of ferrous sulfate/g of sample DW (mg FSE/g DW).

2.10. LC-DAD-ESI/MSn profiling of phenolic compounds

LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) was used for the phenolic profile determination, according to Bessada et al. (2016) protocol. The obtained extract was lyophilized then dissolved in methanol:water (80:20, v/v) at 10 mg/mL concentration. Compounds were detected using a DAD with (370, 330, and 280 nm) as favored wavelengths and MS in negative mode, via a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled to ESI source. The compounds identification was based on their chromatographic behavior and UV–vis, and mass spectra, comparing with standards and reported data in literature via Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The quantification was based on the UV–vis signal using the calibration curve for the available standards or the most similar for the non-available ones.

Values were expressed as mg/g of extract.

2.11. Statistical analysis

Results were presented in mean \pm standard deviation. Comparisons between groups were performed by one-way ANOVA (analysis of variance) followed by post-hoc Tukey's HSD (Honestly Significant Difference) test. $p < 0.05$ was considered statistically significant. All statistics were carried out using R statistical software (version 3.6.0).

3. Results and discussion

3.1. Phytochemical analysis

In this work, we sought to investigate the antioxidant, anti-inflammatory, and analgesic activities of *S. chudaei* infusion from the region of Tamanrasset (south of Algeria). It is well-known that establishing a correlation between phytochemical composition and potential biological activities has an essential role to figure out the potential compounds responsible for those activities. Hence, we have performed profiling and quantification of *S. chudaei* infusion phenolic compounds.

The infusion prepared at 5% rate yielded $15.48 \pm 0.14\%$ (w/w) of extract. Table 1 presents the Total Phenolic and Total Flavonoid contents determined spectrophotometrically. In what concerns TPC, *S. chudaei* infusion showed 2018.48 ± 65.93 mg GAE/100g DW. Hammoudi et al. (2017b) reported for the same species 187.2 ± 4.68 and 292.1 ± 3.81 mg GAE/100g DW for 80% ethanol-water and Soxhlet extracts, respectively. Moreover, *S. chudaei* infusion TPC, determined in this study, is superior or close regarding TPC of some other *Salvia* species infusions and aqueous extracts. This is the case of *S. officinalis* 431 ± 37 mg GAE/100g DW (Hernández-Saavedra et al., 2016), *S. tomentosa* 349.72 ± 1.27 and *S. fruticosa* 396.31 ± 1.89 mg GAE/100g DW (Erdogan-Orhan et al., 2010), *S. cadmica* 940.93 ± 6.97 mg GAE/100g DW (Kocak et al., 2016), and *S. jurisicii* 14.27 ± 0.09 mg GAE/g DW despite the 24 h maceration time and the use of ultrasound bath (Alimpić et al., 2017). Even when methanol-water 70% and ethanol were used as solvents, which are known to have a high recovery, the phenolic compounds rate was 1981.56 ± 32.13 mg GAE/100g (Poyraz et al., 2017). As exception, the aqueous maceration of *S. officinalis* during 24 h obtained 32 mg GAE/g DW (Bahri et al., 2019), which was superior to the values from the presented work.

Concerning Total Flavonoids Content, *S. chudaei* infusion showed 1955.59 ± 126.12 mg ECE/100g DW. Several other *Salvia* infusions and aqueous extracts showed less TFC values, this is the case of: *S. officinalis* 892 ± 26 mg CE/100g DW (Hernández-Saavedra et al., 2016), *S. fruticosa* 107.14 ± 2.47 mg CE/g DE (Altay, 2015), *S. aramiensis* 118.85 ± 4.47 mg CE/g DE (Karatoprak et al., 2020); methanolic extracts of *S. sclarea*, *S. atropatana*, *S. ahendica*, *S. hydrangea*, *S. xanthocheila*, *S. macrosiphon*, *S. glutinosa*, *S. chloroleuca*, and *S. ceratophylla* ranged from 2.2 ± 0.2 to 36.2 ± 0.5 mg CE/g DE (Loizzo et al., 2014), *S. officinalis* 11.2 mg CE/g DE (Ghorbanpour, 2015).

