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HPLC–DAD phytochemical profile, antioxidant, anti–inflammatory and analgesic properties of *Sanguisorba minor* L.: An *in vitro* and *in vivo* assessment

Perfil fitoquímico HPLC–DAD, propiedades antioxidantes, antiinflamatorias y analgésicas de *Sanguisorba minor* L.: Una evaluación *in vitro* e *in vivo*

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ABSTRACT

Sanguisorba minor L. is a plant species of the Rosaceae family to treat several illnesses. The purpose of this investigation is to verify the amount of flavonoids and polyphenols in the extract of the plant *S. minor* and to evaluate its antioxidant, anti–inflammatory and analgesic activities. Phytochemical profile of this plant extract was determined by HPLC–DAD. This latter was used to evaluate polyphenols in the ethanolic extract of *S. minor,* in addition to the previous study, an anti–inflammatory effectiveness of the extract was evaluated *in vitro* using the bovine serum albumin (BSA) denaturation test. The paw edema induced by carrageenan model and Xylene induced edematous foot model were used to evaluate the *in vivo* anti–inflammatory efficacy. Finally, the extract's analgesic qualities were evaluated using the acetic acid–induced writhing test. The ethanolic extract exhibited a high content of total phenols (623.33 ± 0.01 µg EAG·mg^{-1} extract), but a relatively low concentration of flavonoids (8.71 ± 0.04 µg·mg^{-1} extract). The ethanolic extract's IC_{50} value were 11.00 ± 0.00 µg·mg^{-1} of DPPH radical showed that it was a significant antioxidant; 3 ± 0.00 µg·mg^{-1} for OH radical scavenging ; and 7 ± 0.00 µg·mg^{-1} for FRAP assay. Methyl gallate and other chemicals, including gallic acid, rutin, syringic acid, ferulic acid, caffeic acid were identified by the HPLC–DAD analysis, suggesting that the sample contained a high concentration of phenolics. The extract's powerful ability to scavenge and reduce radicals indicated that it exhibited high antioxidant capacity. At 150 and 300 mg·kg^{-1} the ethanolic extract of the *S. minor* showed notable anti–inflammatory action with inhibition percentages of 87.22 ± 0.38%, and 57.65 ± 0.42, respectively. At a concentration of 150 mg·kg^{-1}, the ethanol extract exhibited the greatest analgesic activity (84.30%), showing a more pronounced inhibitory effect on abdominal cramps compared to the 300 mg·kg^{-1} concentration. The extract of the plant of *S. minor* is rich in polyphenols, demonstrating significant antioxidant, anti–inflammatory, and analgesic properties.

Key words: *Sanguisorba minor* L.; HPLC–DAD; antioxidant activity; anti–inflammatory activity, analgesic test

RESUMEN

Sanguisorba minor L., una especie vegetal de la familia de las rosáceas, se utiliza para el tratamiento de diversas enfermedades. El objetivo de esta investigación es determinar mediante HPLC–DAD, el perfil fitoquímico del extracto de *S. minor* y evaluar sus propiedades antioxidantes, antiinflamatorias y analgésicas La eficacia antiinflamatoria del extracto se evaluó *in vitro* mediante la prueba de desnaturalización de la albúmina sérica bovina (BSA). El modelo de edema de pata inducido por carragenina y el modelo de pie edematoso inducido por xileno, se utilizaron para evaluar la eficacia antiinflamatoria *in vivo.* Finalmente, se evaluaron las propiedades analgésicas del extracto mediante la prueba de retorcimiento inducido por ácido acético. El extracto etanólico exhibió un alto contenido de fenoles totales (623,33 ± 0,01µg EAG·mg^{-1} extracto), pero una concentración relativamente baja de flavonoides (8,71 ± 0,04 µg·mg^{-1} extracto). Los valores de CI_{50} de los extractos etanólicos fueron 11,00 ± 0,00 µg·mg^{-1} de radical DPPH mostraron que era un antioxidante significativo; 3 ± 0,00 µg·mg^{-1} para la eliminación de radicales OH; y 7 ± 0,00 µg·mg^{-1} para el ensayo FRAP. El metil galato y otros químicos, incluyendo ácido gálico, rutina, ácido siríngico, ácido ferúlico y ácido cafeico fueron identificados por el análisis HPLC–DAD, sugiriendo que la muestra contenía una alta concentración de compuestos fenólicos . La potente capacidad del extracto para eliminar y reducir radicales libres indicó que exhibió una alta capacidad antioxidante. A dosis de 150 y 300 mg·kg^{-1}, el extracto etanólico de *S. minor* mostró una notable acción antiinflamatoria, con porcentajes de inhibición de 87,22 ± 0,38 % y del 57,65 ± 0,42%, respectivamente. La concentración de 150 mg·kg^{-1} del extracto etanólico, exhibió mayor acción analgésica (84,30%), observándose mayor efecto inhibidor de los calambres abdominales en comparación con la concentración de 300 mg·kg^{-1}. El extracto de la planta de *S. minor* es rico en polifenoles, lo que demuestra importantes propiedades antioxidantes, antiinflamatorias y analgésicas.

