




Teucrium pseudochamaepitys L.: chemical composition, acute toxicity, antioxidant, anti-inflammatory, and analgesic properties

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Received: 16 July 2023 / Accepted: 14 November 2023 / Published online: 15 December 2023
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Abstract

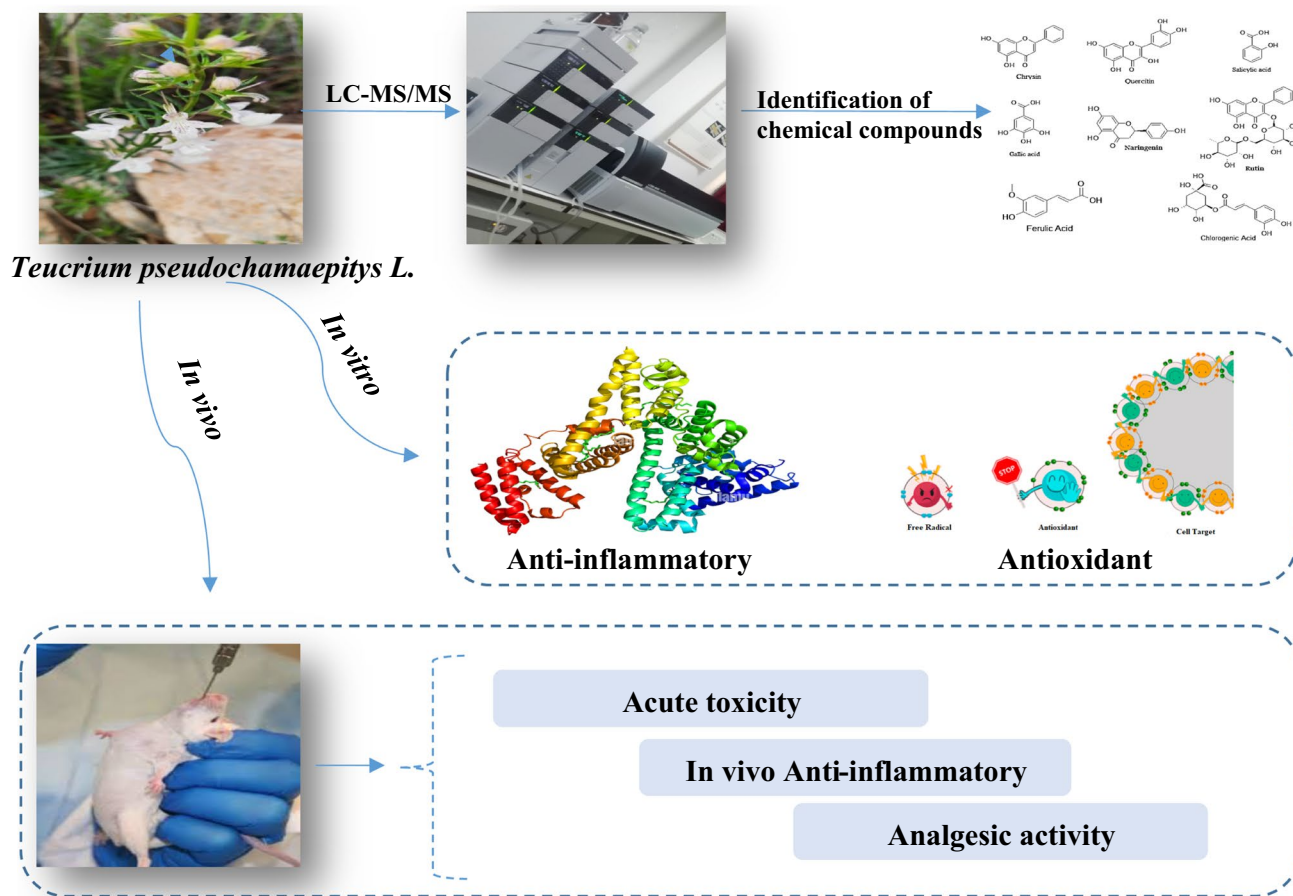
Most of the species of the *Teucrium* genus are known for their strong anti-inflammatory and antioxidant activities due to their richness in phenolic compounds. Thus, one species of the *Teucrium* genus belonging to the Lamiaceae family named *Teucrium pseudochamaepitys* L. was chosen in this study. The main objective of this study was to establish the pharmacological properties of *T. pseudochamaepitys* L. aqueous decoctions using both in vitro and in vivo experiments. This research represents the first attempt to validate the medicinal effects of this plant. The chemical composition was determined using SPE LC-MS/MS analysis, revealing the presence of flavonoids and phenolic acids. The biological activity and chemical composition of the aqueous extract of *T. pseudochamaepitys* (TpAE) were investigated for the first time. Scavenging of DPPH, ABTS⁺, reducing power, and phenanthroline assays was employed in order to measure the antioxidant activity of TpAE. The results showed that TpAE exhibited significant antioxidant activity, with the best performance of the phenanthroline method. In order to evaluate in vitro anti-inflammatory effect, the inhibition of albumin denaturation by TpAE was investigated. TpAE confers high potential anti-inflammatory activity with a percentage of inhibition very close to that of the reference diclofenac. Acute toxicity, anti-inflammatory, and analgesic activities were performed in order to investigate in vivo biological activity of TpAE. Oral administration of TpAE at a single dose of 2000 mg/kg did not cause any symptoms

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of intoxication in all treated animals. On the other hand, the oral administration of TpAE at different concentrations inhibits the development of paw edema and produces significant inhibition in acetic acid-induced writhing test. Our study revealed that TpAE is not toxic but contains bioactive compounds with antioxidant, anti-inflammatory, and analgesic activity.

Graphical abstract



Keywords *Teucrium pseudo-chamaepitys* L. · SPE LC-MS/MS · Analgesic · Acute toxicity · Anti-inflammatory

Introduction

Numerous wild plant species across the globe possess medicinal properties and continue to be utilized within the realm of alternative medicine. The natural world has long been acknowledged and continues to be regarded as the oldest and most abundant source of a diverse array of medicinal resources. The primary factor driving the widespread interest in herbal medicines is the perception among individuals that these alternative treatments are devoid of adverse effects, offer cost-effective solutions, and are significantly safer compared to conventional medications (Muhammad et al. 2012). These sources are of significant importance as they contain numerous bioactive compounds that can be utilized in specific

therapeutic applications owing to their analgesic and anti-inflammatory properties, among other beneficial attributes (Karaköse 2022; Şen et al. 2022). Inflammation is a significant global issue, given its debilitating symptoms, which lead to widespread pain and reduced productivity. In contemporary times, there has been a notable surge in the attention garnered by the pharmaceutical and scientific communities toward utilizing medicinal plants as analgesic agents. This heightened interest is accompanied by a pressing requirement for novel anti-inflammatory medications, particularly those sourced from natural substances (Boujbiha et al. 2023). Furthermore, the inclusion of experimental research is imperative in order to authenticate the ethnopharmacological understanding pertaining to herbal medicine. Phytochemical and pharmacognostic

evaluations are essential procedures for assessing the safety and efficacy of medicinal plant utilization.