The comparison of TPC and TFC values of *S. chudaei* infusion with other reported results allows having an idea about the richness in phenolic compounds of *S. chudaei* infusion. Nevertheless, it is important to identify the type of phenolic compounds present in the sample, here identified by LC-DAD-ESI/MSn. Table 2 shows the results obtained with

Table 1
TPC, TFC, DPPH[•], and FRAP values of *S. chudaei* infusion.

Bioactive compounds	TPC mg GAE/100g DW	2018.48 \pm 65.93
	TFC mg ECE/100g DW	1955.59 \pm 126.12
Antioxidant activity	DPPH [•] μ mol TE/g DW	81.31 \pm 7.79
	FRAP μ mol FSE/g DW	437.53 \pm 17.32

Mean \pm SD, n = 3, mg GAE: gallic acid equivalent; ECE: epicatechin equivalent; TE: Trolox equivalent; FSE: ferrous sulfate equivalent; DW: dry weight.

Table 2

Retention time (Rt), maximum absorption wavelengths in the visible region (λ_{max}), mass spectral data, concentration, and tentative identification of *Salvia chudaei* infusion phenolic compounds.

Peak	Rt (min)	λ_{max} (nm)	[M-H] ⁺ (m/z)	MS ² (m/z)	Tentative identification	Concentration (mg/g) of extract
1	6.59	321	353	191(11),179(45),173(100),135(5)	4-O-Caffeoylquinic acid (Cryptochlorogenic acid)	1.783 ± 0.005
2	7.53	283	403	241(70),197(100),179(5),135(5)	Syringic acid hexoside derivative	0.51 ± 0.02
3	10.07	323	179	135(100)	Caffeic acid	0.74 ± 0.03
4	13.66	340	637	285(100)	Kaempferol-O-diglucuronide	1.42 ± 0.03
5	15.45	285	595	287(100)	Eriodictyol-O-glucuronide	0.81 ± 0.02
6	16.2	330	621	269(100)	Apigenin-O-diglucuronide	1.40 ± 0.02
7	17.29	319	521	359(100),197(5),179(5),161(5),135(5)	Rosmarinic acid hexoside	6.58 ± 0.05
8	18.66	342	593	285(100)	Kaempferol-O-deoxyhexoside-hexoside	1.235 ± 0.003
9	18.95	341	461	285(100)	Kaempferol-O-glucuronide	1.24 ± 0.06
10	21.97	328	359	197(100),179(34),161(19),135(5)	Rosmarinic acid	18.3 ± 0.5
					TPA	27.9 ± 0.5
					TF	6.1 ± 0.1
					TPC	34.0 ± 0.5

*It was not possible to identify any phenolic compounds in the sample. Standard calibration curves: apigenin-7-O-glucoside ($y = 10683x - 45794$, $R^2 = 0.996$, LOD = 136.95 µg/mL and LOQ = 414.98 µg/mL), caffeic acid ($y = 388345x + 406369$, $R^2 = 0.9939$, LOD = 8.57 µg/mL and LOQ = 25.97 µg/mL), chlorogenic acid ($y = 168823x - 161172$, $R^2 = 0.9999$, LOD = 0.83 µg/mL and LOQ = 2.50 µg/mL), naringenin ($y = 18433x + 78903$, $R^2 = 0.9998$, LOD = 18.66 µg/mL and LOQ = 56.55 µg/mL), rosmarinic acid ($y = 191291x - 652903$, $R^2 = 0.999$, LOD = 3.70 µg/mL and LOQ = 11.22 µg/mL), quercetin-3-O-glucoside ($y = 34843x - 160173$, $R^2 = 0.9998$, LOD = 17.01 µg/mL and LOQ = 51.54 µg/mL), and syringic acid ($y = 376056x + 141329$, $R^2 = 0.9995$, LOD = 2.37 µg/mL and LOQ = 7.18 µg/mL). TPA: total phenolic acids, TF: total flavonoids, TPC: total phenolic compounds.

such methodology.