Palabras clave: *Sanguisorba minor*, HPLC–DAD, actividad antioxidante, actividad antiinflamatoria, Parueba analgésica

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INTRODUCTION

The body uses inflammation as a defense against aggression that is chemical, infectious, physical or biological [1]. Because of the pathogen's aggressiveness, persistence, anomalies in the control and generation of inflammatory cells, this defense immunological response can occasionally be detrimental. Numerous diseases in humans, including diabetes, cancer, arthritis, asthma and allergies, are linked to these inflammatory processes [2].

The development and maintenance of inflammation are closely linked to oxidative stress, which can lead to beginning and/or maintenance of variety diseases. Oxidative stress results from an imbalance in redox homeostasis. It results in excessive formation and/or poor elimination of free radicals, which are extremely reactive nitrogen or oxygen molecules, which are highly reactive molecules of either oxygen or nitrogen [3].

This imbalance causes oxidative damage to the cell's different molecules (nucleic acids, proteins, and lipids), resulting in necrosis or apoptosis, which kills cells. Aging and the pathogenesis of numerous illnesses, including heart diseases, cancer and inflammatory diseases, are both influenced by oxidative stress [4]. Using non –steroidal anti–inflammatory drugs can have a number of harmful side effects, including kidney, asthma and gastrointestinal problems [5].

However, teratogenic, mutagenic and carcinogenic effects [6] accompany prolonged usage of artificial antioxidants such as butylated hydroxytoluene in the agri–food, cosmetics and pharmaceutical sectors. To stop these oxidative and inflammatory diseases from developing, it is vital to find novel therapeutic medicines with minimal adverse effects. Plants represent an inexhaustible reservoir of secondary metabolites. These plants are used in traditional medicine without any precise scientific rules. In order to do this, research is concentrated on increasing the usefulness of traditional medicine, confirming the effectiveness of the plants utilized, and developing scientific guidelines for their various applications. The edible perennial herb *Sanguisorba minor*, commonly known as salad, has reddish–green flowers and pinnate leaves [7].

In Asia, North Africa and Europe, *S. minor* is a common herbaceous plant that belongs to the Rosaceae family. It is usually found in semi–dry and dry meadows in Mediterranean areas. This plant is used as culinary additive in fruit juices, cheese, ice drinks and a number of traditional foods [8, 9, 10]. It is known, this plant is widely used in certain illnesses like fever, diarrhea, conjunctivitis, duodenal ulcers, intestinal infections, bleeding, and burns [9, 10, 11, 12].

Plants in the sanguisorba genus [13, 14, 15] expressed numerous pharmacological properties, including hemostatic, antibacterial, anti–inflammatory, antioxidant, hypoglycemic, neuroprotective, anti–alzheimer, and anticancer activities. This is the background to this research, determinate by HPLC–DAD, the phytochemical profile, and antioxidant, anti–inflammatory, and analgesic properties of ethanolic extract of *Sanguisorba minor*.

MATERIALS AND METHODS

Plant materials

In March 2023, aerial portions of *S. minor* were gathered for the Guenzet Setif Mountains (Northern Algeria). Pr. Laouer Hocine of the University Ferhat Abbas Setif 1, El Bez, Algeria's Department of Biology and Vegetal Ecology's Laboratory of Natural Resources Valorization confirmed the species identification. The laboratory received a voucher specimen with the number SNV/0035/2024. For two weeks, the collected samples were allowed to dry outdoors.

