



Production of secondary metabolites in callus cultures of *Scutellaria baicalensis* L. and assessment of their anti-inflammatory and antioxidant efficacy in ulcerative colitis rats

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Abstract

Baikal skullcap or Chinese (*Scutellaria baicalensis* L.) is an interesting plant with promising medicinal properties; however, traditional cultivation methods are time-consuming, and yield variations can be significant; callus culture is considered one of the solutions to overcome these limitations because the callus culture provides an effective, alternative for the consistent production of secondary metabolites. For callus production of *S. baicalensis* L., the in vitro germinating seedlings were cultured on MS medium containing 1.0 mg/L 6-benzyladenine (BAP) and 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Three culture lines were established, and the best growth index represented in fresh and dry weight was obtained from line No. 1. *S. baicalensis* L. callus extract was performed on the best callus line in the stationary phase for in vitro assays. The chemical analysis, antioxidant tests, proline, flavonoids, phenolics, and macronutrient content were assessed. Therefore, this paper aims to evaluate the effectiveness of secondary metabolites in *S. baicalensis* L. callus and to study its biological effect on recurrent ulcerative colitis (UC). Conventional treatment of UC has focused on suppressing immunological responses instead of addressing which are (UC) underlying causes. Recurrent UC is caused by oxidative stress and inflammation that lead to chronic inflammation of the inner lining of the colon and rectum. According to the findings, secondary metabolites in *S. baicalensis* L. callus cultures increased antioxidant activity. This improvement in oxidative activity was positively correlated with the potential to reduce UC in vivo.

Key message

Scutellaria baicalensis callus cultures as plant raw materials have the potential to be used as a source of natural compounds with anti-inflammatory and antioxidant properties in rats with ulcerative colitis.

Keywords *Scutellaria baicalensis* L. · Ulcerative colitis · Inflammatory cytokines · Secondary metabolites · Callus culture · Oxidative stress

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Introduction

Medicinal plants represent a large national income for a limited number of countries, the most important of which are China and India. Those plants used in the manufacture of medicines, perfumes, cosmetics, food, and pesticides represent a real hope to support the economy for those looking for a promising future, especially with the increasing trend the modern world to transform into all that is natural (Milentyeva et al. 2023).

Tibetan medicine uses Baikal skullcap or Chinese skullcap *S. baicalensis* L., sometimes called Chinese skullcap or Baikal, as a significant medicinal herb. 10% of the flavone group's flavonoids are found in the roots, indicating that it is rich in flavonoids (Dinda et al. 2017; Le Violeta and El Amine 2022; Zhao et al. 2022; El Amine 2022). The plant is included in the European Pharmacopoeia, 9th edition (Kawka et al. 2019). A matrix effect and a multicomponent synthesis were shown by both quantitative and qualitative evaluations of *S. baicalensis* constituent components and phyto-preparations based on the instrumental analysis, as stated by Baygildieva et al. (2018) and Shen et al. (2021). As stated by Zhang et al. (2021) and Song et al. (2021), there is potential for using *S. baicalensis* L. extract in treating and rehabilitating respiratory illnesses, including COVID-19. Its extracts may also avoid or suppress premature aging in older people as part of novel medications and functional foods (Muderrisoglu and Yesil-Celiktas 2019).

The antibacterial, anti-inflammatory, anticancer, antioxidant, and immunomodulatory qualities of *S. baicalensis* L. favorably impact human health (Bie et al. 2017; Orzechowska et al. 2020; Milentyeva et al. 2021; Ibrahim et al. 2022). Chronic (UC) is defined by intestinal inflammation, bloody diarrhea, and stomach pain (Walsh et al. 2016). These symptoms substantially lower UC patients' standard of living (Duijvestein et al. 2018). The prevalence of UC is rising annually, increasing the risk of colorectal cancer (Duijvestein et al. 2018). According to Xie et al. (2015); Citi (2018); Mehandru and Colombel (2021), the pathophysiology of UC is multifaceted, including the involvement of oxidative stress, intestinal mucosal injury, inflammation, and microbial imbalance. It is still unclear exactly what causes UC, even though immunological, environmental, and genetic variables are linked to its pathogenesis (Loddo and Romano 2015). Because human UC and experimentally generated colitis in animals are similar, the former has become a vital tool for researching disease pathomechanism and testing new drugs for antiulcerative properties (Owusu et al. 2020).

Human inflammatory bowel disease (IBD) and acetic acid-induced UC may have different etiologies, but they

both have similar pathophysiological traits and are drug-sensitive. According to Abdelmonaem et al. (2021), this is a recognized paradigm for examining (IBD). For example, colonic abnormalities that arise in rats after intrarectal injection of acetic acid AA, including mucosal inflammation, ulceration, bleeding, and weight loss, are also often seen in humans with (IBD) (Ercan et al. 2020). UC's pathophysiological process includes both inflammation and oxidative stress as key elements (Wang et al. 2019). Free oxygen radicals are considered a causative factor in (IBD) because they cause mucosal damage pathogenesis and initiate the process of apoptosis (Cagin et al. 2016). Sulfasalazine comprises 5-aminosalicylate (5-ASA) and is bound to sulfapyridine, which colonic bacteria break down into sulfapyridine and 5-ASA moieties. According to Lu and Zhao (2020), sulfapyridine is anticipated to induce adverse effects, while the 5-ASA moiety is considered the therapeutic agent in treating UC. However, administering natural ingredients may be beneficial and a potential alternative for novel UC therapy since certain negative consequences are associated with current medications as a treatment (Ali et al. 2017; Pastrelo et al. 2017; Tian et al. 2017).

There is a global trend toward utilizing natural plant resources to produce secondary metabolites; nevertheless, callus and cell culture systems represent a potential renewable source of valuable medicinal compounds, flavors, fragrances, and colorants, which cannot be produced by microbial cells or chemical synthesis (Sharma and Shahzad 2013). Secondary metabolite extraction from fresh plant tissues still has problems such as low metabolite yield, seasonal availability, fast deterioration, and inconsistent product quality (Ram et al. 2011). Callus culture offers a strategy to minimize all these problems for the continuous production of potentially valuable compounds for human use. Moreover, callus culture could be an effective substitute for producing secondary metabolites (Debnath and Goyal 2020). In light of this, this is the first study to use a contemporary biotechnological technique to produce secondary metabolites from *S. baicalensis* L. callus culture, assess their effectiveness in treating acetic acid-induced colitis, and look into changes in a few oxidative stress and inflammatory markers that happen during UC.

Materials and methods

Materials

Plant seeds of S. baicalensis L. were obtained from the Shanxi Province (Northwest China). Acetic acid was purchased from El-Nasr Pharmaceutical Co. (Egypt). Corn starch, sugar, and unsaturated fat were purchased at a

nearby store and added to the rats' diet. The quantitative measurements were conducted using spectrophotometry kits acquired from the Biodiagnostic Company (Egypt). Sunlong Biotech in China provided the ELISA kits. Further chemical substances were brought by Merck Chemicals (Darmstadt, Germany).

