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# Compositional Analysis and Bioactivity Assessment of the *Anemone baicalensis* Rhizome: Exploring the Potential for Substituting *Anemones raddeanae* Rhizoma

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**Abstract:** *Anemone baicalensis*, a plant abundant in Northeast China, has garnered attention for its potential medicinal properties. While its aerial parts (ABAP) have demonstrated significant antioxidant activity, the rhizome (ABR) remains less explored, particularly in comparison to the well-documented *Anemones raddeanae* Rhizoma, a valuable traditional Chinese medicine. This study investigates the chemical composition and bioactivity of ABR, comparing it with ABAP and evaluating its potential as a substitute for *Anemones raddeanae* Rhizoma. Phytochemical analyses, including qualitative and quantitative assessments, were conducted on ABR extracts using various solvents. Antioxidant activity was evaluated through multiple assays, and stability studies were performed on methanol and 80% ethanol extracts. UHPLC-ESI-Q-TOF-MS was employed to identify chemical constituents. Acute toxicity and hepatoprotective effects were assessed in vivo using a rat model. The results revealed that ABR and ABAP share nearly half of their chemical components, with ABR containing a higher diversity of triterpenoid saponins. The 80% ethanol extract of ABR exhibited the highest extraction yield, rich in phenolics and triterpenoids, and demonstrated superior antioxidant activity and stability. In vivo studies showed that ABR significantly reduced liver injury markers (ALT, AST, $\gamma$-GT, and MDA), enhanced antioxidant enzyme activity (CAT), and increased albumin concentration, comparable to the effects of *Anemones raddeanae* Rhizoma. Acute toxicity tests indicated low toxicity, supporting its safety for medicinal use. ABR shows significant potential as a substitute for *Anemones raddeanae* Rhizoma, particularly due to its rich triterpenoid content and hepatoprotective effects. While ABAP outperforms ABR in terms of antioxidant activity, ABR's unique chemical profile and liver-protective capabilities highlight its value in drug development. This study provides a scientific foundation for the further exploration of ABR as a sustainable alternative in traditional medicine.

**Keywords:** *Anemone baicalensis*; *Anemones raddeanae* Rhizoma; component analysis; antioxidant activity; hepatoprotective activity

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## 1. Introduction

*Anemone* plants of the Ranunculaceae family are widely distributed worldwide, with significant populations in Northeast and Southwest China [1]. The rhizomes of these plants

often serve as the focus of research [2], particularly those of *Anemone raddeana*, its rhizome also known as *Anemones raddeanae* Rhizoma. This plant is considered a valuable traditional Chinese medicine and is listed in the 2020 edition of the *Chinese Pharmacopoeia* [3], underscoring its importance in both traditional and modern medical applications. In the realm of composition research, *Anemones raddeanae* Rhizoma is at the forefront, with scholars successfully isolating various components such as triterpenoid saponins, coumarins, and volatile oils [4]. It holds considerable medicinal value and occupies an irreplaceable position in the pharmaceutical field. However, given the preciousness of this medicinal resource, we must recognize the critical need for its sustainable use. Each harvest represents the potential loss of a plant, reminding us of the necessity to utilize medicinal plants judiciously while actively exploring conservation and regeneration strategies. At present, the medicinal resources of *Anemones raddeanae* Rhizoma primarily rely on wild collection. However, due to excessive harvesting and environmental degradation, wild populations are at risk of depletion. Consequently, the species has been included in the List of Key Protected Wild Plants in Beijing (https://www.beijing.gov.cn/zhengce/zfwj/zfwj2016/szfwj/202306/t20230609_3128229.html, accessed on 19 January 2025). Artificial cultivation has gradually emerged as an important supplement, although the technology for its cultivation is still under development and not yet fully mature. Therefore, finding alternative plants is crucial, as it can alleviate resource pressure, protect ecosystems, and provide new directions for scientific research. Discovering alternative resources is expected to promote the development of traditional Chinese medicine while ensuring sustainability. Given the scarcity of *Anemones raddeanae* Rhizoma, it is essential to identify suitable substitutes. Naturally, attention has turned to other species within the same genus, seeking alternatives with comparable chemical compositions and medicinal properties. Through comparative studies, the potential of these alternative species can be more accurately evaluated, thereby laying a solid foundation for the sustainable utilization of medicinal resources.