Preparation of extract

According to the procedure, outlined by [16] about 50 g of powdered aerial parts of *S. minor* were extracted using pure ethanol (100%). A first filtrate was realized using Wathman number 1 paper, the same maceration was repeated for four weeks, at the end evaporated with a rotavap (Buchi rotavap R-205 Switzeland) at a temperature of 45°C, and subsequently dried at 40°C to obtain *S. minor* crude extract (SMCE).

In vitro studies

Phytochemical analysis, total flavonoids and phenolics contents

The amount of polyphenols was measured using the colorimetric Follin–Ciocateau technique (Shimadzu UV1800 spectophotometer, Japan). About 500 µL of ten–fold diluted Folin–Ciocalteu reagent was combined with 100 µL of either the ethanolic extract or the standard which is gallic acid solution from (Sigma Aldrich), with concentration ranging from 20 $\mu g \cdot mL^{-1}$ to 160 $\mu g \cdot mL^{-1}$. Furthermore, 400 µL of a sodium carbonate (Na_2CO_3) solution containing 75 $g \cdot mL^{-1}$ was added 4 min later. After the reaction mixture was incubated for one hour (h) and 30 min in the stove at 28°C (Memmert UM200, Germany) the absorbance at 765 nm was measured (Shimadzu UV1800 spectrophotometer,Germany).

The findings were presented as equivalent to micrograms of gallic acid per milligrams of dry extract (µg GAE·mg^{-1} DE). In addition, the extract's total flavonoids concentration was ascertained using the aluminum chloride ($AlCl_3$). About 500 µL of aluminum chloride solution (2%) was combined with an aliquot of 500 µL of either the quercetin standard (from Sigma Aldrich) with concentrations ranging from 5 to 50 $\mu g \cdot mL^{-1}$ or diluted extract.

The absorbance at 430 nm was determined after 10 min incubation at room temperature (Shimadzu UV1800 spectrophotometer, Germany) [17]. Utilizing the quercetin curve calibration, the concentrations of flavonoids was determined as equivalent to micrograms of quercetin per milligrams of dry extract (µgQE·mg^{-1} DE).

HPLC conditions

The HPLC (Agilent 1260,USA) was carried out using an Agilent 1260 series ; a Zorbax eclipse plus C8 column (4.6 mm×250 mm i.d.,5 µm) was utilized. The mobile phase consisted of water (A) and 0.05 % tricloroacetic acid in acetonitrile (B) at a flow rate of 0.9 $mL \cdot min^{-1}$ comprising the mobile phase. The mobile phase was coded in a linear gradient as follows: 0-1min (82% A); 1-11 min (75%A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min

(82% A). The multi wavelength detector was spotted at 280nm. Each sample's injection volume is fixed at 5 µL. The differents standards compounds utilized in the HPLC–DAD is: gallic acid, chlorogenic acid, caffeic acid, methyl gallate , syringic acid, rutin, ellagic acid, coumaric acid, ferulic acid, naringenin, rosmarinic acid, daidzein, querctin, kaempferol, hesperetin, catechin and vanillin.

Antioxidants activity

DPPH radical

The antioxidant capacity of the extract was assessed using the 2,2–diphenyl–1–pycrilhydrazil (DPPH) radical experiment. It is a stable free radical that is reduced by antioxidant molecules, by acceptation of an electron or hydrogen. In its original radical state, DPPH exhibits a rich purple hue, transitioning to yellow in its reduced form. About 50 µL of different concentrations of SMCE (0.05 to 1 mg·mL^{-1}) was combined with 1.25 mL of a methanolic mixture of 2,2–diphenyl–1–pycrilhydrazil. Following 30 min the resultants mixture was measured at an absorbance 517 nm [18]. The % inhibition of DPPH free radical is calculated using the formula below:

$$Radical\ \ scavenging\ \ capacity = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Reducing power assay