Methods

Induction of callus cultures

S. baicalensis L. seeds were sterilized with 1.0 NaOCl (Clorox containing 5.25% sodium hypochlorite) for 20 min and cultured on MS medium (Murashige and Skoog 1962) free of PGRs containing 3% (w/v) sucrose and 0.01% (w/v) myo-inositol. The pH was set to 5.7 ± 0.2 before adding 2.7 g/L gelling agent (phytagel), and all jars were then incubated for 16/8 h (light/dark) at 26 ± 2 °C. Results were recorded after one week of culture. Then, the in vitro germinating seedlings have been used as an explant. The explants were cultured on MS media with 1.0 mg/L 2,4-D + 1.0 mg/L BAP. Moreover, incubated under full dark Fig. 1. According to Adil et al. (2018), The following equation recorded fresh callus weights (g/jar) and callus production percentages:

$$\% \text{ callus formation (x)} = \frac{\text{No. of explants forming callus}}{\text{Total cultivated explants}} \times 100$$

Induced callus was subculture on fresh media of the same composition every 25 days three times.



Fig. 1 Effect of MS medium with 1.0 mg/L of BAP and 1.0 mg/L of 2,4-D on callus formation of *S. baicalensis* L. under dark conditions (initiation stage)

Callus line selection and growth curve establishment for biomass accumulation

Screening and selection of the best line from callus cultures can be checked visually by the naked eye through the different growth behaviors of callus cultures after three successive subcultures; this was done based on the external appearance of callus cultures. In the fourth subculture, clones with similar appearances are selected and transferred as calluses to new media with the same hormonal content. Three callus lines were selected to conduct the following experiments, where biomass (fresh weight) will determine which line is optimal. After the seventh subculture, 0.5 g was taken from each line and subcultured on fresh MS medium supplemented with the same composition of the PGRs. Callus biomass was determined for the three cultured lines every 5 days for 35 days to establish the growth curve. The callus was carefully removed from the cultivated jars and placed into another jar with a known weight to calculate the initial and final fresh weight (FW) of the callus (mg). By Kitipongpatana et al. (1998), the Growth Index (I) was computed as follows:

$$\text{Growth index (I)} = \frac{(M_{\max} - M_0)}{M_0}$$

where $M_{\max}$: denoted final weight of callus (mg), M_0 – denoted the initial mass of callus (mg).

The callus was ground into a powder in a mortar and stored at 4 °C for later use after being dried at 50 °C to a constant weight. Because the maximum secondary metabolite content is obtained in the stationary phase tissues (Abou El-dis et al. 2021), the dry callus produced from the best callus line in the stationary phase was utilized to conduct chemical analysis and in vivo investigation.

Extraction Procedure of callus culture of *S. baicalensis* L.

Callus of *S. baicalensis* L. was soaked in water for one hour. Then, it was concentrated to a density of 1.05–1.15 g/cm³ after being boiled at 100 °C. The extract is dried for five hours at 50 °C in a vacuum chamber (Ni et al. 2018; Hanafi et al. 2023).

Macronutrients content quantification of callus culture of *S. baicalensis* L.

Ascorbic acid content (vitamin C) was evaluated by 2,4,6-dichlorophenol indophenol titration method (AOAC 1990). Macro-nutrient contents: For estimating N, P, K, and Ca contents in callus culture of *S. baicalensis* L., samples were dried at 70 °C until a constant weight was reached

and then they were digested by Cottenie et al. (1982) and Suliman and Saleh (2022). Phosphorus concentration was estimated spectrophotometrically at 650 nm according to Worsfold et al. (2016) and Abdelkader et al. (2024). The flame photometer PFP7 was used to analyze the potassium concentration, similar to the technique used by Cottenie et al. (1982). The standard methods of American Public Health Association (APHA 2012) were followed in evaluating Mn, Cu, and Zn (ppm). Nevertheless, ICP (Perkin Elmer Model 4300DV) was used to measure the iron (Fe). The sulfate (SO_4) concentration was measured using the turbidity technique described by Sparks et al. (1996).

Assessment of total phenolics (TP), total flavonoids (TF) contents, and antioxidant activities of callus culture of *S. baicalensis* L.

Total phenolics Following Waterhouse (2002), a spectrophotometer was used to quantify the dry matter's total phenolic content. A Folin–Ciocalteu reagent and 15 ml of a saturated sodium carbonate solution were added after 1 ml of the sample was combined with 70 ml of distilled water to quantify the phenolics, which were separated using 80% ethanol. A spectrophotometer was used to evaluate the mixture's absorbance at 765 nm after it had been incubated for 30 min at room temperature. According to Nouman et al. (2014), gallic acid generated the calibration curve.

Total flavonoids Pai et al. (2015) stated that a 2% AlCl_3 solution in methanol was used to assess the total flavonoid concentration. 1.5 mL of AlCl_3 and 1.5 mL of the diluted extract (1:5) were mixed in methanol. 30 °C was the incubation temperature for the samples for 10 min. A standard of quercetin was used to record the absorbance at 368 nm.

Proline Proline was extracted from the callus culture of *S. baicalensis* L. according to Abdelkader et al. (2024) and evaluated with a Japanese Shimadzu 240 UV/VIS instrument at 520 nm.

Antioxidant assays 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: The free radical scavenging capacity of the extracts was evaluated using DPPH. A standard curve was generated using the Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The data was quantified by measuring the callus culture's Trolox equivalent (mg TE)/gm. By Brand-Williams et al. (1995) and Suliman et al. (2024), the DPPH test was conducted. In order to prepare the stock solution, 24 mg of DPPH were dissolved in 100 mL of methanol and left for 5 min without light. The resulting solution was then kept at –20 °C until it was required. Using a spectrophotometer, the solution was created by combining 10 mL of stock solution with 45 mL of methanol, yielding an absorbance

of 515 nm. Trolox has been demonstrated to have a linear standard curve between 25 and 800 μmol .

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay: ABTS + test was conducted using the approach assessed by Re et al. (1999) and Mohammed et al. (2024a). A concentration of 7 μM of dissolved ABTS was used in distilled water. The ABTS solution was mixed with 2.45 mM potassium sulfate to create the ABTS + radical effect, which was then kept in the dark and at room temperature for 16 h to react. The solution containing ABTS + was infused and then diluted with distilled water to reach an absorbance of 734 nm. It was then allowed to equilibrate at 30 °C. The detector was read with a blank value. The absorbance was measured precisely six minutes after combining 30 μL of extracts with 3 mL of diluted ABTS + solution. After dilution correction, the results were represented as $\mu\text{m TE}/100$ g of dry weight (dw). Three duplicates ($n=3$) of each experiment were performed.

High-performance liquid chromatography analysis (HPLC) of secondary metabolites of *S. baicalensis* L.

For the HPLC analysis, equipment from the Agilent 1260 series was used. A 5 μm thick Zorbax Eclipse Plus C8 column measuring 4.6 mm in diameter by 250 mm in depth was used for the separation procedure. The mobile phase consisted of two solutions: (A) water and (B) a solution of 0.05% trifluoroacetic acid in acetonitrile at a flow rate of 0.9 ml/min. Following is a list of the minutes: 0 min (82% A), 0–1 (82% A), 1–11 (75% A), 11–18 (60% A), 18–22 (82% A), and 22–24 (82% A). At 280 nm, the multiwavelength detector was visible. An injection volume of 5 μL was used for each sample solution. A steady 40 °C temperature was maintained inside the column.

In vivo experiment

Animals Eleven-week-old male Sprague–Dawley rats weighing 200 ± 10 g were provided by the National Care Unit of the National Research Centre (NRC) in Cairo, Egypt. Water and healthy, balanced food were provided to the animals before the experiment. Their adaptation period lasted seven days, accommodating them in plastic cages. The ambient temperature was regulated to 23 °C, and relative humidity was maintained between 40 and 60%. It had no contaminants and a twelve-hour light/dark cycle, which allowed for ideal development and behavior.