*Anemone baicalensis*, a plant with a height of 13–28 cm and a rhizome diameter of approximately 1 mm (Figure 1), is widely distributed in Northeast China. Its abundance in this region facilitates easy harvesting, collection, and storage, making it an ideal candidate for fundamental research [5]. Notably, both *Anemone baicalensis* and *Anemones raddeanae* Rhizoma belong to the genus *Anemone*, which positions *Anemone baicalensis* as a highly promising substitute for *Anemones raddeanae* Rhizoma. Importantly, the *Anemone baicalensis* aerial part (ABAP) have demonstrated significant antioxidant activity [6], highlighting its potential medicinal value. In plants of the *Anemone* genus, the rhizome is often the primary medicinal portion, containing a variety of pharmacologically active compounds. In this study, we focused on the *Anemone baicalensis* rhizome (ABR) to analyze its chemical composition and biological activity. Our objectives were to investigate the differences and connections between the chemical composition and antioxidant activity of ABR and ABAP, as well as to explore the similarities between the chemical components of ABR and *Anemones raddeanae* Rhizoma. This research provides a scientific basis for developing ABR as a potential alternative to *Anemones raddeanae* Rhizoma, offering new insights into the sustainable utilization of medicinal resources.



**Figure 1.** Morphology of ABR.

## 2. Results and Discussion

### *2.1. Qualitative Phytochemical Analysis*

In this study, an extensive qualitative phytochemical analysis of ABR was performed, as presented in Table S1. According to the analysis, ABR does not contain alkaloids, cyanogenic glycosides, anthraquinones, or flavonoids. However, there were inconsistencies in the results for tannins, cardiac glycosides, and coumarins, requiring further verification. Additionally, a comparative study with ABAP [6] showed no detection of alkaloids and flavonoids in ABR. This finding highlights significant differences in the chemical composition of different parts of *Anemone baicalensis*, offering valuable insights into its pharmacological activity and potential applications. Importantly, at the chemical composition level, ABR shows high similarity with *Anemones raddeanae* Rhizoma; both contain terpenoids, amino acids, coumarins, phenolics, volatile oils, and other active ingredients [4]. This similarity provides chemical evidence supporting the feasibility of ABR as a potential alternative to *Anemones raddeanae* Rhizoma, indicating its research value in traditional medical applications.

### *2.2. Extraction Yields*

The study investigated the extraction efficiency of various solvents for ABR, as illustrated in Figure 2. Notably, both the 80% ethanol extract and the water extract exhibited the highest extraction rate of 14.51%, significantly surpassing the rates of the methanol and ethanol extracts. It is worth noting that the extraction rate of the ethanol extract was the lowest among all the solvents tested, at only 8.12%. These findings reveal distinct differences in the extraction efficiency of ABR's active components with various solvents, suggesting the necessity of selecting the appropriate extraction solvent based on the properties of the target components in practical applications. A comparative study with ABAP [6] revealed that the extraction trends of these four solvents for ABAP were consistent with those for ABR, but the extraction rate for ABAP was generally higher. This emphasizes the differences in chemical content between ABAP and ABR and suggests that the aerial parts may contain more abundant chemical components, possibly due to higher chlorophyll content. In the extraction and separation process of *Anemones raddeanae* Rhizoma, 75% ethanol is typically used as the solvent [7], underscoring the advantages of aqueous ethanol in extracting the effective components of this genus and supporting its reliability in ensuring extraction rate and purity.

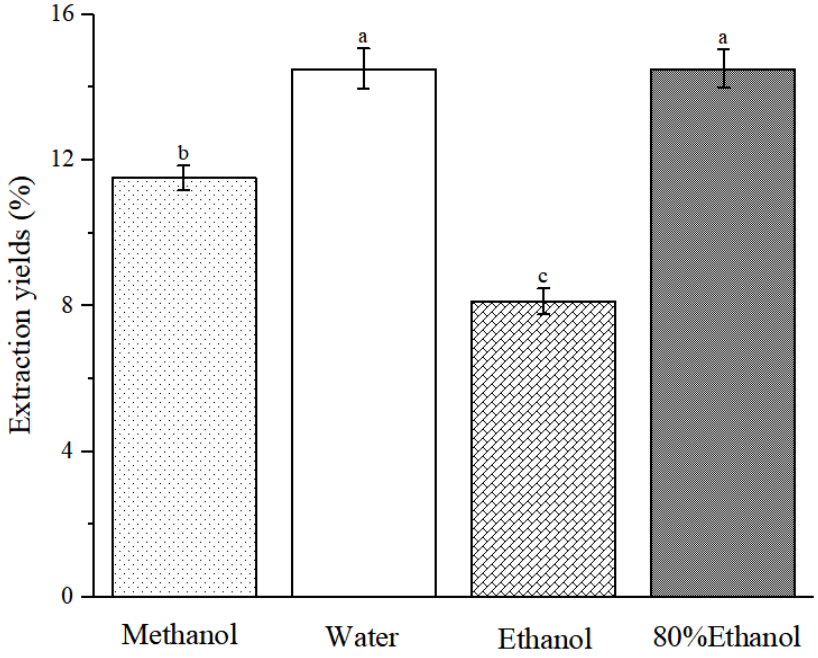



**Figure 2.** The extraction yields of ABR were obtained using four different solvents. [a–c] Columns with differing superscripts signify a statistically significant difference ($p < 0.05$).