The technique described was used gauge the action of the decreasing power [19]. The development of this technique aimed to assess the ability of extract to reduce the ferric ion (Fe^{3+}) found in potassium ferricyanide $K_3[Fe.(CN)_6]$ complex (red color) in ferrous ion (Fe^{2+}) which turns blue or green, the color change is proportional to the antioxidant activity. The mixture contain 50 µL of potassium ferrocyanide 10%, 40 µL of sodium phosphate buffer (0.2M, pH:6.6) and 10 µL of various concentrations (0.005 to 1 mg·mL^{-1}) of the SMCE, following a 20 min incubation period at 50°C in a water bath (Memmert WNB22, Germany). Thereafter, 10% of TCA (tricloroacetic acid) was added. Lastly, a volume of 10 µL of ferric chloride ($FeCl_3$, 0.1%) and 40 µL of the upper layer's H_2O were added to the mixture. At 700 nm, the mixture's was measured. The concentration providing 0.5 absorbance was deduced from a linear regression curve of each extract and standard.

Iron–chelating assay

The method used to evaluate the ferrous ion chelating ability of the SMCE is based on the measurement of the pink chromophore resulting from ferrous ion–ferrozine complex formation [20]. A volume of 40 µL of SMCE or Ethylenediaminetetra acetic acid (EDTA) as standard was combined with the same volume 40 µL of methanol and $FeCl_2$ (0.02%). After five minutes 0.08 mL of ferrozine (0.5 mM) was added to beginning the complexion reaction which proceeded for 10 min at room temperature. The absorbance of the complex Fe^{+2}-ferrozine was registred at 562 nm. The following formula was used to quantify the chelating capacity as an inhibition percentage:

$$Chelation\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Phosphomolybdate test

The reduction of Mo (VI) to Mo (V) is the basis of the phosphomolybdenum technique, it used to assesses the antioxidant activity of SMCE by spectrophotometries (Shimadzu UV1800 spectrophotometer, Germany) [21]. This method is quantitative to determine the antioxidant capacity of the extract by the reduction of molybdate ions, and the formation of a phosphate / Mo(V) complex followed by the degradation of the green color. At different concentrations of ethanolic extract and standard as ascorbic acid with various concentration (0.01 to 0.5 mg·mL^{-1}), 100 µL of each concentration was shaken with 1 mL of the reagent solution which contain 0.28 M sodium phosphate, 0.04 M ammonium molybdate and 0.6 M sulfuric acid. The mixtures were incubated to 95°C in a water bath for 90 min. At 765 nm, the sample and standard absorbance was measured.

The radical hydroxyl scavenging

Following the method outlined, the radical hydroxyl scavenging was evaluated in the SMCE by generating hydroxyl radicals in a Fenton reaction [22]. A solution containing 1 mL of 0.2 M of sodium salicylate, 1 mL of 0.15 M of $FeSO_4$, 0.7 mL of 0.6 M of hydrogen peroxide was added in each different concentration of SMCE. After 1 h of incubation at 37°C in the stove (Memmert UM200, Germany), the absorbance was measured at 562 nm. The % inhibition of the OH scavenging capacity was calculated using this formula:

$$Inhibition\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

In vitro anti–inflammatory activity

The anti–inflammatory effect was assessed [23] with minor adjustments. 0.1 mL of aspirin and SMCE at various concentrations (0.025 to 0.5 mg·mL^{-1}) were combined with 1 mL of BSA (bovine serum albumin 0.2%) solution prepared in Tris–HCl (pH 6.6). The mixture was incubated for 15 min at 37°C, and later was heated at 72°C in water bath for 5 min. After cooling , the supernatant was aspirated and the absorbance was read at 660 nm .

The *in vivo* studies

Animals

Mice (*Mus musculus*) and rats (*Rattus norvegicus*) aged two months and weighing (PS600.R2, Germany) between (25–30 and 150–200 g respectively) were given from the Institute Pasteur in Algiers. Before the research started, they were acclimated in an animal facility for two weeks under typical lab settings (25 ± 2°C, 12–hour dark/light cycle). The animals had access to tap water and were fed a typical commercial meal.

In vivo anti–inflammatory investigation

A rat model of carrageenan–induced paw edema was used to evaluate the inflammation inhibition properties of SMCE [24]. In this research, 24 female rats split up into four equal groups: the 1st group was used as the positive control and was given 20 mg·kg^{-1} of the reference medication indomethacin. The 2nd group was used as the negative control and was given tap water. The test groups receiving (150–300 mg·kg^{-1}) of SMCE are represented by the 3rd and 4th groups. In this research, animals had been fasting for 16 hours.