The Medical Research Ethical Committee (MREC) authorized the study (Approval No. 077802022). Egyptian legislation, the Helsinki Declaration, the IACUC recommendations, the World Health Organization's (WHO) guidelines for the ethics of scientific research, the UK's Animals (Scientific Procedures) Act of 1986, relevant guidelines, and EU

Directive 2010/63/EU (Publication No. 85-23, revised 1985) were all followed in their care.

Induction of UC Rats were administered 1 mL of AA; 5% intrarectally (IR), equivalent to 5% v/v in 0.9% saline, as a model of UC. 6 cm of the tube was placed into the colon via the rectum. To guarantee uniform exposure and retention and stop intracolonic solution leakage, the Trendelenburg posture was then held for 45 s. Subsequently, the acidic solution was neutralized by flushing the catheter with 0.5 mL of saline (Rehman et al. 2022). This method of inducing colitis was favored in scientific environments due to its simplicity and reproducibility, and the characteristics of the model, which include internal colon inflammation and ulcers as well as external symptoms like diarrhea, weight loss, and rectal bleeding, closely resemble those of UC in humans. The animals had access to water constantly, but their food cages were removed sixteen hours before the induction of colitis. Before the rats were induced into colitis, they were given a mild anesthetic to reduce their discomfort and ensure the colitic agent was evenly disseminated.

Diet formation Following Ezzat et al. (2017), the rats' diet was composed of casein (150 g/kg), unsaturated fat (100 g/kg), sucrose (220 g/kg), maize starch (440 g/kg), cellulose (40 g/kg), salt combination (40 g/kg), and vitamin mixture (10 g/kg). The AIN-93 M diet recommendations by Reeves et al. (1993) were followed in preparing the salt and vitamin mixtures.

Experimental design Forty-two rats were divided into seven groups (n = 6/group). The groups were ordered as follows:

- Normal control group: Rats were given a standard 1 mL of saline orally daily and a balanced diet for 14 days.
- Group (1): Rats received a balanced diet and administered callus cultures of *S. baicalensis* L. (400 mg/kg BW/d) orally for 14 days (Kim and Lee 2019).
- Group (2): Rats received a balanced diet and administered callus cultures of *S. baicalensis* L. (800 mg/kg BW/d) orally for 14 days (Kim and Lee 2019).
- Group (UC): Ulcerated rats received a balanced diet for 14 days and were administered 1 mL of 5% acetic acid (5% v/v, in 0.9% saline) orally on day 14.
- Sulfasalazine group: Ulcerated rats received a balanced diet and were administered sulfasalazine (500 mg/kg/rat) for 14 days. However, on day 14, they were given orally two hours before receiving 1 mL of 5% acetic acid (5% v/v, in 0.9% saline) (Owusu et al. 2020).
- Group (3): Ulcerated rats received a balanced diet and were administered callus cultures of *S. baicalensis* L. (400 mg/kg BW/d) orally for 14 days. However, on day 14, they were given orally two hours before receiving

1 mL of 5% acetic acid (5% v/v, in 0.9% saline) (Kim and Lee 2019).

- Group (4): Ulcerated rats received a balanced diet and were administered callus cultures of *S. baicalensis* L. (800 mg/kg BW/d) orally for 14 days. However, on day 14, they were given orally two hours before receiving 1 mL of 5% acetic acid (5% v/v, in 0.9% saline) (Kim and Lee 2019).

Determination of body weight and food consumption Every protocol group was given the synthetic diet, which supplied each rat with about 20 gm/day. The body weight was determined using a computerized weighing scale to assess their weekly weight fluctuations. Water was supplied every day for twenty-four hours until the trial was over. We also looked for any blockage or leaking in the fluid bottles. Conversely, computerized weighing scales and metabolic cages were used to quantify and compute food consumption.

Blood samples collection The animals were terminated by intramuscular injection of 35 mg/kg of ketamine hydrochloride after being starved for twelve hours after the end of the 14-day trial. Blood was drawn from the posterior vena cava following the animals' weight and sacrifice. The samples were centrifuged using a Sigma Labor Centrifuge GMBH, West Germany, model 2-153360 osterode/Hertz, at 4000 rpm for 15 min before storing at -20°C .

Colon homogenates preparation for tissue analysis Colon samples were taken from all the animals, then immediately transferred and dried, and their length was measured. Subsequently, a 10% homogenate was obtained by homogenizing the colons using a glass-teflon homogenizer in an ice-cold Tris-HCl buffer 0.01 M at a pH of 7.4. The homogenate was centrifuged for 30 min at 10,000 rpm and 4°C to yield a supernatant. To provide enzymes for biochemical and enzyme examination, the liquid component was then stored at -80°C .

Disease activity index (DAI) assessment Following acetic acid's 24-h induction of colitis, the disease activity index (DAI) was assessed (Wang et al. 2019). The severity of colitis was assessed using a scoring system that considered into account the following factors: diarrhea (0, normal; 2, loose stools; 4, watery diarrhea); blood in the feces (0, no bleeding; 2, mild bleeding; 4, severe bleeding); and weight loss (0, $<1\%$; 1, $1-5\%$; 2, $5-10\%$; 3, $10-15\%$; 4 $>15\%$) (Song and Park 2014).

Cytokines quantitation Tumor necrosis factor- α (TNF- α) and interleukins (IL-1, IL-6, IL-10, and IL-1 β) were measured using ELISA kits.

Assessment of lipid peroxidation, oxidative stress markers, enzymatic and non-enzymatic antioxidant levels Malondialdehyde (MDA) concentration was measured according to Satoh (1978) approach, whereas nitric oxide (NO) concentration was measured using an ELISA kit. The present investigation assessed the activity levels of many key enzymes, including glutathione peroxidase (GPx) (Rotruck et al. 1973), glutathione S-transferase (GST) (Habig et al. 1974), glutathione reductase (GR) (Goldberg and Spooner 1983), and superoxide dismutase (SOD) (Kakkar et al. 1984). Following Ellman (1959), reduced glutathione (GSH) was measured.

Statistical analysis

Data was presented as mean $\pm$ SD. The in vitro experiments were conducted in five biological replicates; the in vivo experiment was conducted in six biological replicates. Total phenolics (TP), total flavonoids (TF) contents, antioxidant activities, and macronutrient content were conducted in three replicates. A one-way ANOVA test was used to assess the mean and difference values of the studied groups and treatments, and a Tukey post-hoc test was then performed. Version 22.0 of the SPSS program (Chicago, IL, USA) was used to perform the investigation. Means followed by the same lower-case letter do not differ significantly at $p \leq 0.05$.

Results and discussion

Callus cultures induction and line selection

Seeds survival percentage (70%) was recorded without contamination after 7–10 days of culturing, and all seeds germinated within a month after culturing on MS medium free of PGRs. For three consecutive subcultures, the optimum medium to generate callus from *S. baicalensis* L. was MS medium with 1.0 mg/L of 2,4-D and 1.0 mg/L of BAP in dark conditions. The following studies were carried out on three callus lines Fig. 2. As mentioned by (Naz et al. 2015; Park et al. 2023), the processes by which explants differentiate or dedifferentiate are regulated by the amounts of auxin and cytokinin. BAP was introduced to stimulate cell division and control cell morphogenesis (George 2008; Sofian et al. 2018; Long et al. 2022).