Teucrium genus is one of the large and important genera of Lamiaceae family. It includes more than 300 species, widely distributed in the world, especially in the Mediterranean area that contains about 96% species of this genus (Moreira et al. 2014). Based on the ethnomedicine practices observed in various nations, *Teucrium* is recognized as a prominent resource for alleviating a range of female ailments. Its therapeutic properties encompass anti-nociceptive, anti-inflammatory, anti-bacterial, hypolipidemic, anti-rheumatoid, hypertensive, and hypoglycemic effects (Abdollahi et al. 2003). The therapeutic properties of *Teucrium* have been ascribed to specific bioactive substances that were isolated from them. These substances include phenolic acids and their derivatives (Mihailović et al. 2020), iridoids and flavonoids (Rizk et al. 1986), volatile oils (such as myrtenal, α -pinene, and sabinene) (Kabouche et al. 2007). Furthermore, the inclusion of experimental research is imperative in order to authenticate the ethnopharmacological understanding pertaining to herbal medicine. Phytochemical and pharmacognostic evaluations are essential procedures for assessing the safety and efficacy of medicinal plant utilization.

Teucrium pseudochamaepitys, is a specie of *Teucrium* genus that is growing spontaneously in Algeria. Its leaves are mucronate, divided into three–five linear segments. Its inflorescences are terminal with two flowers per whorl, which have white color, spotted with pink or purple color (Quézel et al. 1962). According to the literature, no study has reported the chemical composition and biological activities of *Teucrium pseudochamaepitys* extracts; therefore, we chose this specie in our study. Furthermore, the inclusion of experimental research is imperative in order to authenticate the ethnopharmacological understanding pertaining to herbal medicine. *Teucrium pseudochamaepitys* L. has been used traditionally for the treatment of several illnesses. However, there needs to be more available data on investigating its phytocomponents in relation to their potential anti-inflammatory and antipyretic properties. Thus, the aim of this study was to investigate the chemical analysis, the acute toxicity, analgesic, anti-inflammatory, and antioxidant properties of the aqueous extract obtained from *Teucrium pseudochamaepitys* (TpAE). The antioxidant activity of the TpAE was determined using four different methods, which are: DPPH, ABTS, reducing power, and phenanthroline tests. In vitro anti-inflammatory activity was performed on TpAE using inhibition of albumin denaturation method. In order to evaluate in vivo biological activity of TpAE, acute toxicity, anti-inflammatory, and analgesic activities were carried out on male and female albino mice. Chemical composition of TpAE was determined in this study using SPE LC-MS/MS analysis.

Experimental

Plant and decoction preparations

The aerial part of the plant *Teucrium pseudochamaepitys* was harvested from the northeast of Algeria Setif, Bougaa region (36° 09' 00" north, 5° 26' 00" east), during its flowering season (May 2021). The scientific identification of the plant has been performed by Dr. Halis Youcef researcher in Scientific and Technical Research Centre for Arid Areas Touggourt-Algeria. The plant was dried in the shade, protecting it from moisture and light, at room temperature for 15 days, and a voucher specimen has been deposited in the herbarium of our laboratory under the code: 202105Bo/TeuPs. In order to prepare the aqueous extract of *Teucrium pseudochamaepitys* (TpAE) following the decoction method, 106.281 g of the aerial part of the plant material was cut into small pieces and boiled in 1000 mL of distilled water for 30 min. After that, the decoction was frozen and lyophilized, and the resulting powdered extract was labeled TpAE. The final lyophilized powder was redissolved in water and stored at 4 °C until phytochemical and pharmacological tests.

Experimental animals

In this investigation, we used two 20–30-day-old albino mice sexes (*Mus musculus*) (25 g \pm 5 g). These mice were procured from Pasteur Institute in Algeria (Elevage Centre, Kouba, Algeria). The mice were housed in cages under standard conditions of the pet shop of ASSB faculty (FSB/USTHB) with a temperature of 20–24 °C; humidity of 50–65%; and 12 h of lighting per day, mice had free access to water and standard rodent chow for 16 h prior to experiments. All in vivo trials were conducted following preset guidelines of National Research Council (NRC, 1996), Washington, DC, USA. Every effort was made to minimize the number of animals used. The experimental procedures were approved by the University Animal Experimentation Ethics Committee (Approval Ref No. 162/2011/8).

SPE LC-MS/MS analysis

A qualitative analysis of constituents present in aqueous fractions of *Teucrium pseudochamaepitys* (TpAE) was performed using UPLC–ESI–MS–MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology equipped with binary bump Nexera XR LC-20AD.

Separation was achieved with an Ultra-force C18 column (I.D. 2.5 mm \times 100 mm, 1.8 μ m particle size; Restek), and the oven temperature was 25 °C. The chromatographic separation was carried out using a mixture of 30% (water,

0.1% formic acid) as mobile phase A and 70% methanol as mobile phase B. The flow rate was established at 0.3 mL/min, and the injection volume was 6 µL passed through a Millex-LCR (PTFE) filter with 0.22 mm pore sizes. The separation was performed at room temperature, and the run lasted for 25 min.

The MS/MS used the ESI conditions as follows: 230 KPs CID gas; –6.00 kV conversion dynode; 350 °C interface temperature; 250 °C DL temperature; 3.00 L/min nebulizing gas flow, 400 °C heat block; 15.00 L/min drying gas flow. The ion trap mass spectrometer was used in both negative and positive ions with MRM mode (multiple reaction monitoring). To achieve high extraction efficiency for all target analytes, the process of isolating and preconcentrating polyphenolic compounds from TpAE was carried out using a previously established solid-phase extraction (SPE) method. Accurate identification was made according to their typical fragments and through comparison with standards available in the developed database.

Phenolic compounds analysis

Total phenolic content

The spectrophotometric measurement of TPC in the TpAE was taken using the Folin–Ciocalteu procedure with a few modifications (Singleton and Rossi 1965). A concise depiction of the experimental procedure involved the addition of 100 µL of Folin–Ciocalteu reagent (diluted at a ratio of 1:10) and 75 µL of sodium carbonate solution (7.5%) to 20 µL of each extract. Following a 2-h incubation period in a light-restricted environment at ambient temperature, the absorbance at a wavelength of 765 nm was quantified using a microplate reader (PerkinElmer, Enspire), with a control serving as the control. The quantification of the polyphenol content was conducted using gallic acid equivalents (µg GAE/mg extract).

Total flavonoid content

The estimation of TFC was conducted using the aluminum colorimetric method with certain modifications (Topçu et al. 2007). A total of 130 µL of methanol was carefully dispensed into a microplate consisting of 96 wells. Subsequently, 50 µL of TpAE was added to the microplate. Additionally, 10 µL of potassium acetate (1 M) and 10 µL of aluminum nitrate at a concentration of 10% were introduced into the microplate. Following a 40-min incubation period at ambient temperature, the absorbance was quantified using a microplate reader (PerkinElmer, Enspire) set to a wavelength of 415 nm. TFC concentration was determined by employing a standard calibration curve for “quercetin” across various concentrations. The findings were quantified in terms

of micrograms of quercetin equivalent per gram of extract (µg QE/mg extract).