Xylene–induced edematous

The ear edema induced by Xylene method was used to assess topical anti–inflammatory properties. About 18 female mice split up into three equal groups. All three groups received topical administration. The 1st group received 0.5 mL distilled water as the negative control, the 2sd group received the 0.5 mg indomethacin as the positive control and the 3rd group received 2 mg of the ethanolic extract. Edema was induced in all mice by applying 0.03 mL of pure Xylene per ear. The same ear area was then treated with the same volume (0.03 mL) of indomethacin and SMCE, and then applied to the same ear surface, while the control group was given only Xylene.

In the oral anti–inflammatory activity method [25], the 24 female mice were split up into four groups, each of which had 6 mice. The group 1 received distilled water as considered negative control, the group 2 received the positive control (Indometacin, 50 mg) and the 3 and 4 groups received the 2 doses of SMCE (150–300 mg·kg^{-1}). After 1 h of treatment with positive and negative controls with both doses of SMCE, we applied 30 µL of Xylene to induce edema in all mice. After 1 h , 1 h:30 min and 2 h a digital caliper was used to measure the thickness of the ear for all groups, utilizing this formula:

$$I\,(\%) = \frac{(\Delta T - \Delta E)}{\Delta T} \times 100$$

ΔT represents a difference in ear edema thickness in the negative control, ΔE corresponds to the difference in the positive control or SMCE.

Evaluation of analgesic activity

The analgesic efficacy of the SMCE was assessed using the method outlined by [26] against pain induced by acetic acid. For this test, all groups received ethanolic extract and the standard orally: the positive control was given aspirin at concentration of 100 mg·kg^{-1}, the negative control was given distilled water, and both the 150-300 mg·kg^{-1} of SMCE were given as a single dose to the experimental group. After one hour, 10 mL·kg^{-1} of 0.6 % acetic acid was injected intraperitoneal to each mouse . In a cage take each mouse individually and count the number of twitches from time 0 to 30 min at 5 min intervals. The following formula was used to determine the pain inhibition percentage:

$$I\,(\%) = \frac{(C_{nc} - C_{tr})}{C_{nc}} \times 100$$

C_{nc} noted the negative control group's average and C_{tr} noted the mean number of twitches among groups that received different doses of aspirin and SMCE.

Statistical analysis

The results from the *in vitro* experiments were displayed as mean ± standard error of the mean, while the data from the *in vivo* trials were displayed as mean ± standard. The GraphPad Prism version 6 was utilized to assess the results using Dunnett's test and One–Way analysis.

RESULTS AND DISCUSSION

Total flavonoids and phenolics contents

Since ancient times, humans have utilized medicinal plants to treat various ailments, including inflammatory conditions. These plant's complex combinations of secondary metabolites, such as flavonoids and phenolic chemicals, are frequently responsible for these positive benefits [27]. A key challenge for scientists lies in identifying the specific chemical compounds responsible for the observed pharmacological activities [28]. In this investigation the quantitative analysis revealed that the ethanolic extract of the plant *S. minor* had high levels of total phenolics compounds, 623.33 ± 0.01 µgGAE·mg^{-1} extract and the total flavonoids present in the SMCE were 8.71 ± 0.04 µgQE·mg^{-1} of extract, which may justify about diversity in phenolic compounds characterized in the HPLC–DAD profile.

HPLC–DAD phytochemical analysis

HPLC–DAD phytochemical analysis showed that the ethanolic extract contains 17 different phenolic compounds (TABLE I and FIG. 1). The major compound present in the SMCE is methyl gallate with a concentration of 24574.54 µg·g^{-1}, followed by gallic acid 16778.74 µg·g^{-1}

Following HPLC–DAD, it is found the existence of seventeen phenolic compounds in *S. minor* aerial part amongst, the phenolic acids such as methyl gallate as a major compound, gallic acid, syringic acid, caffeic acid, ellagic acid, chlorogenic acid, ferulic acid and rutin.