The beneficial effects of 2,4-D in conjunction with BAP are corroborated by Hemmati et al. (2020). Cytokinin can enhance the influence of auxin in callus formation by regulating gene expression, which directly impacts auxin activity in this process. (Rao et al. 2006; Schaller et al. 2015; Park et al. 2023). MS medium containing BAP, even with NAA or 2,4-D, can induce callus formation from *Origanum vulgare* and *Clitoria ternatea* in vitro, and low levels

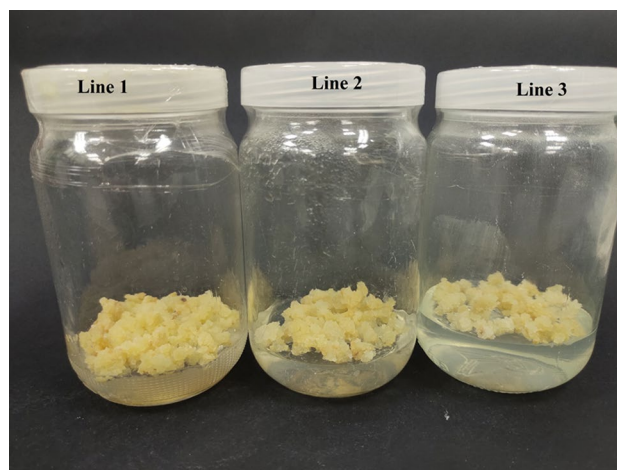


Fig. 2 Three callus lines were selected from third subculture of callus *S. baicalensis* L. growing in dark conditions on MS medium with 1.0 mg/L of BAP and 1.0 mg/L of 2,4-D

of 2,4-D was more effective (Ahlawat et al. 2013; Khatak et al. 2014; Zakaria et al. 2024). Research revealed that callus development and cell division are enhanced when auxins and cytokines are balanced in the culture media (Sagharyan et al. 2020). The exogenous application of 2,4-D controls the activation of auxin gene expression and affects ethylene and protein biosynthesis, as recorded by Tan et al. (2007). Many hormones' interactions with their signaling pathways may control the synthesis of secondary metabolites. It has been demonstrated that the equilibrium between auxins and cytokinins in *Bidens pilosa* callus enhances the accumulation of phenolic compounds (Ramabulana et al. 2021).

Growth curve establishment

One of the key growth characteristics in callus cultures is the growth curve, which can be used to evaluate the optimal time for subculturing by evaluating the growth processes that occur at different stages of the culture (Abbade et al. 2010; Benítez-García et al. 2014). The callus should be subcultured on a new medium every 20–25 days, depending on the studied plant, because the culture medium changes during the deceleration phase; as the nutrient concentration decreases, the waste increases, as Santos et al. (2016) recorded. Furthermore, the stationary phase promotes the formation of secondary metabolites in tissues (Abou El-dis et al. 2021). Figure 3 depicts the sigmoidal growth curve of *S. baicalensis* L. in the seventh subculture, which included lag, exponential, linear, deceleration, and stationary phases. Before the fifth day of the culture, all lines had a lag period, after which the activity of the culture's development increased. This implies that up until the ninth day, all lines persisted

Fig. 3 Callus of *S. baicalensis* L. in the seventh subculture on MS medium with 1.0 mg/L of BAP and 1.0 mg/L of 2,4-D

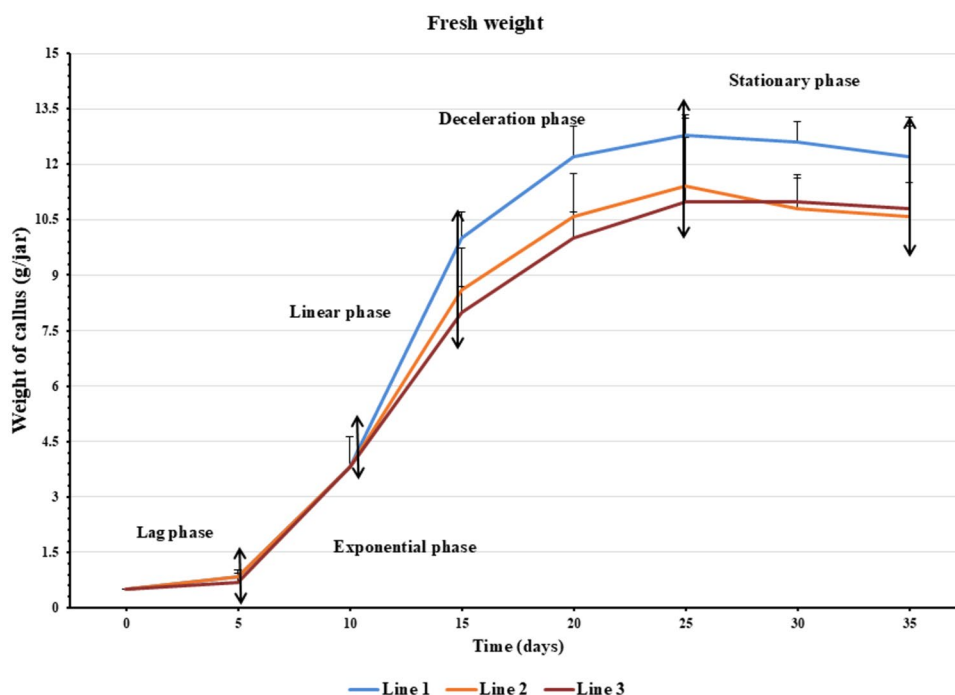


Table 1 Growth index during the stationary phase of different lines of *S. baicalensis* L. callus in the seventh subculture on MS medium with 1.0 mg/L of BAP and 1.0 mg/L of 2,4-D

Indicators	Line numbers		
	1	2	3
Growth index (I) for fresh weight	24.6 ^a ± 0.44	21.8 ^b ± 1.3	21 ^c ± 0.7
Growth index (I) for dry weight	24.7 ^a ± 0.4	21.8 ^b ± 1.3	21.1 ^c ± 0.7

For n = 5, the data show mean ± SD. Using different letters resulted in a statistically significant change ($P \leq 0.05$); however, using comparable letters in each row suggested no meaningful alteration

through the growth transition to the exponential phase. There is a rapid elevation in the growth and multiplication rate in the period from 11 up to 15 days, represented by the linear phase. The gradual deceleration phase, which continued for ten days, extended from the fifteenth day to the twenty-fifth day. A stationary phase with little biomass fluctuation began on the 25th day. By day 25, it was obvious that growth was decreasing; in this stage, there was an improvement in the secondary metabolite biosynthesis in tissues, and this phase established the growth index for choosing the optimal callus line.

Data in Table 1 indicates that Line 1 of *S. baicalensis* L. is the best callus line based on biomass generation in the stationary phase of the three cultured lines in the seventh subculture. Regarding fresh weight, line 1 exhibited the best growth index among the three lines.

Table 2 Content of macronutrients of the callus culture of *S. baicalensis* L.

Macronutrient content	<i>S. baicalensis</i> L.
Vitamin C mg/100 g	26.58 ± 2.29
Total nitrogen (%)	2.41 ± 0.21
Phosphorus (%)	0.48 ± 0.02
Potassium (%)	2.78 ± 0.13
Calcium (%)	0.37 ± 0.04
Fe (ppm)	4.93 ± 0.61
Mn (ppm)	2.89 ± 0.27
Cu (ppm)	1.52 ± 0.11
Zn (ppm)	1.74 ± 0.05

Macronutrients content of callus culture of *S. baicalensis* L.