The compounds identification was achieved through the UV–vis behavior and mass spectra fragmentation in negative ion mode, comparing with standards, when available, and Xcalibur® data system. According to literature review, no work on the profiling of phenolic compounds concerning *S. chudaei* from the Tamanrasset region (southern Algeria) or elsewhere, has been reported previously. Ten compounds from two classes, phenolic acids and flavonoids, were identified. Among the phenolic acids, one hydroxybenzoic acid (syringic acid hexoside derivative (2)) and four hydroxycinnamic acids (4-O-caffeoylquinic acid (1), caffeic acid (3), rosmarinic acid hexoside (7), and rosmarinic acid (10)) were detected. For the flavonoids class, five compounds were putatively identified, being three flavonols (kaempferol-O-diglucuronide (4), kaempferol-O-deoxyhexoside-hexoside (8), and kaempferol-O-glucuronide (9)), one flavanone (eriodictyol-O-glucuronide (5)), and one flavone (apigenin-O-diglucuronide (6)).

Quantitatively, the rosmarinic acid was the major compound with an amount of 18.3 ± 0.5 mg/g of extract, followed by another rosmarinic acid derivative, the rosmarinic acid hexoside with 6.58 ± 0.05 mg/g of extract, representing together about 73% of identified phenolics. 4-O-caffeoylquinic acid, kaempferol-O-diglucuronide, and apigenin-O-diglucuronide are present in considerable amounts of 1.783 ± 0.005, 1.42 ± 0.03, and 1.40 ± 0.02 mg/g of extract, respectively. The less abundant were eriodictyol-O-glucuronide, caffeic acid, and syringic acid hexoside derivative with 0.81 ± 0.02, 0.74 ± 0.03, and 0.51 ± 0.02 mg/g of extract, respectively. The phenolic compounds extracted are almost all in the glycosylated form; glycosylation increases the solubility in water, the stability, and the promotion of the biological activity of various natural compounds including phenolics (Moon et al., 2017; Torres et al., 2011).

Several studies about phenolic compounds in *Salvia* species, using different extraction solvents (water, methanol, and ethanol), are in agreement with the present results. They reported rosmarinic acid as the dominant phenolic compound. This is the case of *S. cadmica* (Kocak et al., 2016), *S. officinalis* (Pedro et al., 2016), *S. brachyantha* and *S. aethiopsis* (Tohma et al., 2016), and *S. farinacea* Var. *Victoria Blue* (Afonso et al., 2019). Nevertheless, some researchs have highlighted other abundant compounds than rosmarinic acid: luteolin-glucuronide in the infusion of *S. officinalis* (Martins et al., 2015; Schnitzler et al., 2008), and decoction and methanol/water extract of *S. officinalis* (Martins et al., 2015), kaempferol in an ethanolic extract of *S. microstegia* (Tohma et al., 2016). These molecules may function as genus markers of this species. Several factors can affect the chemical composition of

plants, for example the harvesting season, geographical origin, and the extraction method (Moon et al., 2017). The reported findings indicate that *S. chudaei* can be a good source of rosmarinic and caffeic acid, as well as apigenin and kaempferol.