### *2.3. Quantitative Phytochemical Analysis*

#### 2.3.1. Total Carbohydrate Content (TCC)

Carbohydrates are a primary energy source for humans, and their value in drug development is increasingly recognized [8]. Glycosides, a significant form of carbohydrates, have garnered attention due to their notable antioxidant activity [9]. This study focused on ABR, evaluating the carbohydrate content in each extract using different solvents (Table 1). The TCC ranged from 522.50 ± 3.37 to 700.30 ± 5.02 mg glucose equivalents (GE)/g extract. Notably, the TCC in methanol and 80% ethanol extracts was significantly higher than that in the water extract. This finding aligns with previous research on ABAP [6], further affirming methanol and 80% ethanol's superiority in extracting glycosides. Additionally, ABR's higher glycoside content may be attributed to a stable growth environment and richer nutrient supply in the rhizome, which are conducive to glycoside synthesis and accumulation. Similar studies have found that the *Polygonatum cyrtonema* rhizome is richer in carbohydrates than its aerial part [10]. This similarity underscores the rhizome's potential value for chemical component accumulation and drug development.

#### 2.3.2. Total Protein Content ($TP_{ro}C$)

Plant proteins are nutrient-dense and safe for most, especially in meeting the dietary needs of groups like diabetic patients and those with kidney disease [11]. This study examined the water extract of ABR and discovered that its $TP_{ro}C$ was an impressive 191.04 ± 1.80 mg bovine serum albumin equivalents (BSAE)/g extract (Table 1). However, when comparing the protein content of ABR with ABAP, an intriguing phenomenon emerged: Although ABR's protein content is relatively high, it is only half that of ABAP [6]. In-depth analysis suggests that the aerial parts, directly exposed to the complex and volatile atmospheric environment—facing challenges like light, temperature, and humidity—may need to synthesize more proteins to enhance structural stability, physiological function, and adaptability. In *Polygonatum cyrtonema*, the protein content in its rhizome is lower than in the aerial part [10], consistent with our findings.

**Table 1.** TCC, $TP_{ro}C$, TAC, $TP_{he}C$, TPAC, TFC, $TT_{an}C$, GC, CTC and $TT_{ri}C$ of ABR and ABAP extracted using various solvents.

| Medicament Portions | Extracting Solvents | TCC (mg GE/g Extract) | $TP_{ro}C$ (mg BSAE/g Extract) | TAC (mg BHE/g Extract) | $TP_{he}C$ (mg GAE/g Extract) | TPAC (mg CAE/g Extract) | TFC (mg QE/g Extract) | $TT_{an}C$ (mg TAE/g Extract) | GC (mg GAE/g Extract) | CTC (mg GAE/g Extract) | $TT_{ri}C$ (mg GRE/g Extract) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ABAP | Methanol | $424.65 \pm 3.08^{f}$ | N.T. | $4.36 \pm 0.03^{b}$ | $30.92 \pm 0.15^{c}$ | $15.38 \pm 0.86^{b}$ | $13.73 \pm 0.47^{b}$ | $22.72 \pm 0.11^{c}$ | NONE | NONE | $86.85 \pm 2.43^{a}$ |
| | Water | $355.38 \pm 2.86^{h}$ | $493.08 \pm 3.15^{a}$ | $4.12 \pm 0.03^{c}$ | $34.53 \pm 0.24^{a}$ | $11.62 \pm 0.79^{c}$ | $4.04 \pm 0.21^{d}$ | $27.25 \pm 0.38^{a}$ | $4.33 \pm 0.11^{f}$ | NONE | $12.30 \pm 1.05^{f}$ |
| | Ethanol | $433.95 \pm 5.17^{e}$ | N.T. | $5.71 \pm 0.01^{a}$ | $19.06 \pm 0.43^{f}$ | $8.97 \pm 0.53^{d}$ | $6.14 \pm 0.12^{c}$ | $16.31 \pm 0.20^{d}$ | NONE | NONE | $73.20 \pm 1.62^{b}$ |
| | 80% Ethanol | $389.32 \pm 3.24^{g}$ | N.T. | $3.00 \pm 0.00^{d}$ | $31.57 \pm 0.58^{b}$ | $16.45 \pm 0.20^{a}$ | $15.44 \pm 0.31^{a}$ | $25.93 \pm 0.18^{b}$ | $6.29 \pm 0.15^{d}$ | NONE | $42.97 \pm 0.75^{c}$ |
| ABR | Methanol | $700.30 \pm 5.02^{a}$ | N.T. | NONE | $19.80 \pm 0.13^{e}$ | $7.79 \pm 0.23^{f}$ | NONE | $15.70 \pm 0.12^{e}$ | $7.37 \pm 0.34^{b}$ | NONE | $36.01 \pm 1.31^{d}$ |
| | Water | $522.50 \pm 3.37^{d}$ | $191.04 \pm 1.80^{b}$ | NONE | $16.04 \pm 0.14^{h}$ | $3.14 \pm 0.11^{h}$ | NONE | $15.39 \pm 0.09^{f}$ | $4.50 \pm 0.14^{e}$ | NONE | NONE |
| | Ethanol | $525.36 \pm 4.37^{c}$ | N.T. | NONE | $17.11 \pm 0.08^{g}$ | $8.59 \pm 0.44^{e}$ | NONE | $14.63 \pm 0.15^{g}$ | $7.27 \pm 0.15^{c}$ | NONE | $1.24 \pm 0.16^{g}$ |
| | 80% Ethanol | $587.48 \pm 3.18^{b}$ | N.T. | NONE | $21.76 \pm 0.32^{d}$ | $7.53 \pm 0.19^{g}$ | NONE | $14.39 \pm 0.08^{h}$ | $8.53 \pm 0.28^{a}$ | NONE | $29.27 \pm 0.55^{e}$ |