The results in Table 2 show the percentages of macronutrients present in *S. baicalensis* L., which indicate that the callus culture contains high values of total nitrogen (%), phosphorus (%), potassium (K%), calcium (Ca), iron (ppm), manganese (ppm), copper (ppm), and zinc (ppm). The nutritional elements in callus culture are very essential due to their great importance in the formation of plant cells and their rapid growth (Hazrati et al. 2022). These elements also have an effective and necessary role in the formation of active substances inside the plant in order to complete the vital processes inside the plant cell. From this standpoint, the extent of the connection between these micronutrients

Fig. 4 Secondary metabolites of callus culture *S. baicalensis* L. by high-performance liquid chromatography (HPLC)

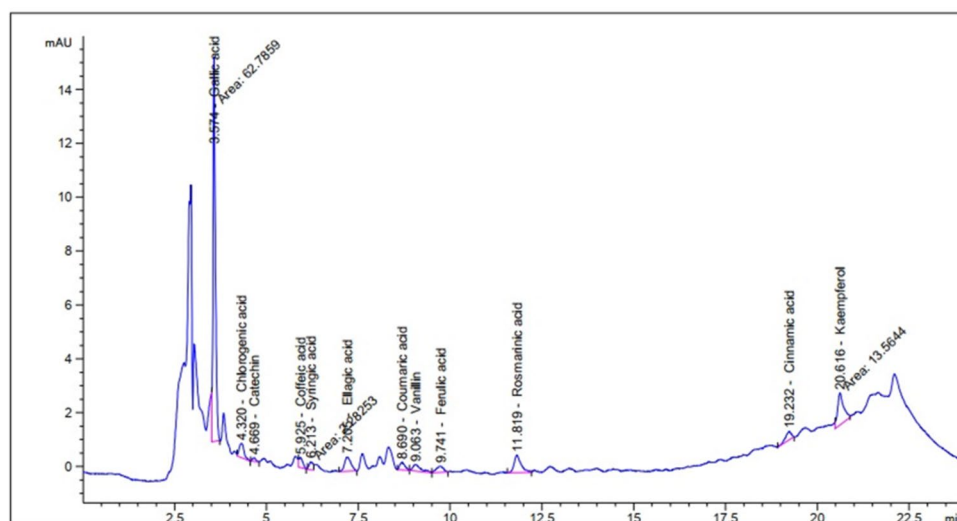


Table 3 Determination of total phenolics, total flavonoids, Proline, and antioxidant assays

Assays	<i>S. baicalensis</i> L.
Total Phenolics (mg/g Dw)	62.92 ± 5.21
Total Flavonoids (mg/g Dw)	2.81 ± 0.41
Proline (mg/100 g FW)	32.37 ± 2.92
DPPH (mg/g Dw)	2.8 ± 0.23
ABTS (mg/g Dw)	1.69 ± 0.16

Values are mean ± SD, (n = 3)

and the construction of active substances inside the plant becomes clear and this was observed in Table 4 and Fig. 4 that provide all the active compounds presented in the callus sample. Moreover, these mineral salts are considered important for the body and general health where their effect reflected in the nutritional parameters of the *in vivo* study (Table 5). Moreover, N and K elements had integral roles of in plant physiology, photosynthesis, sugar metabolism, and nutrient transport. The balance of N and K is critical for effective photosynthetic carbon assimilation and overall plant growth, with imbalances leading to significant physiological repercussions (Shah et al. 2024).

Total phenolics, total flavonoids, proline and antioxidant assays

Among the polyphenols found in fruits and vegetables, flavonoids are the most prevalent. As Mohammed et al. (2024b) stated, flavonoids have high antioxidant capabilities, which may demonstrate their antioxidant activity by chelating metals, scavenging free radicals and reactive oxygen species (ROS), and suppressing the oxidation of low-density lipoproteins (LDLs). There is a potential for using flavonoids

found in *S. baicalensis* L. radix to treat cerebral injuries brought on by free radical attacks and to act as effective free radical scavengers (Chen and Jin 2023). Table 3 Analyzes showed higher antioxidant activity by the DPPH method. Correlation analyses found substantial values in the connection among the phenolic substances under investigation and the reduction in the strength of the extracts. A moderate positive relationship was noticed between the phenolic compound content and antioxidant activity using the DPPH method for the sample. As a result, analyses of *S. baicalensis* L. show that the plant possesses elevated concentrations of total phenolics and flavonoids known for their potent antioxidant qualities and ability to fend off free radical-induced cell deterioration. These findings can be applied to additional research, particularly that about pharmacokinetics. This outcome aligns with studies assessed by Vergun et al. (2019) and Abdoon et al. (2024).

High-performance liquid chromatography analysis of secondary metabolites of callus culture of *S. baicalensis* L.

Table 4 and Fig. 4 provide the quantitative findings of the HPLC profiles. A sample was chosen at random for HPLC analysis. The active chemicals and compounds therein emerged as a result of the investigation. Numerous substances, including gallic acid, kaempferol, rosmarinic acid, ellagic acid, and caffeic acid, are excessively in the callus. Gallic acid (GA), also known as 3,4,5-trihydroxybenzoic acid and chlorogenic acid, is a naturally occurring secondary metabolite that is often isolated from a range of fruits, plants, and nuts (Ozay et al. 2024). Because of its powerful anti-inflammatory qualities, gallic acid has drawn more attention recently. Despite the abundant nature of the gallic acid plant source, its low extraction rate limits its use in

Table 4 High-performance liquid chromatography analysis of secondary metabolites of callus culture *S. baicalensis* L

Secondary metabolites	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	62.79	5.49	274.29
Chlorogenic acid	4.87	0.69	34.73
Catechin	0.89	0.22	10.97
Coffeic acid	2.78	0.24	12.22
Syringic acid	2.28	0.17	8.38
Ellagic acid	6.40	0.68	33.78
Coumaric acid	2.75	0.10	5.11
Vanillin	3.84	0.16	7.85
Ferulic acid	3.06	0.19	9.49
Rosmarinic acid	8.67	0.95	47.43
Cinnamic acid	3.55	0.07	3.42
Kaempferol	13.56	1.00	50.21

development. Not only can gallic acid be derived from a diverse range of plants, but it can also be synthesized chemically and biologically in enormous amounts. Traditional Chinese medicine uses the Chinese skullcap plant—native to China and parts of Russia—to alleviate various symptoms, including inflammation, infections, headaches, and allergies (Tanaka and Kashiwada 2021). Pharmacological investigations have shown that the gallic acid contained in this plant is quickly absorbed and excreted after oral administration. It could also possess antifungal and antiviral qualities. The findings indicate that GA's anti-inflammatory actions are mainly mediated via the MAPK and NF-κB signaling pathways. As such, it diminishes the synthesis of inflammatory cytokines, chemokines, adhesion molecules, and cell infiltration, attenuating the inflammatory response (Bai et al. 2021).

Ellagic acid traps free radicals and lessens oxidative stress by delaying, inhibiting, or preventing the oxidation of other substances. It has also been observed that ellagic acid is present in several commercial items with antioxidant properties. These compounds provide several advantages due to their anti-mutagenic, antibacterial, antioxidant, and HIV-inhibiting qualities (Sepúlveda et al. 2011).

Plants contain the phenolic chemical rosmarinic acid. It significantly aids plant growth and defense systems. It has antimicrobial, antioxidant, anti-inflammatory, anti-cancer, and anti-angiogenic qualities. Controlling free radical damage to cells lowers the chance of developing several kinds of cancer (Singh et al. 2022).