TABLE I
HPLC–DAD phytochemical analysis of *Sanguisorba minor* ethanolic extract

	Area	Conc (µg·mL^{-1})	Conc (µg·g^{-1})
Gallic acid	5071.57	335.57	16778.74
Chlorogenic acid	2442.18	280.38	14018.97
Catechin	/	/	/
Methyl gallate	11049.80	491.49	24574.54
Caffeic acid	633.98	42.61	2130.44
Syringic acid	981.07	55.81	2790.34
Rutin	889.86	121.10	6055.03
Ellagic acid	272.96	18.79	939.44
Coumaric acid	17.68	0.52	25.94
Vanillin	78.37	2.46	122.98
Ferulic acid	1549.91	74.20	3709.97
Naringenin	44.55	3.46	172.86
Rosmarinic acid	14.12	1.25	62.37
Daidzein	5.28	0.28	13.96
Quercetin	11.70	0.78	39.11
Cinnamic acid	3.62	0.06	2.77
Kaempferol	15.89	0.94	46.84
Hesperetin	5.41	0.22	11.04

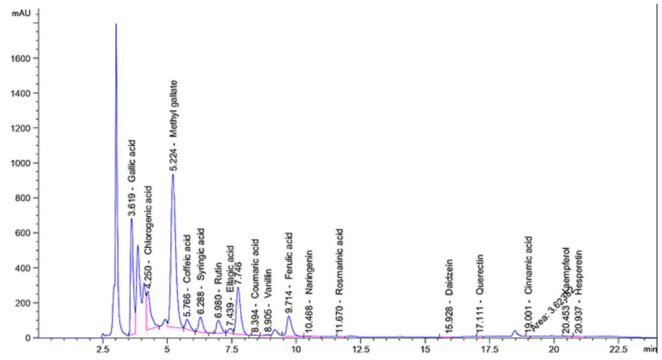

FIGURE 1. Chromatogram of *Sanguisorba minor* aerial part extract

Evaluation of antioxidant activity

As there is a multitude of factors that may influence the antioxidant activity of an extract, it is important to test more than one method to assess the extract antioxidant potential, to consider the different antioxidant pathways involved.

In this study antioxidant properties were assessed using several techniques, including 2,2–diphenyl–1–pycrilhydrazil (DPPH) assay, iron–chelating test, phosphomolybdate assay, and OH assay. Results shown in TABLE II were expressed as inhibition concentration IC_{50} and as $A_{0.50}$ for ferric reducing antioxidant power expressing the concentration that records an absorbance equal to 0.5.

Given the fact that the extract is rich in terms of phenolic compounds, its antioxidant ability was investigated in vitro as a first step towards understanding the eventual antioxidant, anti–inflammatory relationship of the plant. The findings showed a significant antioxidant activity with IC_{50} values varying in an interval from 11 ± 0.00 to 71 ± 0.00 µg·mL^{-1}, in different in vitro systems, including the DPPH assay, Iron–chelating activity, phosphomolybdate assay, OH assay and reducing power assay. This high antioxidant activity may be due to the existence of significant amounts of powerful phenolics compounds in the SMCE. Indeed, methyl gallate is a polyphenol naturally occurring secondary metabolite in many medicinal herbs and pharmacologically bioactive as a potent antioxidant compound [29] was the major compound in the extract. Another antioxidant phenolic acid characterized in the extract is chlorogenic acids (CGA), are common caffeoylquinic acid isomers (3–, 4–, and 5–CQA) that are well known because of its powerful antioxidant and capacity to scavenge radicals [30]. Regarding the phytochemical profile of the *S. minor* plant in previous study [31] they found fewer phenolic compounds than the results obtained in this new study ,which may be due to changes in climates or soil.

In vitro anti–inflammatory activity

The in vitro inhibition activity of SMCE was assessed using the bovine serum albumin product; the findings are shown in FIG. 2. At a concentration of 500 µg·mL^{-1} SMCE could prevent BSA denaturation with a maximum inhibition rate of 81.11%.

At 500 µg·mL^{-1}, acetylsalicylic acid is an nonsteroidal anti–inflammatory drug (NSAID), widely available and affordable globally, making it an excellent standard for comparison in experimental studies. It was utilized as positive control, reduced inflammation by 76.04%. The SMCE demonstrated a strong inhibitory effect of BSA denaturation.