$^{a–h}$ Columns with differing superscripts signify a statistically significant difference ($p < 0.05$). N.T. indicates no test.

#### 2.3.3. Total Alkaloid Content (TAC)

Alkaloids, widely distributed bioactive components in nature, pose potential risks in clinical applications and food due to their diverse toxic effects [12]. In this study, four extracts of ABR were rigorously tested, revealing that none contained alkaloids (Table 1). This finding aligns closely with prior qualitative experiments, providing strong evidence of ABR's significant safety advantages. Although a small amount of alkaloids were detected in ABAP, its acute toxicity was relatively low [6], supporting the overall safety of the *Anemone baicalensis* plant.

#### 2.3.4. Total Phenolic Content ($TP_{he}C$)

Phenolics, known for their unique chemical structure and wide-ranging biological activities, hold significant value in fields like food, medicine, and chemical industry, especially due to their strong antioxidant properties [13]. A recent study revealed that consuming a smoothie rich in phenolics on a regular basis significantly boosted the activity of catalase (CAT) and glutathione peroxidase in the livers of obese mice fed a high-fat diet. This led to the effective prevention of metabolic disorders and improvement in non-alcoholic fatty liver disease [14]. This research not only highlights the health benefits of phenolics but also suggests new avenues for developing functional foods and drugs. In this study, the $TP_{he}C$ of ABR extract ranged from 16.04 ± 0.14 to 21.76 ± 0.32 mg gallic acid equivalents (GAE)/g extract (Table 1). Notably, the $TP_{he}C$ was highest in the 80% ethanol extract, followed by methanol, ethanol, and water extracts. This finding highlights ethanol's advantages as an extraction solvent for phenolic components, as ethanol's good solubility in phenolics makes it effective for this purpose. Studies have shown that aqueous ethanol more easily extracts higher concentrations of phenolics [15,16], supporting the preference for 80% ethanol as an extraction solvent. Additionally, the $TP_{he}C$ in ABAP is higher than in ABR [6], which can be explained by the fact that the aerial parts of plants usually contain higher phenolic concentrations than underground parts. This is because the aerial part faces more challenges and threats from the external environment during growth. Direct exposure to the natural environment makes it more susceptible to bacterial infections, insect damage, and physical harm. To combat these hazards, plants enhance their defense mechanisms by synthesizing and accumulating phenolic compounds, which have antibacterial and insect-resistant properties and reduce oxidative stress, thus protecting the aerial parts and ensuring normal growth and development [17].

#### 2.3.5. Total Phenolic Acid Content (TPAC)

Research indicates that oral intake of phenolic acids can effectively counteract increases in body mass index and blood glucose levels induced by a high fructose diet. This process also restores metabolic hormone levels and antioxidant enzyme activities disrupted by such a diet, exhibiting a strong protective effect against metabolic syndrome induced by high fructose intake [18]. This study evaluated the TPAC of various ABR extracts, which ranged from 3.14 ± 0.11 to 8.59 ± 0.44 mg caffeic acid equivalents (CAE)/g extract (Table 1). The findings reveal an interesting phenomenon: the 80% ethanol, ethanol, and methanol extracts all showed similarly high phenolic acid content, indicating these organic solvents have comparable effectiveness in extracting phenolic acids. In contrast, the TPAC of the water extract was relatively low, highlighting the advantages of organic solvents in phenolic acid extraction. Both ABAP and ABR showed the same trend, with 80% ethanol and methanol extracts containing higher TPAC [6].

#### 2.3.6. Total Flavonoid Content (TFC)

Flavonoids are well known for their diverse range of biological effects, such as their abilities to combat cancer, exhibit antioxidant activity, fight viruses, and reduce inflammation, which have garnered significant research interest [19]. Experimental results revealed that flavonoids were absent from these solvent extracts (Table 1). This finding corroborates the accuracy and reliability of many prior qualitative tests and prompts deeper investigation into the distribution pattern of flavonoids in plants. While flavonoids are present in ABAP [6], they were not found in ABR. This comparison highlights an important pattern: flavonoids are typically abundant in the flowers, fruits, and leaves of plants but are relatively scarce or absent in rhizomes. The aerial parts, being directly exposed to light, facilitate the synthesis and accumulation of flavonoids. In contrast, the rhizome, located underground, has limited light exposure, resulting in reduced flavonoid synthesis. Studies on *Farfugium japonicum* have also demonstrated that flavonoid contents are influenced by light exposure, with significantly higher concentrations found in aerial parts than in rhizomes [20]. This study further supports the notion that flavonoid distribution in plants is closely linked to light conditions.