In vivo study results

Nutritional parameters

From the beginning until the end of the research period, the normal control, (UC), Sulfasalazine, and groups (1, 2, 3, and 4) all recorded their weights, as well as their initial and final weights, total food intake, and feed efficiency Table 5. Over the 14 days, the weight of the normal control increased gradually. All the nutritional parameters did not differ statistically significantly between experimental groups (1) and (2) as opposed to the normal control group. In comparison to the group (UC), no statistically substantial modifications were observed in the initial body weights of the Sulfasalazine group, group (3), and group (4). Furthermore, there was no appreciable variation in the initial body weights between the (UC) and normal control groups. Several research studies have supported this conclusion (Shahid et al. 2022; Salem et al. 2024). Moreover, the (UC) group demonstrated a substantial decrease in all nutritional parameters compared to the normal control group. The colonic inflammatory response that resulted from intrarectal acetic acid injection in rats was shown as weight loss and loose stool, either with or without occult blood. According to Owusu et al. (2020), fast fluid loss from diarrhea and intestinal bleeding, decreased appetite, and malabsorption may contribute to nutritional deficiencies and weight loss in colitis patients. Furthermore, TNF-α and IL-6 release neuropeptides that suppress appetite and cause cachexia, greatly contributing to colitis's weight loss (Hunschede et al. 2017). As a consequence, fluid loss from bleeding and diarrhea may be the cause of the weight loss.

Table 5 Effects of secondary metabolites in callus cultures of *S. baicalensis* L. on nutritional parameters

Groups Parameters	Normal control	Group (1)	Group (2)	Ulcerative colitis group (UC)	Sulfasalazine	Group (3)	Group (4)
Initial body weight (g)	206 ± 2	203.7 ± 2.16 ^a	203.5 ± 1.64 ^a	204.8 ± 1.47 ^a	205.2 ± 1.94 ^a	203.3 ± 1.21 ^a	203 ± 1.1 ^a
Final body weight (g)	225.8 ± 2.48	223.2 ± 2.93 ^a	224.2 ± 2.32 ^a	206.5 ± 1.87 ^b	218.5 ± 2.59 ^c	213.3 ± 1.51 ^d	214 ± 1.26 ^e
Body weight gain (g)	19.8 ± 0.48	19.5 ± 0.77 ^a	20.7 ± 0.68 ^a	1.7 ± 0.4 ^b	16.7 ± 0.65 ^c	10 ± 0.3 ^d	11 ± 0.16 ^e
Total food intake (g)	1669.2 ± 5.64	1668.7 ± 5.2 ^a	1667.5 ± 5.01 ^a	1647.5 ± 6.72 ^b	1665.3 ± 4.97 ^c	1656.7 ± 5.16 ^d	1656.3 ± 5.13 ^e
Feed efficiency ratio	0.012 ± 0.09	0.012 ± 0.15 ^a	0.012 ± 0.14 ^a	0.001 ± 0.06 ^b	0.01 ± 0.13 ^c	0.006 ± 0.058 ^d	0.007 ± 0.03 ^e

For n = 6, the data show mean ± SD. Using different letters resulted in a statistically significant change ($P < 0.05$); however, using comparable letters in each row suggested no meaningful alteration. Feed efficiency ratio = body weight gain/total food intake

Through the elimination of the colitis-induced symptoms caused by acetic acid, as evidenced by decreased rectal bleeding, enhanced stool consistency, reduced epithelial erosion, and raised colon length, oral ingestion of *S. baicalensis* L. callus cultures with dosages of 400 and 800 mg/kg BW/d, respectively, restored the normal weight to groups (3 and 4) as opposed to group UC as they significantly increased the nutritional parameters due to their macronutrients content of the callus culture results which presented in Table 2. According to these results, *S. baicalensis* L. callus cultures may protect against colitis that is generated experimentally (Owusu et al. 2020; Salem et al. 2024).

Effect of secondary metabolites in callus cultures of *S. baicalensis* L. on colon length

A well-established rat model of UC caused by acetic acid has many of the same signs and symptoms as UC in humans. These include mucosal ulcerations, colonic reduction, bloody stools, melena, severe diarrhea, inflammatory cell infiltration, and weight loss (Shahid et al. 2022). According to Bastaki et al. (2022), acetic acid causes UC in the colon by releasing protons onto the epithelium, leading to intracellular epithelial acidification and severe epithelial damage. Every animal's colon was inspected to see how severe the colitis caused by acetic acid was. After UC was induced, the main symptoms were seen, including bleeding, ulceration, mucosal erosion, mucosal necrosis, inflammation, and diarrhea. Rat models of UC have been shown to have similar deficits (Bezerra et al. 2017).

Compared to rats in the group (UC), treatment with oral administration at 400 and 800 mg/kg BW/d significantly

protected against and suppressed the primary symptoms of UC. It could be caused by secondary metabolites from *S. baicalensis* L. callus cultures, which prevent acetic acid-generated inflammatory mediators from being released or synthesized, or by anti-inflammatory action, enhanced mucous content, or suppression of apoptotic damage. According to Saldanha et al. (2019), polyphenols may rehabilitate conditions linked to oxidative stress and inflammatory illnesses. Our results show that polyphenols reduce acetic acid-induced UC compared to earlier research (Ju et al. 2018; Saldanha et al. 2019).

The results presented in Fig. 5 demonstrated that the group (UC) had a pronounced colon shortening, a sign of colon inflammation, unlike the normal control group. Colon shortening was significantly improved by the sulfasalazine group, group (3), and group (4) therapy as opposed to the group (UC). This finding implies that *S. baicalensis* L. callus cultures' secondary metabolites may be able to slow the progression of UC. Additionally, group (4), which received treatment with 800 mg/kg BW/d of *S. baicalensis* L., had the highest recovery of colon shortening.

Effect of secondary metabolites in callus cultures of *S. baicalensis* L. on disease activity index (DAI).

Weight loss, rectal bleeding, and diarrhea intensity are the three factors that make up this score, which calculates the overall severity of the condition. With a DAI of 0%, the normal control group had no symptoms of UC Fig. 5. As anticipated, Table 6 demonstrates a substantial reduction in body weight in the group (UC). Enhanced neutrophil infiltration, extensive mucosal and submucosal layer necrosis,

Fig. 5 Effects of secondary metabolites in callus cultures of *S. baicalensis* L. on DAI

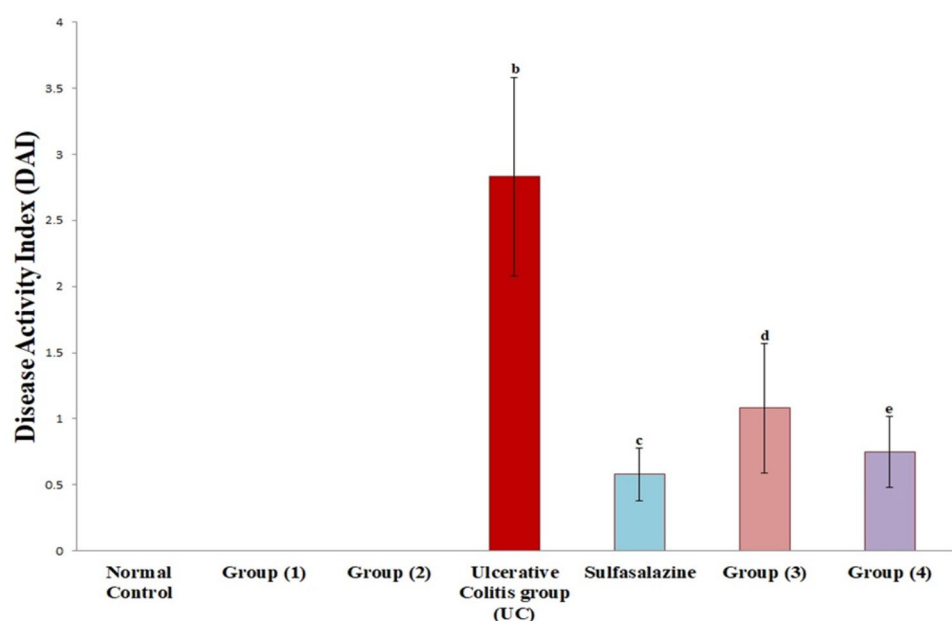


Table 6 Effects of secondary metabolites in callus cultures of *S. baicalensis* L. on inflammatory cytokines