3.2. Antioxidant activity

Two colorimetric methods, DPPH• and FRAP, generally used to evaluate the antioxidant capacity of natural products, were performed with the same objective in this study. DPPH• assay is widely used to assess the ability of the compounds as free-radical scavengers or hydrogen donors to form stable non-radical DPPH• molecules. The results from the antioxidant activity evaluation are presented in Table 1, with *S. chudaei* infusion DPPH• activity of 20.35 ± 1.95 mg TE/g DW (81.31 ± 7.79 µmol TE/g DW). The DPPH• scavenging activity was higher than the reported by Kocak et al. (2016) for the infusion, and even for ethyl acetate and methanol extracts of *S. Cadmica* (40.51 ± 0.05, 5.93 ± 0.13, and 54.71 ± 0.05 µmol TE/g DW, respectively). Also, the reported results of Erdoğan et al. (2014) were inferior, for the methanolic extract of *S. fruticosa* leaves from three regions, with values ranging from 450.51 ± 1.19 to 287.57 ± 2.16 (µmol TE/100g DW). The determined value of *S. chudaei* infusion in this activity was higher than the ones determined in many plant infusions traditionally used in Andes/South America: *Adesmia melanthes* Phil. (1523 µmol TE/L); *Chuquiraga atacamensis* K. (77 µmol TE/L); *Senecio nutans* (609 µmol TE/L); *Fabiana densa* (1026 µmol TE/L); *Fabiana squamata* (74 µmol TE/L); *Lampaya medicinalis* (1045 µmol TE/L); *Azorella compacta* (459 µmol TE/L); *Opuntia ignescens* (6 µmol TE/L); *Baccharis tola* (67 µmol TE/L); *Parastrephia lepidophylla* (265 µmol TE/L); *Acantholippia deserticola* (222 µmol TE/L); and *Parastrephia lucida* (541 µmol TE/L) (Rojo et al., 2009). However, *S. officinalis* from Brazilian market, extracted with a solvent solution of acetone/ultrapure water/glacial acetic acid (70:28:2, v/v) had higher DPPH• activity (2.59 ± 0.17 g TE/100g DW) (Fernandes et al., 2016) than the infusion in evaluation.

The FRAP assay consists in TPTZ-Fe (III) complex reduction to TPTZ-Fe (II) by the compounds present in tested samples. The reducing power is a useful determinant of potential antioxidant activity. *S. chudaei* infusion showed a value of 437.53 ± 17.32 µmol FSE/g DW, this value seems better than the ones previously reported by other authors. According to Grzegorzczuk-Karolak and Kiss (2018) a FRAP activity between 890 and 1575 µmol FSE/g extract were obtained for decoction, infusion, and ethanolic extracts of *S. viridis*. Alimpić et al. (2017) evaluated the decoction and ethanolic extract of *S. jurisicii* (277.14 ± 8.6,

and 279.44 ± 8.45 $\mu\text{mol FSE/g}$ extract, respectively) obtained lower values than the reported in this work. Another study of nine *Salvia* species, using methanol as extraction solvent, showed a lower FRAP activity: *S. sclarea* L. (160.1 ± 1.5 $\mu\text{mol FSE/g}$ extract); *S. atropatana* (Boiss.) Buhse Bunge (20.6 ± 7.3); *S. ahendica* (Boiss.) Buhse (8.9 ± 4.1); *S. hydrangea* DC. (195.9 ± 5.4); *S. xanthocheila* Boiss. ex Benth. (14.4 ± 4.6); *S. macrosiphon* Boiss. (10.2 ± 3.4); *S. glutinosa* L. (422.0 ± 9.8); *S. chloroleuca* Rech.f. & Aell. (12.9 ± 0.8) and *S. ceratophylla* L. (290.7 ± 6.0 $\mu\text{mol FSE/g}$ extract) (Loizzo et al., 2014).

In both tests, DPPH[•] and FRAP, *S. chudaei* infusion exhibit excellent values for antioxidant activity, taking into account the reported results in the literature. It is also well-known that this feature has a strong correlation with TPC (Fernandes et al., 2016; Grzegorzczuk-Karolak and Kiss, 2018; Kamatou et al., 2010), demonstrating the strong involvement of phenolics in antioxidant activity. Indeed, rosmarinic acid, non-exhaustively, could be responsible for the high antioxidant power of the sample. Rosmarinic acid extracted from *Perilla frutescens* leaves has displayed a higher DPPH[•] radical scavenging activity (SC50 = 5.5 ± 0.2 $\mu\text{g/mL}$) than *P. frutescens* leaves extract (10.8 ± 0.1 $\mu\text{g/mL}$). Additionally, rosmarinic acid SC50 compared to standards was slightly close to that obtained by Trolox (5.1 ± 0.1 $\mu\text{g/mL}$) and higher than that of butylated hydroxytoluene (BHT) (6.6 ± 0.3 $\mu\text{g/mL}$) (Zhu et al., 2014). Moreover, rosmarinic acid showed the greatest activity potential among various phenolic acids tested in many antioxidant methods; caffeic acid has also shown an excellent antioxidant activity (Sevgi et al., 2015). Lu and Foo (2001) working on *S. officinalis* polyphenols have found that caffeic acid and rosmarinic acid derivatives exhibited potent antioxidant activity than flavone glycosides, and suggested that this strong activity could be due to their catechol moieties and conjugated unsaturation presence.