#### 2.3.7. Total Tannin Content ($TT_{an}C$), Gallotannin Content (GC) and Condensed Tannin Content (CTC)

Tannins are prevalent in plants and can be categorized into hydrolyzed tannins and condensed tannins based on their chemical structure. These tannins are recognized for their exceptional antioxidant and anti-inflammatory properties, making them valuable resources in various biological activity studies. To evaluate the tannin content in ABR extract, key indicators such as $TT_{an}C$, GC, and CTC were analyzed in this study (Table 1). $TT_{an}C$ ranged from $14.39 \pm 0.08$ to $15.70 \pm 0.12$ mg tannic acid equivalents (TAE)/g extract, while GC ranged from $4.50 \pm 0.14$ to $8.53 \pm 0.28$ mg GAE/g extract. CTC was not detected in ABAP and ABR, confirming the absence of condensed tannins in *Anemone baicalensis*. Environmental factors significantly influence the distribution and content of tannins in plants, with changes in light, temperature, and moisture affecting the synthesis and accumulation of tannins. In Polygonaceae plants, the tannin content is higher in the aerial parts than in the rhizome, a pattern also observed in *Anemone baicalensis*. As the primary organ for photosynthesis and gas exchange, the aerial part exhibits more active physiological activities and a higher metabolic rate, leading to the accumulation of more secondary metabolites, including tannins, during growth.

#### 2.3.8. Total Triterpenoid Content ($TT_{ri}C$)

Triterpenoids, a class of natural products found widely in nature, serve as effective antioxidants. In this study, the $TT_{ri}C$ of ABR extract was determined (Table 1) and ranged from $1.24 \pm 0.16$ to $36.01 \pm 1.31$ mg ginsenoside Re equivalents (GRE)/g extract. The methanol extract exhibited the highest level of triterpenoid content, with the 80% ethanol extract closely following, mirroring the outcomes observed with ABAP. Comparing the triterpenoid content of different solvent extracts revealed that the solvent significantly affects the extraction rate and final product content. Methanol and ethanol are commonly used organic solvents, and their strong solubility and permeability to plant cell walls likely contribute to the high triterpenoid content in the extract. This finding not only validates previous conclusions that *Anemone* plants are rich in triterpenoids but also highlights the importance of further exploration in this promising research field.

In this research, the chemical composition of ABR was analyzed in depth, with fifteen types of phytochemicals identified (Table S1), notably characterized by the absence of alkaloids in ABR. This contrasts sharply with the study of ABAP, indicating that the

absence of alkaloids in ABR implies a potentially lower toxicity level, making it more suitable for medicinal use and enhancing drug safety. Alkaloids have not been detected in *Anemones raddeanae* Rhizoma, which reinforces the similarity between it and ABR. Crucially, ABR is also rich in triterpenoids, making it a promising alternative to *Anemones raddeanae* Rhizoma. This discovery broadens the scope of medicinal plant resources and provides a robust scientific basis for further drug development and clinical application. A study also demonstrated that plants of the same genus, *Anemone rupicola*, contain a variety of active ingredients, including phenolics, saponins, and glycosides [21]. Notably, the chemical composition analysis of ABR in this study strongly aligns with the findings on active ingredients in *Anemone rupicola* and other *Anemone* species.