Group Parameters	Normal control	Group (1)	Group (2)	Ulcerative colitis group (UC)	Sulfasalazine	Group (3)	Group (4)
TNF- α (pg/ml)	58.37 $\pm$ 1.89	56.37 $\pm$ 0.95 ^a	57.15 $\pm$ 1.79 ^a	79.45 $\pm$ 1.22 ^b	61.38 $\pm$ 0.9 ^c	63.12 $\pm$ 0.96 ^d	62.5 $\pm$ 1.3 ^e
IL-1 (pg/ml)	27.93 $\pm$ 2.06	27.45 $\pm$ 1.78 ^a	27.5 $\pm$ 2.06 ^a	71.05 $\pm$ 2.59 ^b	36.37 $\pm$ 1.84 ^c	39.83 $\pm$ 3.55 ^d	37.7 $\pm$ 2.27 ^e
IL-6 (pg/ml)	34.8 $\pm$ 1.29	34.18 $\pm$ 1.61 ^a	34.35 $\pm$ 1.43 ^a	69.67 $\pm$ 2.37 ^b	39.78 $\pm$ 0.74 ^c	41.93 $\pm$ 0.99 ^d	40.27 $\pm$ 0.68 ^e
IL-10 (pg/ml)	57.2 $\pm$ 0.69	56.77 $\pm$ 1.42 ^a	57.07 $\pm$ 0.51 ^a	78.12 $\pm$ 1.25 ^b	50.27 $\pm$ 0.88 ^c	48.47 $\pm$ 1.6 ^d	49.53 $\pm$ 0.9 ^e
IL-1 β (pg/mg protein)	87.47 $\pm$ 1.56	86.45 $\pm$ 0.74 ^a	87.12 $\pm$ 1.89 ^a	138.37 $\pm$ 11.24 ^b	98.95 $\pm$ 0.5 ^c	103 $\pm$ 3.18 ^d	99.23 $\pm$ 0.79 ^e

For n = 6, the data show mean $\pm$ SD. Using different letters resulted in a statistically significant change ($P < 0.05$); however, using comparable letters in each row suggested no meaningful alteration

submucosal ulceration, enhanced vascular dilatation, and edema were the hallmarks of the colonic inflammation induced by intrarectal acetic acid injection. As shown in Fig. 5, the group (UC) had higher ratings for other criteria, such as diarrhea and rectal bleeding. In contrast, the groups receiving Sulfasalazine, group (3), and group (4) therapies had significantly lower scores for these parameters.

Regarding the overall disease activity index (DAI), the UC group had the highest DAI, while the sulfasalazine group substantially lowered the DAI. Oral administration of *S. baicalensis* L. callus cultures at 400 (group 3) and 800 mg/kg BW/d (group 4) defended the rats from serious colonic ulceration. Reduced rectal bleeding, better stool consistency, and lessened body weight loss were the clinical signs of DSS-induced colitis that were reversed, resulting in the restoration of the high DAI score (Owusu et al. 2020; Salem et al. 2024). However, the group (4) treated with *S. baicalensis* L. at 800 mg/kg BW/d showed a greater improvement in the DAI score than the group (UC).

Biochemical parameters

Gajendran et al. (2019) describe UC as one of the (IBD) that is most frequent and negatively impacts lifestyle. The precise etiology of UC is still unknown despite the involvement of many genetic and immunological components in its development. The production of UC is linked to several inflammatory mediators and ROS (Arafa et al. 2020; De Oliveira et al. 2021). The well-researched UC known as "acetic acid-induced UC" is brought on by widespread acidification of the intracellular environment, which erodes mucosal barriers and damages the colonic epithelium. This damage triggers the release of monocytes, macrophages, and inflammatory cytokines, and an easily employed experimental model (Rashidian et al. 2020; Motawea et al. 2020). This acetic acid-induced UC model could provide a good fit since it is linked to oxidative and inflammatory processes that resemble the pathophysiology of clinical (IBD) (Esiringü et al. 2014; Wu et al. 2020).

A positive feedback loop created by oxidative stress produces inflammatory cytokines, which trigger chemotactic signals that lead to the invasion of neutrophils and lymphocytes. Oxidative stress usually enhances several cellular signaling pathways associated with inflammation, according to Khalil et al. (2019) and Mohammed et al. (2023). Unlike the normal control group, the UC group had greater TNF- α , IL-1, IL-6, IL-10, and IL-1 β Table 6. This is explained by the excessive generation of ROS, which substantially influences the development and manifestation of colon problems (Abdel-Moneim et al. 2011; Chen et al. 2019; Mohammed et al. 2024c). Compared to the group (UC), the injection of sulfasalazine improved the colonic damage, reflected by a substantial reduction in TNF- α , IL-1, IL-6, IL-10, and IL-1 β levels. Additionally, all inflammatory cytokines levels were lower in groups (3 and 4) that received *S. baicalensis* L. (400 and 800 mg/kg BW/d, respectively) than in the group (UC).

In the pathogenesis of inflammatory bowel disease, interleukins and tumor TNF- α are crucial players (Guan and Zhang 2017; Nemeth et al. 2017). Colonic damage is caused by their manipulation of the mucosal immune system, modification of epithelial integrity, and the integration of neutrophil and macrophage infiltration and activation (Rehman et al. 2022). One theory is that the anti-inflammatory cytokine effects of the *S. baicalensis* L. callus are suppressed by its high antioxidant capacity (Soliman et al. 2022).

When considered together, the current research findings suggest that AA injection causes colonic injury, and oxidative stress is a major factor in the pathophysiology of this damage. Furthermore, as opposed to sulfasalazine as a medication, *S. baicalensis* L. at two doses, a plant-based substance, exhibited no adverse effects and minimized the severity of UC in our experimental model, as shown by decreased DAI, better mucosal integrity, and increased weight gain. *S. baicalensis* L. acts on several targets to attenuate UC in the animal model. This is shown by declined oxidative stress parameters and inflammatory markers of innate immunity. The findings we obtained agreed with the research assessed by Abdelmonaem et al. (2021) and Rehman et al. (2022).

According to Li et al. (2023), inflammation and oxidative stress are substantial in developing ulcerative colon disease by causing intestinal tissue damage and microbial dysbiosis. Moreover, Wang et al. (2016) and Bardelčíková et al. (2023) suggest that they may be significant initiators in the progression of UC to colon cancer. Several pathological alterations lead to cellular damage if the delicate equilibrium between oxidants and antioxidants in tissues cannot be controlled (Curieses Andrés et al. 2023).

The colon tissue of the group (UC) showed diminished levels of GSH, SOD, CAT, GPx, GR, and GST Table 7. Moreover, higher quantities of colonic NO content, MDA, and oxidative stress biomarkers were found in the colon tissue during the study. In contrast to the normal control group, these findings show that oxidative stress is present and that the antioxidant system is not functioning properly. Compared to the group (UC), the injection of sulfasalazine substantially lowered colonic MDA levels and normalized SOD levels, indicating improvement in colonic damage. The previous findings of Liu et al. (2020), which discovered that SLZ reduced acetic acid-induced UC in rats by inhibiting oxidative stress, are consistent with our outcomes. Furthermore, *S. baicalensis* L. was given to groups (3 and 4) at 400 and 800 mg/kg BW/d, respectively. These therapies effectively reduced the accumulation of lipid peroxides and free radicals in colon tissues as opposed to the group (UC).