Reactive oxygen species (ROS) play a crucial role in the inflammatory response. They proceed as inflammatory mediators and signaling molecules produced by polymorphonuclear neutrophils (PMNs). Besides, reactive nitrogen species (RNS) can be rapidly formed by combination of nitrogen oxide (NO) and superoxide (O₂⁻). Unfortunately, this reaction is 3–4 faster than the dismutation of superoxide by the superoxide dismutase (SOD) (Mittal et al., 2014). The unbalance between ROS/RNS production and deletion may probably damage biomolecules (DNA, lipids, amino acid ...), perturb cellular thiol levels, and deregulate cellular signaling pathways implicated in various diseases (Fransen et al., 2012; Mungli et al., 2009).

3.3. Acute toxicity

Table 3 displays the results (mortality and toxicity sign) of the determination of acute toxicity of the infusion of *S. chudaei* aerial parts. The different infusion tested doses (2000, 3000, and 5000 mg/kg b.w.) did not show any toxicity sign (salivation, sleep, diarrhea, coma, etc.) in the first 4 h after oral administration. In the 14 days after, no mortality was observed for either male or female mice. This feature allows supposing the safety of *S. chudaei* infusion at the tested doses. Likewise, it is expected that LD50 (lethal dose causing 50% of tested animals death) will be superior to 5000 mg/kg b.w., taking into account the absence of any toxicity sign/mortality. Nevertheless, it will be very

Table 3
Effect of *S. chudaei* infusion on acute oral toxicity in mice.

Dose mg/kg b.w.	Animals	Mortality	Toxicity sign
Control (saline)	3 male	–	–
	3 female	–	–
2000	3 male	–	–
	3 female	–	–
3000	3 male	–	–
	3 female	–	–
5000	3 male	–	–
	3 female	–	–

interesting to complete this test with a sub-acute oral toxicity test including biochemical and histological studies.

3.4. Anti-inflammatory activity

The anti-inflammatory effect of *S. chudaei* infusion was evaluated via carrageenan test, one of the most popular tests to screen the anti-inflammatory properties of drugs and plant extracts. Table 4 shows the effect of three doses of *S. chudaei* infusion orally administered on mice paw induced inflammation. The doses (250, 500, and 1000 mg/kg b.w.) of *S. chudaei* infusion and diclofenac® (50 mg/kg b.w.) reduced significantly ($p < 0.001$) the paw edema after 4 h of administration, comparing to the control. The lower edema percentage was obtained with the more concentrated infusion (1000 mg/kg b.w.) with an inhibition percentage of 58%, showing a strong anti-inflammatory effect. This effect was better than the effect obtained with diclofenac® (50 mg/kg b.w.) that allowed an inhibition percentage of 53%. The other assayed infusion doses (250 and 500 mg/kg b.w.) allowed obtaining lower percentages but not so far from the mentioned values (45 and 48%, respectively). Many *Salvia* species have been screened for their anti-inflammatory activity (Akram et al., 2015; Kamatou et al., 2010). Choi et al. (2018) reported that some caffeic acid derivatives, especially rosmarinic acid methyl ester, extracted from *S. miltiorrhiza* exhibit potent anti-inflammatory effects through the inhibition of inflammatory mediators (COX-2: cyclooxygenase-2, NO: nitric oxide, and iNOS: inducible nitric oxide synthase) and the induction of HO-1 (heme oxygenase-1) which have for mission to inhibit the oxidative stress. On the other hand, Kamatou et al. (2010) tested the anti-inflammatory potential of sixteen *Salvia* species, reporting a poor inhibition of the 5-lipoxygenase enzyme (IC50 > 100 $\mu\text{g/mL}$), with exception of one species that displayed a moderate inhibition. The administration of *Rosmarinus officinalis* extract and its rosmarinic acid in similar dose (25 mg/kg b.w.) resulted the same paw edema inhibition of about 60% volume reduction compared with control (Rocha et al., 2015).