### *2.4. Antioxidant Activity In Vitro*

#### 2.4.1. Free Radical Scavenging Ability

Among the various methods for evaluating antioxidant activity in vitro, assessing the capacity of antioxidants to neutralize free radicals is a prevalent and effective method. This method is closely associated with the primary function of antioxidants, which is to neutralize and scavenge excess free radicals in the body, thereby offering high accuracy and sensitivity [22]. In this study, four experiments were carried out to thoroughly evaluate the free radical scavenging ability of the ABR extract, as detailed in Table 2. In the DPPH and ABTS free radical scavenging experiments, compared with standard antioxidants, the extracts of ABAP and ABR exhibit slightly lower antioxidant activity. However, the scavenging ability of ABAP extract is significantly stronger than that of ABR, making it a viable alternative antioxidant. It was noted that the 80% ethanol extract, ethanol extract, and methanol extract exhibited excellent free radical scavenging abilities. This suggests that these solvent extracts contain antioxidant components capable of rapidly and effectively neutralizing DPPH and ABTS free radicals, thus reducing oxidative stress levels. *Anemone cathayensis* also demonstrated significant DPPH radical scavenging activity [23]. Several phenolic compounds were isolated from the root of *Anemone chinensis*, among which *pulsatillanin* A exhibited strong DPPH radical scavenging activity. Additionally, *pulsatillanin* A alleviated lipopolysaccharide-induced oxidative stress in RAW264.7 cells in a dose-dependent manner by reducing ROS production, increasing superoxide dismutase activity, and replenishing glutathione (GSH) levels. Western blot analysis revealed that *pulsatillanin* A exerted its antioxidant effects by activating the Nrf2 signaling pathway [24]. However, in the hydroxyl radical and superoxide anion radical scavenging experiments, compared with standard antioxidants, various ABAP and ABR extracts demonstrated relatively weak scavenging abilities. Triterpenoids, including *hederasaponin* B, *raddeanoside* 20, and *raddeanoside* 21, were isolated from the ethanol extract of *Anemones raddeanae* Rhizoma. These triterpenoids exhibited a slight suppressive effect on superoxide radical generation induced by *N*-formyl-methionyl-leucyl-phenylalanine in a concentration-dependent manner [7]. These results may be due to the distinct characteristics of different free radicals and the mechanisms of action of antioxidants. Hydroxyl radicals and superoxide anion radicals may be challenging for antioxidants to scavenge due to their high reactivity and complex chemical properties. Notably, the ability of ABAP to scavenge free radicals can reach about twice that of ABR [6]. This finding implies that the biological activity differences between different parts of plants should be carefully considered when developing and applying antioxidants.

**Table 2.** The antioxidant capacity of ABR and ABAP was evaluated through assays measuring its ability to scavenge DPPH, ABTS, hydroxyl, and superoxide radicals.

| Medicament Portions | Extracting Solvents | DPPH ($IC_{50}$, μg/mL) | ABTS ($IC_{50}$, μg/mL) | Hydroxyl Radicals (%, 2500 μg/mL) | Superoxide Radicals (%, 2143 μg/mL) |
|---|---|---|---|---|---|
| ABAP | Methanol | 37.21 ± 1.66 $^{c}$ | 71.21 ± 2.05 $^{e}$ | 47.28 ± 0.43 $^{c}$ | 55.01 ± 0.45 $^{b}$ |
| | Water | 93.53 ± 2.35 $^{g}$ | 52.36 ± 0.54 $^{c}$ | 41.12 ± 1.21 $^{e}$ | 20.81 ± 0.34 $^{f}$ |
| | Ethanol | 99.76 ± 2.45 $^{h}$ | 125.03 ± 2.40 $^{j}$ | 40.74 ± 1.62 $^{f}$ | 32.09 ± 0.54 $^{d}$ |
| | 80% Ethanol | 45.14 ± 0.88 $^{d}$ | 58.10 ± 1.36 $^{d}$ | 42.15 ± 1.08 $^{d}$ | 48.72 ± 0.55 $^{c}$ |
| ABR | Methanol | 91.36 ± 2.49 $^{f}$ | 86.13 ± 4.42 $^{h}$ | 14.26 ± 0.60 $^{h}$ | 11.76 ± 1.21 $^{h}$ |
| | Water | 376.72 ± 5.00 $^{j}$ | 113.58 ± 2.09 $^{i}$ | 29.24 ± 1.94 $^{g}$ | 8.56 ± 0.81 $^{i}$ |
| | Ethanol | 81.58 ± 1.89 $^{e}$ | 84.30 ± 1.85 $^{g}$ | NONE | 21.38 ± 0.85 $^{e}$ |
| | 80% Ethanol | 115.61 ± 5.22 $^{i}$ | 74.48 ± 0.46 $^{f}$ | NONE | 14.43 ± 0.34 $^{g}$ |
| Standard Antioxidant | Trolox * | 2.19 ± 0.23 $^{a}$ | 3.16 ± 0.17 $^{a}$ | 86.48 ± 2.68 $^{a}$ | N.T. |
| | BHT * | 10.06 ± 0.32 $^{b}$ | 5.18 ± 0.14 $^{a}$ | 72.33 ± 1.06 $^{b}$ | N.T. |
| | Curcumin * | N.T. | N.T. | N.T. | 74.97 ± 0.53 $^{a}$ |

$^{a–j}$ Columns with differing superscripts signify a statistically significant difference ($p < 0.05$). * Used as a standard antioxidant; N.T. indicates no test.

#### 2.4.2. Ferric-Reducing Antioxidant Power (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

To scientifically and accurately assess the ability of antioxidants, researchers have developed various methods, with reducing ability being a key evaluation index [25]. FRAP and CUPRAC are the most commonly used and widely recognized methods that can reveal the reducing ability of antioxidants from different perspectives (Table 3). The 80% ethanol extract of ABR demonstrated a certain reduction ability in both tests, which was stronger than that of the other extracts. However, compared to trolox, both ABAP and ABR extracts exhibited lower antioxidant activity levels [6], suggesting that the reduction ability of *Anemone baicalensis* is relatively weak.