Antioxidant activity

DPPH radical trapping test

The assessment of the effect of *Teucrium pseudochamaepitys* aqueous extract on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was conducted using the methodology outlined by Blois (1958). In a 96-well microplate, 40 µL of TpAE and standards with varying concentrations were added to 160 µL of a pre-prepared DPPH solution. Standard antioxidants, namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and α-tocopherol, were employed in the study under identical experimental conditions. The measurement of absorbance was taken at a wavelength of 517 nm using a blank solution consisting of DPPH dissolved in methanol. This measurement was taken after a 30-min incubation period. The results were subsequently quantified as the percentage inhibition of DPPH, which was determined using the equation provided below.

$$\% \text{Inhibition} = \left[\frac{(\text{Abs}_C - \text{Abs}_E)}{\text{Abs}_C} \right] \times 100$$

where Abs_C is the absorbance of the control and Abs_E is the absorbance of the extract.

ABTS⁺ scavenging activity

The ABTS⁺ scavenging activity was assessed using the methodology outlined by Re et al. (1999). The cation ABTS⁺ was generated through a reaction involving 7 mM ABTS and 2.45 mM potassium persulfate. Following a 24-h period, the absorbance of the ABTS⁺ solution was manipulated in order to attain a specific absorbance value of 0.700 ± 0.020 at a wavelength of 734 nm. Subsequently, a mixture of 160 µL of ABTS⁺ solution and 40 µL of samples (TpAE, BHT, BHA, and Trolox) at varying concentrations were prepared. The measurement of absorbance was taken at a wavelength of 734 nm following a duration of 10 min in a lightless environment.

Reducing power assay

According to Oyaizu (1986), the reducing power assay was carried out with potassium ferricyanide, and the absorbance was subsequently measured at a wavelength of 700 nm. Various concentrations of TpAE and standards were combined with a 0.2 M phosphate buffer solution at a pH of 6.6, followed by the addition of 1% potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$). The resulting mixture was then incubated at a temperature of 50 °C for a duration of 20 min. Subsequently,

a solution containing 10% trichloroacetic acid and 10 μL of ferric chloride FeCl_3 (0.1%) was introduced. The outcomes were denoted as $A_{0.5}$, which represents the concentration that yields an absorbance of 0.5.

Phenanthroline assay

In this experiment, 10 μL of samples with varying concentrations was introduced into a solution containing 50 μL of a 0.2% ferric chloride (FeCl_3) solution and 30 μL of a 0.5% phenanthroline solution. Subsequently, the absorbance of the mixture was determined at a wavelength of 510 nm following an incubation period of 20 min at a temperature of 30 $^\circ\text{C}$ (Szydłowska-Czerniak et al. 2008). The absorbance levels were reported as $A_{0.5}$ $\mu\text{g}/\text{mL}$.

Acute toxicity study.

In vivo assessment of acute toxicity of TpAE was conducted in mice of both sexes (male mice and female mice) adopting the experimental protocol described by the guidelines of Organization for Economic Cooperation and Development (OECD) 423 for 14 days acute oral toxicity tests. All animals were fed with TpAE at doses 2000 mg/kg (per oral) (OECD 2001). Initially after TpAE administration, an examination of each animal was performed periodically after 4 h for any change in behavior and then daily for 14 days.

Anti-inflammatory activity

Inhibition of bovine serum albumin (BSA) denaturation

In this experiment, a volume of 100 μL of the sample solution (at concentrations of 97.65, 195.31, 390.62, and 1562.5 $\mu\text{g}/\text{mL}$) was combined with 100 μL of a 0.2% w/v BSA solution that was prepared in Tris-buffered saline with a pH of 6.6. The control group was comprised of a solution containing 100 μL of a 0.2% BSA solution mixed with 100 μL of water. On the other hand, the standard group consisted of a solution containing 100 $\mu\text{g}/\mu\text{L}$ of diclofenac sodium dissolved in water, combined with 500 μL of BSA solution. The test tubes were subjected to incubation at a temperature of 37 $^\circ\text{C}$ for a duration of 15 min, followed by immersion in a water bath maintained at a temperature of 72 $^\circ\text{C}$ for a period of 5 min. The absorbance values of the solutions were measured using a microplate reader (PerkinElmer, Enspire) at a wavelength of 660 nm (Kandikattu et al. 2013). The measurement of the inhibition against denaturation of BSA was taken using the following equation:

$$I\% = [A_{\text{CN}} - (A_{\text{TpAE}} - A_{\text{white}})] / A_{\text{CN}} \times 100$$

A_{CN} : 100 μL H_2O + 100 μL BSA, A_{TpAE} : 100 μL TpAE + 100 μL BSA, A_{white} : 100 μL TpAE + 100 μL Tris-phosphate (pH 6.8).

The control group in this study consists of denatured proteins, representing 100% of the sample. The obtained results are then compared to those of diclofenac sodium.

In vivo anti-inflammatory activity

Carrageenan-induced paw inflammation activity of TpAE was carried out in strain of male albino mice according to the method of Winter et al. (1962). In this study, the mice used were fasted for 16 h before the experiment. Then, the animals were divided into four groups each group comprised of 3 animals ($n = 3$). Animals in group 1 (control): mice were fed with normal saline. Animals in group 2 (positive control): the mice received standard Diclofenac[®] (500 mg/kg, b.w). Animals in group 3 and 4 were fed with TpAE 100 mg/kg and 500 mg/kg, separately. All mice were injected with carrageenan suspension (0.05 mL, 1% w/w) into the sub-plantar tissues of the right hind paw one hour after oral administration of all treatments. Following 3 h of anesthesia with diethyl ether, the animals were killed. Two posterior legs were severed at the tarsal joint and weighed on an analytical scale. The percentage of increase in paw weights (%Edema) and the percentage of reduction in edema (%inhibition) of treated mice were calculated using following formulas:

$$\% \text{Edema} = [(WA_{\text{LP}} - WA_{\text{RP}}) / WA_{\text{TP}}] \times 100$$

where WA_{LP} is the weight average of left paw; WA_{RP} is the weight average of right paw

$$\% \text{Inhibition} = [(\% \text{Edema}_{\text{C}} - \% \text{Edema}_{\text{E}}) / \% \text{Edema}_{\text{C}}] \times 100$$

where $\% \text{Edema}_{\text{C}}$: %Edema of control; $\% \text{Edema}_{\text{E}}$: %Edema of extract.

Analgesic activity

The assessment of analgesic efficacy was performed through the use of chemical stimulation-induced pain, utilizing two distinct models, namely the writhing.

Writhing test

The current study was carried out utilizing the torsion approach (manifesting as abdominal cramps) as expounded by Koster (1959) and afterward modified by Vogel (2002). Before beginning the experiment, the mice were subjected to a 16-h fasting period and subsequently divided into 4 groups, each consisting of 3 mice. Each group of mice was administered 0.5 mL of the respective substance. The substances used were divided into two groups: the negative control (physiological water) and the positive control (500 mg/kg Aspirin). Groups 3 and 4 were administered (TpAE at

100 mg/kg and 500 mg/kg). Following oral administration of the appropriate dosage, all mice were subjected to an intraperitoneal injection of 0.6% (1 mL/kg) acetic acid after a lapse of 30 min to elicit pain characterized by cramping. After 5 min, the frequency of cramps in each mouse was assessed through direct observation of the animal for a duration of 15 min. The analgesic effect of TpAE was evaluated by determining the percentage reduction in cramps (percentage protection), which was determined for each group using the formula:

$$\% \text{Protection} = \left[(1 - W_C / W_D) \right] * 100$$

where W_C is the mean value of writhes in treated groups and W_D is the mean value of writhes in control groups.