Taking into account the results of the phytochemical analysis performed in this study revealing the presence of rosmarinic acid as the main compound, and its described anti-inflammatory and antioxidant potential, it is expectable the ability of *S. chudaei* infusion to inhibit paw edema. This activity has rosmarinic acid as the responsible, probably due to its action on the COX-2 inhibition pathway. Many studies involve the flavonoids in the *in vitro* and *in vivo* anti-inflammatory properties. This is the case of apigenin, a compound with a significant presence in the studied sample, that is linked to its ability to inhibit cyclooxygenase and lipoxygenase enzymes (Kim et al., 2004; Narayana et al., 2001).

3.5. Analgesic tests

3.5.1. Acetic acid-induced writhing test

The analgesic effect of *S. chudaei* infusion was evaluated by the acetic acid test and Table 5 summarizes the obtained results. All tested doses (250, 500, and 1000 mg/kg b.w.) showed highly significant ($p < 0.001$)

Table 4
Effect of *S. chudaei* infusion on carrageenan-induced paw edema in mice.

Group (n = 5)	Dose (mg/kg b. w.)	Edema (%)	Inhibition percentage (%)
Control (saline)	0.5 (mL)	42.00 ± 2.12 a	–
Diclofenac®	50	19.79 ± 1.15 bd	52.91
Infusion	250	23.20 ± 1.11 c	44.79
	500	21.64 ± 1.61 bc	48.51
	1000	17.55 ± 1.48 d	58.25

Mean \pm SD, different letters indicate statistically significant differences between groups ($p < 0.05$).

Table 5Effect of *S. chudaei* infusion on constrictions number of acetic acid-induced writhing test in mice.

Group (n = 6)	Dose (mg/kg b.w.)	Mean of constrictions number	Inhibition percentage (%)
Control (saline)	0.5 mL	48.83 ± 3.43 a	–
Paracetamol®	100	24.00 ± 3.16 bc	50.85
Infusion	250	26.67 ± 1.50 b	45.38
	500	21.67 ± 2.94 c	55.63
	1000	15.00 ± 1.67 d	69.28

Mean ± SD, different letters indicate statistically significant differences between groups (p<0.05).

reduction of abdominal constrictions, comparing to the control. The decreasing number of constrictions is dependent of the tested dose, meaning that the highest dose (1000 mg/kg b.w.) has the least number of constrictions (15, with an inhibition percentage of 69%). Paracetamol® 100 mg/kg b.w. showed also a highly significant (p<0.001) reduction regarding the control (51% with 24 constrictions). It is worth noting that the infusion doses 500 and 1000 mg/kg b.w. exhibited higher protection percentages (56% and 69%, respectively) than paracetamol® (51%). The less concentrated infusion sample presented inhibition percentage slightly lower (45.38) than paracetamol®. Nevertheless, the presented values give to *S. chudaei* infusion a potential analgesic effect.

In the acetic acid test, after intraperitoneal injection of the acid, an increase in the number of polymorphonuclear neutrophils into the peritoneal fluid was detected; this may raise the release of several inflammatory mediators, leading to the inflammatory pain in the inflammation site through nociceptive neurons stimulation. The acetic acid activates directly visceral and somatic nociceptors, as a general (non-selective) nociceptive model. It is a sensitive test to evaluate the peripheral analgesic action of drugs (Abbate et al., 1990; Le Bars et al., 2001; Satyanarayana et al., 2004).