The results showed that the SMCE might decrease bovine serum albumin denaturation in a dose–dependent manner. The highly nominated bioactive compounds extracted from *S. minor*, phenols and flavonoids have a great anti–denaturant activity. This activity observed by SMCE was highly to ferulic acid, caffeic acid, vanillin binding ability to humain serum albumin [32]. However, Liu *et al.* [33] showed that rutin and quercetin have a binding affinity towards bovine serum albumin.

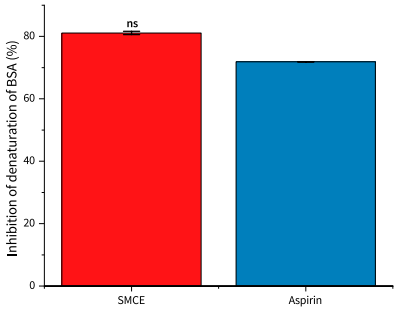

FIGURE 2. The *in vitro* inflammation inhibition activity of ethanolic extract of *Sanguisorba minor*. The data is shown as mean± standard deviation (n=3). ns: no significant difference

TABLE II
In vitro* antioxidant activity of ethanolic extract of *Sanguisorba minor

Extract / standard	*In vitro* antioxidant estimation				
	DPPH assay	Iron–chelating assay	Phosphomolybdate	OH assay	FRAP assay
	IC_{50} (µg·mL^{-1})				
SMCE	11.03 ± 0.00	23.77 ± 0.00	46.83 ± 0.00	63.93 ± 0.00	71.82 ± 0.00
BHT	8.81 ± 0.00	NE	NE	NE	16.67 ± 0.00
EDTA	NE	84.50 ± 0.00	NE	NE	NE
Ascorbic acid	NE	NE	21.20 ± 0.00	66.55 ± 0.00	NE

IC_{50}: sample concentration that inhibited 50% of the activity of free radicals, SMCE: ethanolic extract of *Sanguisorba minor,* BHT: butylated hydroxytoluene, EDTA: ethylene diamine tetra acetic, NE: not evaluated. Values are the mean of triplicates ± SD

In vivo anti–inflammatory activity

According to present research, SMCE significantly reduces inflammation at 150 mg·kg^{-1} in both carrageenan paw edema (TABLE III) and Xylene induced ear edema (FIG. 3). According to the data SMCE significantly and dose–dependently decreased the ear edema reaction caused by Xylene. The results showed that SMCE, at 150 mg·kg^{-1} decreased the carrageenan–induced paw edema impact by 87.23%.

The *in vivo* anti–inflammatory effectiveness of SMCE was investigated using two well–established models: ear edema induced by Xylene and paw edema induced by carrageenan [34]. During the first hour histamine and 5–hydroxytryptamine mediate the rat paw edema brought on by carrageenan, followed by sustained vascular permeability due to kinin release in the subsequent hour. Leukocyte migration into the inflamed site between 2 and 4 h is accompanied by the release of prostaglandins, which are believed to be the primary mediators during this phase. Prostaglandins are essential in fostering inflammation by inducing the formation of inflammatory exudates. The findings demonstrate that SMCE exhibited significant anti–inflammatory effects during the prostaglandin–mediated phase in both models, comparable to indomethacin.

Phlogistic agents that cause ear edema for example Xylene are susceptible to cyclooxygenase inhibitors. This model is commonly used to assess the efficacy of NSAIDs, which exert their anti–inflammatory effects by inhibiting cyclooxygenase and prostaglandin synthesis [35, 36]. Recently [37] summarized the multiple therapeutic functions of gallic acid using both in vivo and in vitro systems. Human cells are protected from the acute and chronic oxidative stress by gallic acid, which is known for its potent antioxidant and free radical scavenging properties , and is an excellent option as a therapeutic agent or dietary supplements [38, 39]. By neutralizing superoxide anion, preventing myeloperoxidase release and activity, and maybe influencing the accumulation of active NADH oxidase, gallic acid may delay the development of inflammation [40]. According to certain research gallic acid efficiently inhibits the synthesis of PGG–2, LPS–induced NO and interleukin–6(IL–6) release without having any harmful effects [41, 42].