Statistical analysis

The data pertaining to phytochemical studies and biological activity tests are presented as the average values obtained from three independent analyses. Values represent the average results of three assays, with the standard deviation indicated. The one-way analysis of variance (ANOVA) test, specifically utilizing the XLSTAT software, was employed to conduct a statistical comparison of the mean values associated with each treatment. The experiments were conducted using concentrations exceeding four distinct values. The IC_{50} and $A_{0.50}$ values were determined through the application of linear regression analysis, while the detection of significant differences was accomplished using one-way computation of variance (ANOVA) with a value level set at $p < 0.05$.

Results and discussion

SPE LC-MS/MS analysis

Once the optimal conditions for UPLC–ESI–MS–MS were determined, the TpAE was subjected to analysis using the MRM mode, employing both negative and positive ions. Figure 1A, B, C, D, E, and F depicts the UPLC–ESI–MS–MS and Mass spectra of the chemicals that have been identified from TpAE. Table 1 presents a comprehensive overview of the retention time (Rt), the mass-to-charge ratio (m/z), and the chemical formula of the identified compounds. These compounds were detected based on their characteristic fragments and were further confirmed by comparing them with the standards stored in the established database.

According to Table 1 and Fig. 1, six of the compounds identified in TpAE are categorized as polyphenols based on the available standards in the existing database: chrysin, quercetin, salicylic acid, gallic acid, naringenin, and rutin (Fig. 2).

The outcomes of a phytochemical investigation conducted on the aerial parts of *Teucrium pseudo-chamaepitys* were analyzed using LC–MS–MS methodology. The obtained data, presented in Table 1, allowed for the preliminary identification of eight compounds out of a total of eleven. This identification was achieved by comparing the obtained results with standards that are currently accessible in the established database (Table 1). Both full and fragmentation analyses were utilized for this purpose. The electron spray ionization mass spectrometry (ESI–MS) technique is commonly employed to analyze ion peaks in the form of $[M-H]^-/[M+H]^+$. The following is an extensive list of the phytoconstituents that have been found: rutin ($C_{15}H_{12}O_5$), chrysin ($C_{15}H_{10}O_4$), quercetin ($C_{15}H_{10}O_7$), salicylic acid ($C_7H_6O_3$), gallic acid ($C_7H_6O_5$), chlorogenic acid ($C_{16}H_{18}O_9$), ferulic acid ($C_{10}H_{10}O_4$), and naringenin ($C_{15}H_{12}O_5$). Since four of them are constituents of the flavonoid family, the *Teucrium pseudo-chamaepitys* aqueous fraction has a heavy amount of flavonoids. Some of these compounds are being investigated for their potential to treat diabetes (rutin) (Habtemariam and Lentini 2015), reduce inflammation and exhibit higher antioxidant capacity (chrysin, naringenin, and salicylic acid) (Zeinali et al. 2017; Venkateswara et al. 2017; Randjelović et al. 2015), and protect cells from free radical damage (quercetin) (Baghel et al. 2012).

Numerous studies have affirmed the presence of phenolic acid and flavonoid compounds in different *Teucrium* species, notably *T. botrys*, *T. compactum*, *T. montanum*, *T. aragonense*, *T. haensekri*, and all the subspecies of *T. polium*. These compounds act as the main contributors to the antioxidant potential and to many other biological activities of plant extracts. Flavones and their glycosides are the most common class of secondary metabolites within the genus of *Teucrium* (Harborne, et al. 1986), notably rutin and quercetin which are the most common flavonoids found in our subjected species (*T. pseudo-chamaepitys*). Diverse flavanols such as cirsiol, apigenin, pectolarigenin, and naringenin were identified to be components of *T. chamaedrys* (Labbe et al. 1989) salvigenin, cirsimaritin, 3'-O-methyleupatonin from *T. heterophyllum* (Fraga et al. 1995). Furthermore, several additional secondary metabolites originating from the molecular identification of different *Teucrium* species in previous studies included caffeoylated phenylpropanoid, terpenoids, and derivatives, volatiles, and phenolic compounds (Bahramikia et al. 2022). Volatile compounds of *T. polium* and *T. montanum* consisted mainly of sesquiterpenes with germacrene D and δ -cadinene as the main constituents (Radulović et al. 2012); nevertheless, they were not found in our plant.