**Table 3.** The antioxidant capacity of ABR and ABAP was determined using FRAP, CUPRAC, and metal chelation assays.

| Medicament Portions | Extracting Solvents | $TEAC_{FRAP}$ | $TEAC_{CUPRAC}$ | Iron Chelation ($IC_{50}$, μg/mL) | Copper Chelation ($IC_{50}$, μg/mL) |
|---|---|---|---|---|---|
| ABAP | Methanol | 0.22 ± 0.00 $^{b}$ | 0.14 ± 0.00 $^{c}$ | 921.30 ± 4.04 $^{b}$ | 473.20 ± 4.85 $^{d}$ |
| | Water | 0.20 ± 0.00 $^{d}$ | 0.13 ± 0.00 $^{d}$ | 2019.96 ± 13.63 $^{f}$ | 347.42 ± 3.84 $^{c}$ |
| | Ethanol | 0.18 ± 0.00 $^{f}$ | 0.06 ± 0.00 $^{f}$ | 1481.40 ± 11.92 $^{d}$ | 1243.17 ± 5.87 $^{h}$ |
| | 80% Ethanol | 0.21 ± 0.00 $^{c}$ | 0.17 ± 0.00 $^{b}$ | 1065.22 ± 10.94 $^{c}$ | 317.04 ± 3.12 $^{b}$ |
| ABR | Methanol | 0.17 ± 0.00 $^{g}$ | 0.02 ± 0.00 $^{h}$ | 2388.66 ± 14.81 $^{g}$ | 1547.81 ± 21.16 $^{i}$ |
| | Water | 0.17 ± 0.00 $^{g}$ | 0.06 ± 0.00 $^{f}$ | >2500 $^{d}$ | 585.59 ± 23.27 $^{f}$ |
| | Ethanol | 0.18 ± 0.00 $^{f}$ | 0.05 ± 0.00 $^{g}$ | >2500 $^{d}$ | 1164.63 ± 35.17 $^{g}$ |
| | 80% Ethanol | 0.19 ± 0.00 $^{e}$ | 0.09 ± 0.00 $^{e}$ | 2096.62 ± 20.14 $^{e}$ | 519.30 ± 16.35 $^{e}$ |
| Standard Antioxidant | Trolox * | 0.91 ± 0.02 $^{a}$ | 0.90 ± 0.02 $^{a}$ | N.T. | N.T. |
| | $EDTANa_2$ * | N.T. | N.T. | 2.85 ± 0.07 $^{a}$ | 32.26 ± 0.16 $^{a}$ |

$^{a–i}$ Columns with differing superscripts signify a statistically significant difference ($p < 0.05$). * Used as a standard antioxidant; N.T. indicates no test.

#### 2.4.3. Metal Chelation

Metal ion chelating ability is an important property of antioxidants, referring to the capacity of antioxidants to combine with metal ions and form stable complexes. It is a crucial method for assessing their antioxidant activity in vitro [26]. In this study, the 80% ethanol extract displayed significant advantages in metal ion chelation (Table 3). Although ABAP and ABR extracts are significantly less effective than $EDTANa_2$ in metal ion chelation, this comparison still demonstrates that they possess certain antioxidant activities. However, compared to ABAP, ABR achieved only half of its chelating capacity [6]. The strong metal ion chelating ability demonstrated by ABAP may be closely related to its high concentration of antioxidant components. This unique property may result from its flavonoids, which effectively chelate metal ions, thus showing potential value in the field of antioxidation.

#### 2.4.4. Hydrogen Peroxide ($H_2O_2$), Singlet Oxygen and Hypochlorous Acid (HClO)

$H_2O_2$, a widely recognized oxidative stress inducer, is commonly employed to assess the protective effect of antioxidants against oxidative damage and to assess their antioxidant activity. Singlet oxygen is a highly reactive molecular form of oxygen that can cause extensive biological damage, making antioxidants that can efficiently scavenge singlet oxygen vital for protecting organisms from oxidative stress. As a strong oxidant, HClO is also included in the evaluation system of antioxidant capacity [27]. The results indicated that methanol and 80% ethanol extracts exhibited certain antioxidant potential. They are capable of effectively scavenging both $H_2O_2$ and HClO, in addition to having some ability to scavenge singlet oxygen (Table 4). Compared to standard antioxidants, the extracts of ABAP and ABR demonstrated relatively lower performance in these experiments. In another study, water and ethanol extracts of *Anemone nemorosa* were found to contain a high concentration of polyphenols and demonstrated significant $H_2O_2$ scavenging capacity [28]. Pretreatment with rosmarinic acid, identified from ABR, significantly inhibited $H_2O_2$-induced ROS generation in N2A cells [29]. Hederacolchiside F, identified from ABR, demonstrated significant antioxidant activities, including DPPH, superoxide, and $H_2O_2$ scavenging, reducing power, and metal ion chelating capabilities [30]. Surprisingly, ABR demonstrated superior HClO scavenging ability compared to ABAP [6], highlighting its potential in the field of antioxidation. Notably, as a plant rich in antioxidant components, different parts of *Anemone baicalensis* exhibit their unique advantages in antioxidant activity. Therefore, an in-depth study of the antioxidant capacity of various parts of *Anemone baicalensis* can lead to the development of more efficient and safe antioxidants.