TABLE III
Effect of ethanolic extract of *Sanguisorba minor* at different doses with indomethacin as positive control on paw edema induced by carrageenan

Groups	Inhibition (%) of inflammation			
	1h	2h	3h	4h
G	64.29	32.56	33.33	65.48
D1 G	24.52***	47.09**	65.7***	87.23***
D2 G	23.96***	38.95*	56.13***	57.65*

The results are expressed using the average of 6 rats ± SEM: ***P<0.001, **P<0.01, *P>0.05 compare with PG: positive group; D1 G: 150 mg·kg^{-1}; D2 G: 300 mg·kg^{-1}

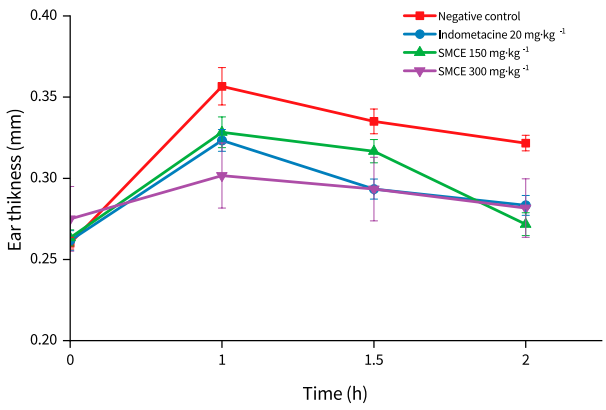

FIGURE 3. Investigation of anti–inflammatory properties by gavage of SMCE with ear edema induced by Xylene in albino mouse (n=6). SMCE was highly active P<0.05

Analgesic activity

The ethanolic extract of *Sanguisorba minor,* demonstrated a considerable dose–dependent analgesic capability against acetic acid induced abdominal construction in mice. The extract significantly (P<0.05) decreased abdominal constriction by 84.30% at 150 mg·kg^{-1} compared with acetylsalicylic acid as the control positive with value of 81.02% (FIG. 4).

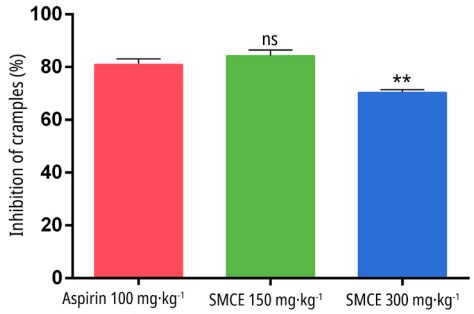

FIGURE 4. Effects of SMCE extract of *Sanguisorba minor* on mice (n=6) peripheral nociception. Mean ± SEM is used to express the results. ns: no significant difference and P<0.01 when compared with acetylsalicylic acid as standard

During the experiment the mice's acetic acid–induced abdominal contractions were considerably (P<0.001) and dose–dependently decreased by SMCE. These results suggests that the SMCE anti nociceptive action either directly suppresses the receptors for inflammatory chemicals or stops their endogenous manufacture. Swiss albino mice were used in a hot plate test to evaluate the analgesic impact of rutin, and the results confirmed that it had an analgesic effect [43]. Additionally it was verified that rutin has both central and peripheral antinociceptive properties, in Peripheral Nervous System, by reducing prostaglandin effects released in response to inflammation and in central nervous system, by directly stimulating opioid receptors at various CNS levels [44, 45]. These findings show that *S. minor* has potential as a source of anti–inflammatory chemicals and merit more research.

CONCLUSIONS

In present study, based on the results, the aerial part of *Sanguisorba minor* L. have proven to have antioxidant and anti–inflammatory activity. The antioxidant properties of the extract were demonstrated by different techniques such as DPPH, total antioxidant capacity, hydroxyl radical scavenging assay, ferrous ion chelating activity and ferric reducing antioxidant power. The extract showed a strong capacity to scavenge free radicals due to its high content of phenolic compounds. The traditional use of the plant *S. minor* to treat inflammatory diseases is proven by the results obtained in this study. This study brings attention to the beneficial substances found in *S. minor* plant, which possess antioxidant, anti–inflammatory, and analgesic activities. These compounds make *S. minor* a potential candidate for inclusion in functional food or pharmaceutical industries.

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