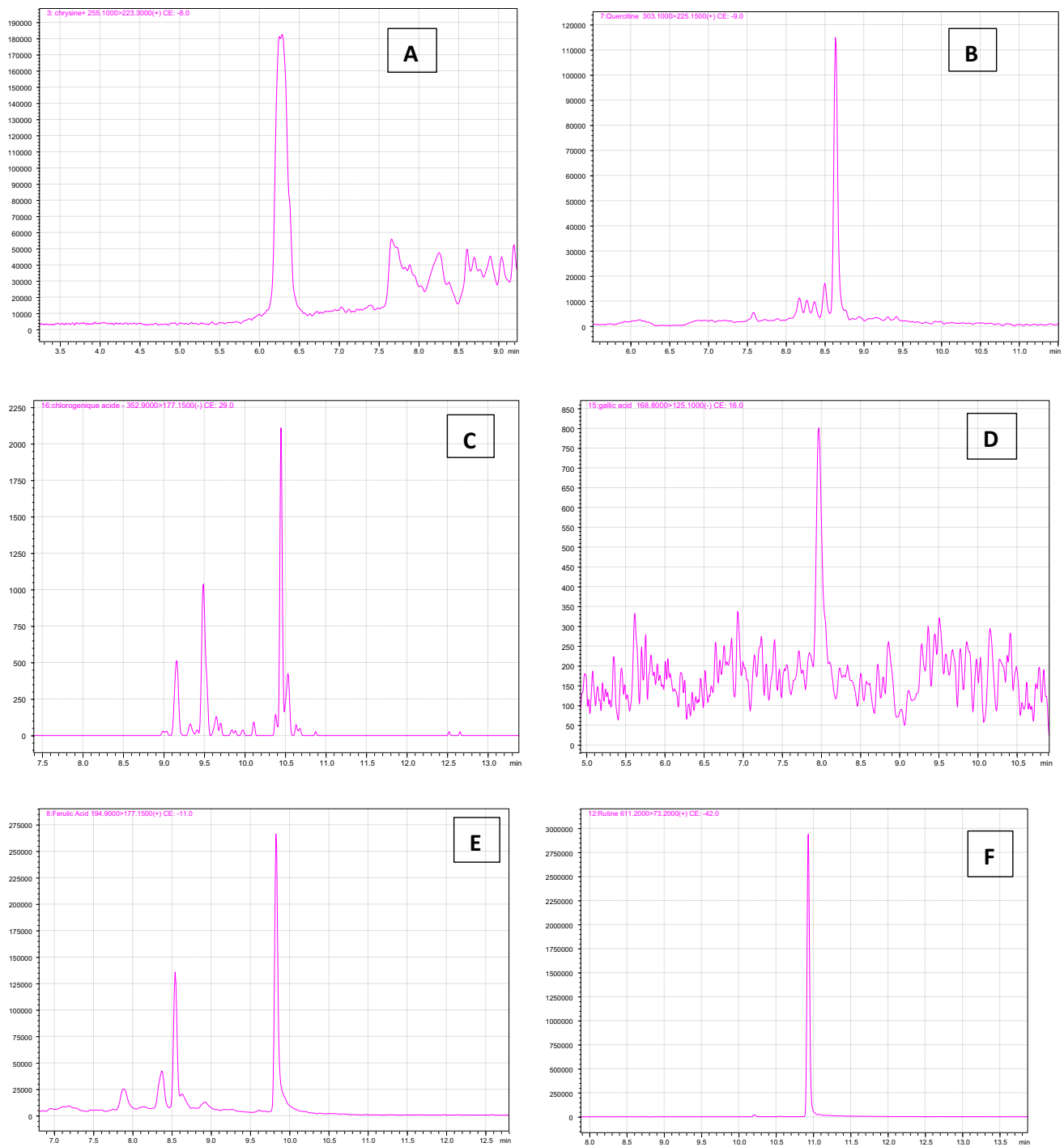


Fig. 1 Chromatograms profiles of *Teucrium pseudo-chamaepitys* (TpAE). **A** Chromatogram of standard chrysin in TpAE; **B** chromatograms of standard quercetin in TpAE; **C** chromatograms of standard

chlorogenic acid in TpAE; **D** chromatograms of standard gallic acid in TpAE; **E** chromatograms of standard ferulic acid in TpAE; **F** chromatograms of standard rutin in TpAE

Total phenolics and flavonoids

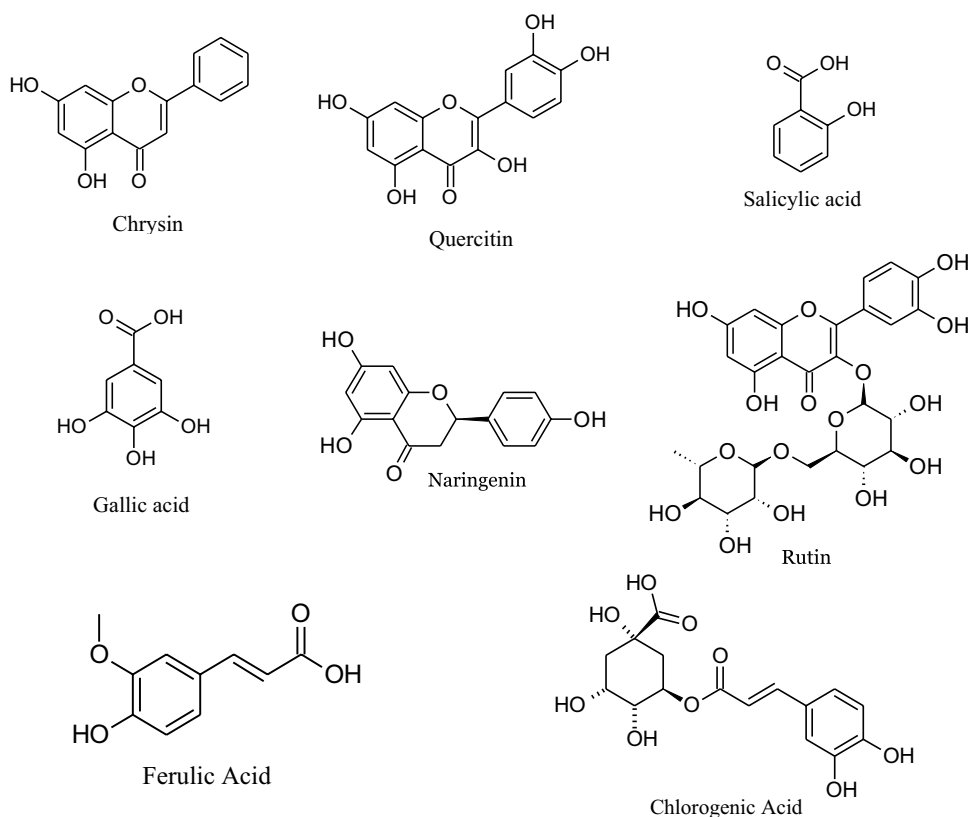
Table 2 presents the quantitative analysis of secondary metabolites found in the aqueous extract of *T. pseudo-chamaepitys*. The quantification of TPC and TFC was

conducted by determining their respective concentrations in $\mu\text{g GAE/mg}$ and $\mu\text{g QE/mg}$ of the extract. This was achieved by employing a calibration curve for each compound (phenol: $y = 0.0034x + 0.1044$, $R^2 = 0.9972$; flavonoid: $y = 0.0048x$, $R^2 = 0.997$), as illustrated in Fig. 3 and

Table 1 The detected molecules in the polyphenol fractions of TpAE according to the LC/MS–MS results

Molecule	Rt (Min)	Molecular formula	Ionization mode (<i>m/z</i>)	Analyzed fragment	TpAE
<i>p</i> -Coumaric acid	ND	C ₉ H ₈ O ₃	[M+H] ⁺	165.100 > 101.200	ND
Kaempferol	ND	C ₁₅ H ₁₀ O ₆	[M+H] ⁺	287.1000 > 255.250	ND
Chrysin	6.286	C ₁₅ H ₁₀ O ₄	[M+H] ⁺	255.100 > 223.300	D
Quercetin	8.638	C ₁₅ H ₁₀ O ₇	[M+H] ⁺	303.100 > 225.150	D
Naringenin	9.9	C ₁₅ H ₁₂ O ₅	[M+H] ⁺	273.100 > 147.130	D
Beta-carotene	ND	C ₄₀ H ₅₆	[M+H] ⁺	537.200 > 23.100	ND
Gallic acid	7.966	C ₇ H ₆ O ₅	[M–H] [–]	168.800 > 125.100	D
Salicylic acid	6.075	C ₇ H ₆ O ₃	[M–H] [–]	137.100 > 93.150	D
Chlorogenic acid	9.97	C ₁₆ H ₁₈ O ₉	[M–H] [–]	352.900 > 177.150	D
Rutin	10.932	C ₂₇ H ₃₀ O ₁₆	[M+H] ⁺	611.200 > 73.200	D
Ferulic acid	9.75	C ₁₀ H ₁₀ O ₄	[M–H] [–]	194.900 > 177.500	D

D detected, ND not detected

Fig. 2 Chemical structures of molecules detected in TpAE**Table 2** TPC and TFC of *Teucrium pseudochamaepitys* aqueous extract

TpAE	TPC (μg GAE/mg)	TFC (μg QE/mg)
	187.32 ± 2.39	152.98 ± 1.96

Fig. 4. The findings of our study indicate that the plant under investigation exhibits a high concentration of polyphenols, as evidenced by the total phenol content of TpAE, which was measured to be 187.32 ± 2.39 μg GAE/mg of

extract. Regarding the overall flavonoid content, the total flavonoids content extract (TpAE) demonstrates a significantly higher concentration, measuring 152.98 ± 1.96 μg QE/mg of extract.

The analysis of total phenolic content and total flavonoid content has revealed that the TpAE exhibits a significant presence of phenolic and flavonoid compounds, surpassing the levels reported in previous studies on the *Teucrium* species (Bendjabeur et al. 2018; Al-Otaibi and AlMotwaa 2022).