3.5.2. Formalin test

The antinociceptive action of *S. chudaei* infusion was determined by measuring the spent time in licking/biting mice paw, after formalin injection, and the results are displayed in Table 6. Three doses (250, 500, and 1000 mg/kg b.w.) of infusion were used to evaluate the neurogenic (early) and inflammatory (late) phases. In both phases, the licking/biting time was dose-dependent, decreasing with the dose increasing. In the early phase, the licking/biting time of the tested doses decreased significantly, comparing to the control (Table 6). The highest protection percentage was exhibited by the 1000 mg/kg b.w. dose (61%). This value is near to the one presented by paracetamol® 50 mg/kg b.w. (63%). In the late phase, a significant decrease in licking/biting time was also observed in all tested doses, comparatively to control, and in a similar way as described for the early phase. The 1000 mg/kg b.w. infusion dose showed a protection percentage of 58%, lower than the paracetamol 50 mg/kg b.w. protection percentage (65%) but an interesting antinociceptive action.

In the formalin test, there is a biphasic nociceptive response: an immediate and intense response characterized by direct activation of sensory fibers (early phase: 0–5 min); a prolonged tonic response phase related to the functional changes in the spinal cords dorsal horn and the production and release of numerous inflammatory mediators in the peripheral tissue (late phase: 15–30 min) (Dubuisson and Dennis, 1977; Tjølsen et al., 1992). In general, drugs acting on the central system inhibit these phases, whereas the peripherally acting ones inhibit only the early phase.

The results obtained from these analgesic tests suggest that *S. chudaei* infusion has peripheral and central analgesic activity. Rosmarinic acid may be the responsible, once it was proven to attenuate both central and peripheral pain involving opioid receptors. It may reduce the liberation

Table 6Effect of *S. chudaei* infusion on early and late phase of the formalin test in mice.

Group (n = 5)	Dose mg/kg b.w.	Early phase		Late phase	
		Licking/biting time (s)	Inhibition percentage (%)	Licking/biting time (s)	Inhibition percentage (%)
Control (saline)	0.5 mL	84.60 ± 3.21 a	–	66.00 ± 2.91 a	–
Paracetamol®	50	31.00 ± 2.25 b	63.35	22.80 ± 3.03 b	65.45
Infusion	250	44.40 ± 2.41 c	47.51	37.00 ± 1.58 c	43.93
	500	39.00 ± 3.39 cd	53.90	29.60 ± 2.07 d	55.15
	1000	33.20 ± 3.63 bd	60.75	27.60 ± 1.92 d	58.18

Mean ± SD, different letters indicate statistically significant differences between groups (p<0.05).

of inflammatory mediators (see anti-inflammatory part (3.4.)) or directly block receptors (Boonyarikpunchai et al., 2014). Moreover, rosmarinic acid orally administered produces a significant and dose-dependent nociceptive response inhibition in mice paw glutamate-induced nociception (Guginski et al., 2009). In addition, rosmarinic acid showed a decrease of inflammatory and oxidative markers (COX-2, PGE-2, NO, IL-1β) in the spinal cord levels (Rahbardar et al., 2017).

Another study reported rosmarinic acid pain reduction only in the formalin test late phase (Falcão et al., 2016). Furthermore, kaempferol glycosides may display also an analgesic effect. After oral administration, they showed a potent analgesic and anti-inflammatory effect in mice (De Melo et al., 2009).

4. Conclusions

This is the first work about phenolic compounds composition, anti-inflammatory, and analgesic effect of the infusion of *Salvia chudaei* Batt. & Trab. The infusion has a remarkable antioxidant and anti-inflammatory activities, with analgesic effects in both central and peripheral models. The rosmarinic acid seems to be the main compound justifying such effects. Thus, the biological activities of *S. chudaei* aerial parts infusion may be due to the ability of phenolic compounds to inhibit inflammatory mediator generation, to act as radical scavenging, and to block directly the nociceptive receptors. The results of this study support the folk use of *Salvia chudaei* to treat inflammation and pain. However, more tests are needed to completely understand the mechanisms of action.

Author contributions

All authors were involved in the writing and design of this study. R.S. performed experiments and drafted the manuscript. S.M. contributed to TPC, TFC, DPPH*, and FRAP experiments. L.B. and I.C.F.R.F. provided LC-MS analysis. S.O. and M.B.P.P.O. designed and supervised the study, and revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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