One well-known possible mechanism linked to acetic acid-induced UC is generating ROS, a mixture of bound enzymatic and non-enzymatic antioxidant molecules. Excess ROS production by oxidizing macromolecules inside the cell, including proteins and lipids, and starting

the destruction of DNA (Al Olayan et al. 2020; Mohammed et al. 2025). Within the phospholipid compartments of the cellular membrane, a peroxidative process known as "lipid peroxidation" takes place that seriously damages the membrane (Al-Megrin et al. 2020; Salama et al. 2023). Furthermore, AA induction elevates nitric oxide production, which is involved in immunological responses and inflammation. A very strong cytotoxic compound called peroxynitrite anion (ONOO^-) is formed when superoxide anions (O_2^-) and nitric oxide (NO) react due to the excessive production of NO (Radi 2013). The current study suggests that the elevated nitric oxide (NO) levels might be attributed to the colonic tissue's enhanced production of inducible NO synthase (iNOS). Moreover, the colon tissue's levels of GSH were markedly lowered by the introduction of alcohol. Al Olayan et al. (2020) state that neutralizing free radicals oxidized into GSSG requires GSH under abnormal oxidative stress circumstances. AA has also been shown to prevent glutathione (GSH) enzymes from being made or recycled, which lowers GSH levels even further (Flora et al. 2012; Iruarizaga-Lejarreta et al. 2017). Biological systems need antioxidant enzymes like SOD and CAT to eliminate oxygen radicals (O_2^-) (Al-Megrin et al. 2020; Soliman et al. 2024). Lipid peroxidation takes place in the tissue and produces MDA as a consequence. Considerable elevations in MDA levels in plasma are associated with UC, and this information is utilized to make key diagnoses of individuals suffering from inflammatory bowel disease (Ali et al. 2017). Total antioxidant activity is the total of endogenous and dietary antioxidants. By preventing lipid peroxidation and

Table 7 Alterations in oxidative stress biomarker, lipid peroxidation, and levels of enzymatic and non-enzymatic antioxidants in several experimental groups

Groups Parameters	Normal control	Group (1)	Group (2)	Ulcerative colitis group (UC)	Sulfasalazine	Group (3)	Group (4)
MDA (nmol/mg protein)	2.75 ± 0.26	2.56 ± 0.25 ^a	2.65 ± 0.17 ^a	6.2 ± 0.4 ^b	3.37 ± 0.18 ^c	3.88 ± 0.11 ^d	3.54 ± 0.11 ^e
NO (nmol/100 mg tissue)	37.77 ± 1.94	36.73 ± 2.04 ^a	36.85 ± 1.3 ^a	76.78 ± 2.61 ^b	40.38 ± 1.5 ^c	47.72 ± 2.19 ^d	43.47 ± 1.42 ^e
GSH (nmoles/mg protein)	4.82 ± 0.12	4.68 ± 0.15 ^a	4.77 ± 0.27 ^a	2.38 ± 0.12 ^b	3.92 ± 0.29 ^c	3.62 ± 0.17 ^d	3.77 ± 0.22 ^e
SOD (nmol pyrogallol oxidized/ min/mg protein)	67.82 ± 2.67	66.85 ± 1.37 ^a	67.27 ± 1.63 ^a	36.13 ± 2.47 ^b	51.82 ± 0.89 ^c	49.6 ± 2.72 ^d	50.9 ± 1.42 ^e
CAT (μmol of H ₂ O ₂ utilized/ min/mg protein)	63.33 ± 1.38	63.03 ± 1.56 ^a	63.13 ± 2.08 ^a	33.1 ± 1.15 ^b	52.55 ± 1.43 ^c	46.05 ± 1.94 ^d	51.15 ± 1.09 ^e
GPx (μg of GSH consumed/ min/mg protein)	107.68 ± 1.67	106.92 ± 1.42 ^a	107.17 ± 1.65 ^a	72.85 ± 1.62 ^b	99.48 ± 0.79 ^c	96.47 ± 1.52 ^d	98.12 ± 1.58 ^e
GR (nmol of NADPH oxidized/ min/mg protein)	97.92 ± 1.52	97.15 ± 1.21 ^a	97.7 ± 1.36 ^a	70.03 ± 1.14 ^b	89.62 ± 0.98 ^c	84.12 ± 0.65 ^d	88.2 ± 1.45 ^e
GST (μmol of CDNB–GSH conjugate formed/min/mg protein)	13.07 ± 0.31	12.82 ± 0.39 ^a	12.9 ± 0.47 ^a	5.35 ± 0.3 ^b	9.43 ± 0.32 ^c	8.8 ± 0.2 ^d	9.12 ± 0.4 ^e

For n = 6, the data show mean ± SD. Using different letters resulted in a statistically significant change ($P < 0.05$); however, using comparable letters in each row suggested no meaningful alteration

facilitating superoxide anion dismutation, SOD shields the cells from ulcerative damage.

Additionally, in colonic tissues, SOD suppresses leukocyte adherence and rolling (Baldo et al. 2017). Moreover, the administration of *S. baicalensis* L. significantly mitigates oxidative stress, demonstrating its strong antioxidant capabilities in colonic tissue. Moreover, flavonoids and polyphenols are thought to be hydrogen atom donors that may protect mammalian cells against ROS and combat free radicals (Kurutas 2015).

Based on our findings, it can be inferred that Table 4 presents the antioxidant compounds found in the secondary metabolites of *S. baicalensis* L. callus culture. These molecules include gallic acid, kaempferol, rosmarinic acid, ellagic, and caffeic acid. These conclusions are based on our research. These substances have been shown to diminish the colon's oxidative stress successfully. Additionally, the group (4) that received 800 mg/kg BW/d of *S. baicalensis* L. was shown to have the best result.

Conclusion and future perspective

Biotechnology tools such as callus culture are an effective and important alternative to consuming natural resources. The current findings highlighted the differential effects medium containing 1.0 mg/L BAP + 1.0 mg/L 2,4-D and incubated in the dark gave the highest stimulation of growth to induce callus from the skullcap Chinese. Moreover, the findings of this work have proven the potential of *S. baicalensis* L. callus culture secondary metabolites as a fruitful and attractive research topic for UC therapy, suggesting bright future possibilities for this discipline. *S. baicalensis* L. callus has anti-inflammatory and antioxidant qualities essential for protecting against ROS the effects appeared were due to the phenolic compounds, flavonoids and antioxidant activity of *S. baicalensis* L. callus and this is confirmed by all in vitro assays we measured in this study. By increasing body weight and enhancing the biochemical parameters, we discovered that treating acetic acid-induced UC in rats with *S. baicalensis* L. (800 mg/kg BW/d) dramatically alleviated UC. These results provide a new, safe, and efficient UC therapy and preventive strategy. Our future perspectives are scaling up a technology for callus and cell suspension cultures of skullcap Chinese through bioreactor application and improving its efficiency for phytoremediation research. In addition, we aim to investigate clinical studies for their effectiveness in humans in the near future.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or nonfinancial interests to disclose.

Ethical approval and consent to participate All authors approve ethics and consent for participation and publication.

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