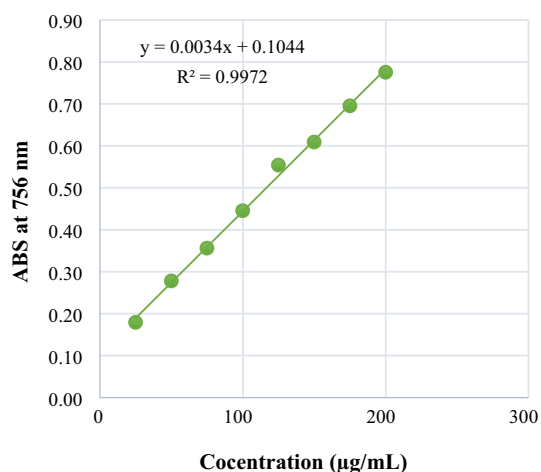


Fig. 3 Gallic acid curve for total phenolic content

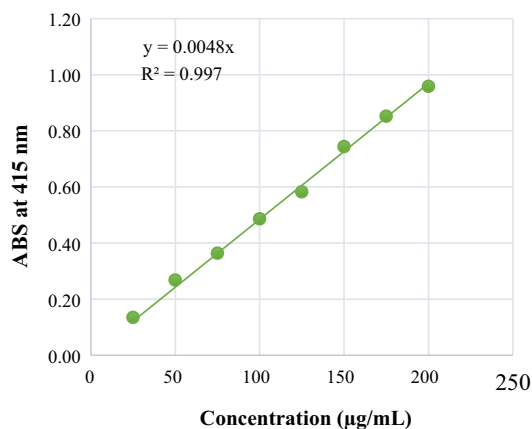


Fig. 4 Quercetin curve for total flavonoid content

Antioxidant activity

The findings related to the antioxidant activity, as assessed through the DPPH, ABTS, RP, and O-phenanthroline tests, are summarized in Table 3. The IC_{50} and $A_{0.5}$ values assess the concentration of TpAE required to achieve a 50% inhibition of free radicals. Nevertheless, there exists an inverse correlation between the antioxidant capacity of a compound and its IC_{50} value. Consequently, a compound with a lower IC_{50} value demonstrates a higher level of antioxidant activity.

It is noteworthy to mention that the aqueous fraction of *T. pseudochamaepitys* demonstrated a sustainable antioxidant activity in all four tests. However, it is important to acknowledge that there exists a correlation between the concentration of polyphenols and the activity of antioxidants. This correlation serves to validate the notion that polyphenols possess significant antioxidant properties, as they are able to effectively hinder the formation of free radicals and counteract the oxidation of macromolecules.

The potential antioxidant capacity of a biochemical substance could be assessed through various in vitro tests that measure its ability to inhibit stable free radicals. In pursuit of this objective, we have conducted an array of antioxidant tests employing various methodologies such as direct, indirect, or competitive procedures, as well as principles of reduction, chelation, or inhibition (Cantos et al. 2002). It is our contention that the implementation of the four examined techniques may effectively guarantee the antioxidant attributes of TpAE, notwithstanding its moderate potential in one of these strategies. The TpAE has demonstrated significant antioxidant activity, as evidenced by the relatively low $A_{0.5}$ and IC_{50} presented in Table 3. These concentrations were comparable to the standard values, with the exception of the ABTS scavenging assays.

It can be inferred that the amount of the compound responsible for the observed effect is relatively lower. This

Table 3 Antioxidant activities of the aqueous extract from *Teucrium pseudochamaepitys*

Products	DPPH (IC_{50} (µg/mL))	ABTS	Reducing power ($A_{0.5}$ (µg/mL))	Phenanthroline
TpAE	95.48 ± 2.02^a	133.71 ± 2.35^a	76.19 ± 0.50^a	23.89 ± 0.10^a
α -Tocopherol*	13.02 ± 5.17^b	NT	34.56 ± 1.32^b	NT
BHA*	6.14 ± 0.41^c	6.75 ± 0.29^b	NT	0.91 ± 0.01^b
BHT*	12.99 ± 0.41^d	13.05 ± 0.33^c	NT	2.19 ± 0.13^c
Ascorbic acid*	NT	NT	5.88 ± 0.95^c	3.01 ± 0.01^d
Tannic acid*	NT	NT	5.45 ± 0.67^d	NT
Trolox*	NT	5.14 ± 0.06^d	5.10 ± 0.14^e	4.47 ± 0.15^e

*Reference compounds: TpAE—*Teucrium pseudochamaepitys* aqueous extract. Values represent the average of three distinct tests \pm SD. Means divided by distinct letters in the same column varied substantially at $p < 0.05$. NT not tested. Values with different subscripts (a, b, c, d, e) in the same column were significantly different compared with the control (* $p \leq 0.001$; very highly significant, ** $p \leq 0.01$; highly significant, * $p \leq 0.05$; significant), and those with the same subscripts were not significantly different ($p > 0.05$)

inference is made under the assumption that the measured activity resulted from the combined effect of multiple molecules. However, despite this, the TpAE still exhibited significant antioxidant capabilities comparable to those of the standard chemicals.

The TpAE exhibited a notable antioxidant capacity, as evidenced by its lower DPPH IC₅₀ and reducing power A_{0.5} values in comparison with extracts derived from *Teucrium* species, *Teucrium montanum*, *Teucrium chamaedrys* (Kadifkova et al. 2005), *Teucrium orientale* (Çakir et al. 2006), whereas the methanolic extract of *Teucrium polium* showed the highest antioxidant activity as measured by DPPH and FRAP assays with IC₅₀ values of 0.41 ± 0.03 mg/mL and 0.21 ± 0.002 mg/mL, respectively (El Atki et al. 2019), which confirms that our findings are more significant than those of previous studies. Indeed, DPPH IC₅₀ from of the essential oil of *Teucrium pseudochamaepitys* was 0.77 ± 0.05 mg/mL (Hammami et al. 2015). Concerning previous antioxidant studies of *Teucrium pseudochamaepitys*, none have utilized four distinct antioxidant techniques to demonstrate how potent and powerfully bioactive the target extract.

The TpAE has demonstrated significant antioxidant activity, suggesting its potential as a promising intervention for addressing various illnesses associated with imbalances in oxidant-antioxidant levels and oxidative stress, which can contribute to the onset of severe diseases.

Acute toxicity

During a 14-day study of acute toxicity evaluation, we found no toxicity, body weight changes, behavioral alterations, or death among rats when TpAE was administered at a dosage of 2000 mg/kg. Therefore, the LD₅₀ of this extract is greater than 2000 mg/kg, which indicated that TpAE is considered as relatively safe according to the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (Europe 2005). This finding is consistent with the results of Chabane et al. (2021), which reported that the oral administration of methanolic extract of *Teucrium polium* at the doses of 1000 to 5000 mg/Kg did not cause any toxicity symptoms.

A prior investigation utilized four *Teucrium* species: *T. carthagenenses* Linn. (chloroform extract), *T. flavum* Linn. (methanolic extract), *T. pumilum* Linn. (chloroform extract), and *T. buxifolium* Lin to establish acute toxicity. Male albumin was used to conduct acute toxicity experiments with a constant volume of 1 mL/kg administered intraperitoneally (ip). In this study, the dose tested ranged from 130 to 200 mg/kg. Most of the extracts exhibited limited toxicity, consistent with our findings (Ulubelen et al. 2000).

Anti-inflammatory activity

Inhibition of bovine serum albumin denaturation

The findings of the in vitro anti-inflammatory effectiveness of the aqueous extract of *T. pseudochamaepitys* are presented in Table 4. It is observed that the level of inhibition of BSA denaturation (0.2%) exhibits a direct relationship with the concentration of various plant extracts. The concentration of 1562.5 µg/mL yielded the highest percentage inhibition, measuring 71.93 ± 2.78. Nevertheless, these values are close to that obtained for diclofenac which demonstrated 86.52% inhibition at 1.5 mg/mL (Fig. 5), commonly used as a standard anti-inflammatory drug, effectively inhibited the denaturation of BSA at an equivalent concentration.

In vivo anti-inflammatory effect

The anti-inflammatory effects of two doses of TpAE (100 mg/kg and 500 mg/kg) were evaluated in an in vivo environment. Carrageenan was used to induce experimental paw inflammation in mice. The induction of paw edema by carrageenan is a widely recognized acute model of inflammation that is commonly employed to evaluate the anti-inflammatory efficacy of various compounds. Table 5 represents the percentage of edema (%Edema), and the percentage of its reduction using TpAE and diclofenac sodium (%inhibition). The distinct phases of paw edema induced by carrageenan are evident. The initial phase, occurring approximately one hour after the stimulus, is characterized by the liberation of histamine, serotonin, bradykinin, and to a lesser extent, prostaglandins. In contrast, the subsequent

Table 4 Effect of *Teucrium pseudochamaepitys* aerial parts aqueous extracts on albumin denaturation

Concentration (µg/mL)	TpAE %inhibition	Diclofenac* %inhibition
1562.5	71.93 ± 2.78 ^a	86.52 ± 0.16 ^b
781.25	57.22 ± 0.07 ^a	78.95 ± 0.32 ^b
390.625	54.53 ± 0.18 ^a	74.34 ± 0.30 ^b
195.3125	38.38 ± 0.07 ^a	71.47 ± 0.29 ^b
97.65625	1.65 ± 0.68 ^a	68.01 ± 0.33 ^b

*Reference compounds: TpAE—*Teucrium pseudochamaepitys* aqueous Extract. Values represent the average of three distinct tests ± SD. Means divided by distinct letters in the same column varied substantially at $p < 0.05$. NT: not tested. The variance analysis of our results was performed on XLSTAT Version 2016 using ANOVA, and the significance of differences was evaluated using Tukey's HSD test. Values with different subscripts (a, b) in the same column were significantly different compared with the control ($*p \leq 0.001$; very highly significant, $**p \leq 0.01$; highly significant, $*p \leq 0.05$; significant), and those with the same subscripts were not significantly different ($p > 0.05$).

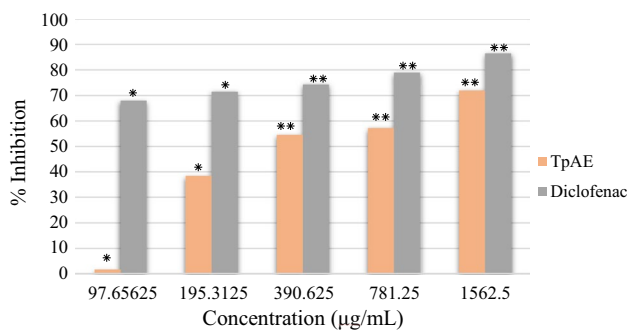


Fig. 5 Effect of *Teucrium pseudochamaepitys* aerial parts aqueous extracts on albumin denaturation. The variance analysis of our results was performed on XLSTAT Version 2016 using ANOVA, and the significance of differences was evaluated using Tukey's HSD test. Values with different subscripts (a, b) in the same column were significantly different compared with the control ($*p \leq 0.001$; very highly significant, $**p \leq 0.01$; highly significant, $*p \leq 0.05$; significant), and those with the same subscripts were not significantly different ($p > 0.05$)

Table 5 The effect of TpAE and diclofenac sodium on carrageenan-induced hind paw edema in mice

	Dose	%Edema	%Inhibition
Normal saline	–	59	–
Diclofenac®	500	17.56	70.23
TpAE	100	23.00	61.10
	500	18.71	68.28

phase (occurring after one hour) is marked by the infiltration of polymorphonuclear (PMN) leucocytes and the sustained synthesis of prostaglandins (Sadeghi et al. 2011).

The present study aimed to evaluate the in vivo anti-inflammatory activity of TpAE by examining its ability to inhibit paw edema induced by carrageenan in albino mice. Conversely, the in vitro anti-inflammatory activity was assessed using the albumin denaturation method. Based on the findings derived from our in vivo experiments, it can be concluded that there exists anti-inflammatory activity (Table 5); the present experiment employs a widely recognized experimental model for investigating acute inflammation, utilizing carrageenan, a chemically approved substance known for its ability to induce the release of mediators of inflammation. The results of this study indicate that TpAE demonstrates a beneficial anti-inflammatory effect, as it displayed significant activity during the later stage of the inflammatory response process. The outcomes of our study indicate that the administration of TpAE at a dosage of 500 mg/kg resulted in a substantial decrease in carrageenan-induced paw edema when compared with diclofenac sodium, a standard drug administered at an equivalent concentration. Table 5 demonstrates a noticeable reduction in paw weight

edema, reaching 68.28%, when juxtaposed with diclofenac sodium, which exhibited an inhibition rate of 70.23%. Therefore, the results acquired in this study provide confirmation of the extract's efficacy as a remedy for inflammation. This effect can be attributed to the combined effect of its various naturally occurring phenolic substances. Moreover, our in vivo anti-inflammatory effect data align with the findings collected on the genus *Teucrium* using carrageenan-induced inflammation method, found by Tariq et al. (1989), Farshchi et al. (2010), Pourmotabed et al. (2010) and El-Ashmawy (2018).

Moreover, in order to preserve 3D proteins structure, the principal mechanism of albumin denaturation consist of maintain of the electrostatic, hydrophobic, hydrogen, and disulfide links (Mizushima and Kobayashi 1968; Barros et al. 2008). Most proteins lose their biological roles after denaturation, which leads to the creation of autoantigens that trigger a variety of autoimmune dysfunctions, including inflammatory illnesses. Thus, it is thought that anti-inflammatory medications that inhibit protein denaturation are efficient (Lekmine et al. 2021). Inflammation is a multifaceted physiological reaction that is contingent upon various contributing factors. The denaturation of cell proteins is considered to be an important contributor (Khan et al. 2022). Therefore, the inhibitory effects of various concentrations of TpAE were investigated to evaluate the in vitro anti-inflammatory properties of the plant. The findings demonstrated a significant inhibitory effect of TpAE on the denaturation of proteins. Based on our results of the in vitro anti-inflammatory activity of TpAE (Table 3), which is show strong anti-inflammatory ability of this extract with an inhibition percentage 71.93 ± 2.78 at concentration of 1562.5 µg/mL, this protective capacity was very closer to the Diclofenac®. Compared to the results found for hydroalcoholic extract of the plant *Teucrium polium* reported by Benchikha et al. (2022), at a 2 mg/mL concentration, the evaluated substance exhibited a noteworthy inhibitory potential for BSA denaturation, estimated to be approximately 97.53%, also different concentration of this plant shows strong anti-inflammatory activity higher than the diclofenac sodium. Thus, these studies suggesting that *Teucrium* plants could be potential candidates as anti-inflammatory agents.

The *Teucrium* genus encompasses many species, such as *T. polium*, *T. oliverianum*, *T. hyrcanicum*, and *T. chamaedrys*. These species are known to possess secondary metabolites that demonstrate a wide range of biological activities, including anti-inflammatory properties both in vitro and in vivo (Stanković 2020). In this study the aqueous extract *Teucrium pseudochamaepitys* L. aerial part shows strong anti-inflammatory activity through in vivo and in vitro tests. Obviously, the phytochemical compounds found in the aqueous extract of *Teucrium pseudochamaepitys* L. aerial parts by the LC-MS/MS analysis contribute to the

anti-inflammatory activity of this plant. Further, as we mentioned previously within the LC-MS/MS analysis results, the major compounds detected on the TpAE belong to flavonoids and phenolic types of compounds. Therefore, the TpAE anti-inflammatory properties may be attributed to the existence of flavonoids and phenolic compounds within the plant. Previous studies have indicated that flavonoids significantly stabilize lysosomal membranes, both in vitro/in vivo (Kariawasam et al. 2017). The study reported the advantageous impacts of chrysin in the suppression of blood levels of NF- κ B (nuclear transcription factor κ B) p65 unit, TNF- α (tumor necrosis factor- α), and interferon- γ (IFN- γ). Additionally, it has been established that chrysin acts as an NF- κ B antagonist and a PPAR- γ agonist. This dual action leads to the reduction of crucial pro-inflammatory enzymes, including cyclooxygenase-2, myeloperoxidase, inducible nitric oxide synthase, prostanooids, and phospholipase A2 (Zeinali et al. 2017). Furthermore, naringenin possesses the ability to generate a sufficient amount of -OH (hydroxyl substitutions), thereby endowing it with the capacity to effectively scavenge reactive oxygen species (ROS). Therefore, it is postulated that naringenin possesses the potential to attenuate or enhance pathological states characterized by oxidative stress or inflammation as key contributing factors (Venkateswara et al. 2017). Additionally, it is worth noting that acetyl salicylic acid, a pharmaceutical agent with a history of over a century in clinical application, is commonly regarded as possessing comparable anti-inflammatory properties to salicylic acid (Randjelović et al. 2015). Therefore, this study proposes that TpAE could act as promising candidates for anti-inflammatory drugs.

Analgesic activity

Acetic acid-induced writhing

The study's findings indicate that administering varying doses of the aqueous extract of *Teucrium pseudochamaepitys* resulted in a significant reduction ($p < 0.001$) in abdominal writhing in mice compared to the negative control group. The administration of all doses of TpAE demonstrated a robust and statistically significant analgesic effect in the animal subjects. This effect was observed through the dose-dependent inhibition of acetic acid-induced writhing, resulting in inhibitions of 64.93% and 70.12% at doses of 100 and 500 mg/kg, respectively. In contrast, aspirin administration at a 500 mg/kg dosage resulted in a writhing inhibition effect of only 67.53%, as indicated in Table 6.

The acetic acid-induced writhing test model is widely regarded as a classical peripheral analgesic animal model and is regularly employed to screen analgesic drugs (Negus et al. 2006). Inhibiting cyclo-oxygenases, lipoxigenases, and other inflammatory mediators facilitates the peripheral

Table 6 Acetic acid-induced writhing of TpAE and aspirin in mice

Treatment	Dose (mg/Kg)	Number of writhing responses	% de protection
Control (physiological water)	–	77	–
Aspirin	500	25	67.53
TpAE	100	27	64.93
	500	24	70.12

analgesic impact. On the other hand, the central analgesic action is potentially facilitated by the suppression of central pain receptors. The administration of acetic acid intraperitoneally during the analgesic effect test resulted in the biosynthesis of leukotrienes and prostaglandins, as well as an increase in cyclooxygenase (COX), lipoxigenase (LOX), serotonin, histamine, bradykinin, substance P, interleukin-1 β (IL-1 β), interleukin-8 (IL-8), and tumor necrosis factor- α (TNF- α) in the peripheral tissue fluid. The augmentation of indigenous substances in the affected area has been documented as the causative agent for pain perception and contortions induced by acetic acid (Sarkar et al. 2019). Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin produce their analgesic effects by inhibiting the biogenesis of prostaglandins and are therefore able to inhibit acetic acid-induced writhing. The extract TpAE demonstrated a noteworthy capacity to inhibit writhing in mice that is comparable to that of conventional aspirin; it is plausible that they exerted their effects via a similar mechanism. As previously discussed, the involvement of flavonoids has been observed in the inhibition of COX-2, as well as the reduction of intracellular calcium levels and mediators of inflammation like TNF- α . Additionally, steroids have been found to inhibit prostaglandins, resulting in the production of an analgesic effect (Imam and Moniruzzaman 2014; Golder et al. 2020). The LC-MS/MS analysis of TpAE indicated the presence of phytochemical constituents, which is chrysin, quercetin, naringenin, ferulic acid, chlorogenic acid, and rutin, as previously mentioned. The previous investigation on phytochemicals in the *Teucrium* genus indicated the presence of monoterpene compounds. More specifically, (–)-linalool, a major compound identified in *Teucrium polium* (Vokou and Bessiere 1985), was reported to possess anti-nociceptive potential based on experimental animal studies. As a result, (–)-linalool has been extensively studied in various painful conditions. The observed effects of this particular monoterpene on nociception in various models indicate its potential involvement with muscarinic, opioid, dopaminergic, adenosinergic, and glutamatergic systems, as well as the participation of ATP[–] sensitive K⁺ channels. These findings suggest a significant reduction in the nociceptive

response (Guimarães et al. 2013). Additionally, it is important to consider the manner in which findings that validate prior research in the respective field are presented, which can be achieved through a thorough examination of the existing literature (Radhakrishnan et al. 2001). The in vivo experiments conducted in this study demonstrate a strong correlation with previous research findings.

Conclusion

The present research represents the first inquiry into the phytochemical and pharmacological features of aqueous decoctions from *Teucrium pseudochamaepitys* L. collected in Algeria. Hence, it can be asserted that the aqueous decoction derived from Algerian *Teucrium pseudochamaepitys* L. has exhibited promoting pharmacological properties as evidenced by its successful outcomes in both in vitro and in vivo experiments involving laboratory animals. The administration of orally delivered TpAE did not result in any observed toxicity or mortality in animals at doses up to 2000 mg/kg BW. Additionally, notable analgesic and anti-inflammatory effects were noticed. Based on the results obtained from this study via LC-MS/MS analysis of TpAE, it can be concluded that the biological activity of TpAE could be attributed to the bioactive compounds present in the *T. pseudochamaepitys* plant, especially flavonoids and phenolic acids. The latter are prospective candidates based on their currently demonstrated biological activity and tolerated non-cytotoxicity. These results open up future horizons for us to separate and purify the compounds present in this extract using various chromatographic techniques to obtain pure compounds, as well as determining the structure of these compounds with the help of several spectroscopic methods, including 1D NMR, 2D NMR, HR-MS, UV-Vis, and IR. Study of the biological activities mentioned in this study of the separated compounds is the most important step to find and determine the responsible molecules for the antioxidant, anti-inflammatory, and analgesic activities.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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