**Table 4.** The antioxidant capacity of ABR and ABAP was evaluated through assays involving $H_2O_2$, singlet oxygen, $\beta$-carotene bleaching, and HClO.

| Medicament Portions | Extracting Solvents | $H_2O_2$ ($IC_{50}$, μg/mL) | Singlet Oxygen (%, 2000 μg/mL) | HClO ($IC_{50}$, μg/mL) | $\beta$-Carotene Bleaching AAC |
|---|---|---|---|---|---|
| ABAP | Methanol | 1143.52 ± 6.17 $^{c}$ | 15.30 ± 1.29 $^{g}$ | NONE | 496.45 ± 2.59 $^{c}$ |
| | Water | 970.74 ± 8.13 $^{b}$ | 5.91 ± 0.39 $^{i}$ | NONE | 443.68 ± 2.12 $^{g}$ |
| | Ethanol | 1522.10 ± 10.39 $^{e}$ | 25.40 ± 1.46 $^{f}$ | NONE | 482.21 ± 2.29 $^{e}$ |
| | 80% Ethanol | 1393.38 ± 8.05 $^{d}$ | 40.20 ± 1.18 $^{b}$ | NONE | 483.12 ± 2.37 $^{d}$ |
| ABR | Methanol | 2035.32 ± 22.95 $^{h}$ | 29.45 ± 1.64 $^{e}$ | 812.45 ± 6.60 $^{b}$ | 135.98 ± 1.67 $^{i}$ |
| | Water | 1801.87 ± 31.30 $^{f}$ | 7.79 ± 0.74 $^{h}$ | >990 $^{e}$ | 65.80 ± 4.41 $^{j}$ |
| | Ethanol | 1970.25 ± 30.83 $^{g}$ | 37.71 ± 0.46 $^{d}$ | 852.51 ± 7.29 $^{c}$ | 282.88 ± 5.63 $^{g}$ |
| | 80% Ethanol | 2491.80 ± 26.05 $^{i}$ | 39.36 ± 0.28 $^{c}$ | 956.60 ± 8.49 $^{d}$ | 458.58 ± 5.43 $^{f}$ |

**Table 4.** *Cont.*

| Medicament Portions | Extracting Solvents | $H_2O_2$ ($IC_{50}$, μg/mL) | Singlet Oxygen (%, 2000 μg/mL) | HClO ($IC_{50}$, μg/mL) | β-Carotene Bleaching AAC |
|---|---|---|---|---|---|
| Standard Antioxidant | Trolox * | N.T. | N.T. | 15.62 ± 0.46 [a] | N.T. |
| | Lipoic acid * | N.T. | N.T. | 26.58 ± 0.52 [a] | N.T. |
| | Gallic acid * | 36.62 ± 0.97 [a] | N.T. | N.T. | N.T. |
| | Ferulic acid * | N.T. | 88.25 ± 1.30 [a] | N.T. | N.T. |
| | BHT * | N.T. | N.T. | N.T. | 875.53 ± 6.33 [a] |
| | TBHQ * | N.T. | N.T. | N.T. | 799.85 ± 3.88 [b] |

[a–j] Columns with differing superscripts signify a statistically significant difference ($p < 0.05$). * Used as a standard antioxidant; N.T. indicates no test.

#### 2.4.5. β-Carotene Bleaching and Nitric Oxide (NO)

β-Carotene bleaching serves as an efficient and practical antioxidant screening method, leveraging the characteristic that β-carotene is easily oxidized by free radicals. When antioxidants effectively remove or slow the generation of free radicals, the bleaching rate of β-carotene is significantly reduced, reflecting the protective ability of antioxidants [31]. As a unique free radical, NO has the capability to react with other free radicals and scavenge them, thereby playing an essential antioxidant role in vivo [32]. The results showed that the 80% ethanol extract performed excellently in the β-carotene bleaching experiment, outperforming other extracts (Table 4, Figure 3). Similarly, the 80% ethanol extract and water extract also demonstrated good NO scavenging abilities (Figure 4). The 80% ethanol extract of ABR exhibited performance comparable to ABAP [6] in inhibiting the bleaching rate of β-carotene and scavenging NO; however, compared to the standard antioxidants BHT and BHA, its activity remains relatively weak. According to previous report, hederacolchiside A1, isolated from *Anemones raddeanae* Rhizoma, has been shown to effectively reduce plasma NO levels in patients exposed to PM2.5 [33]. These findings are consistent with our results.

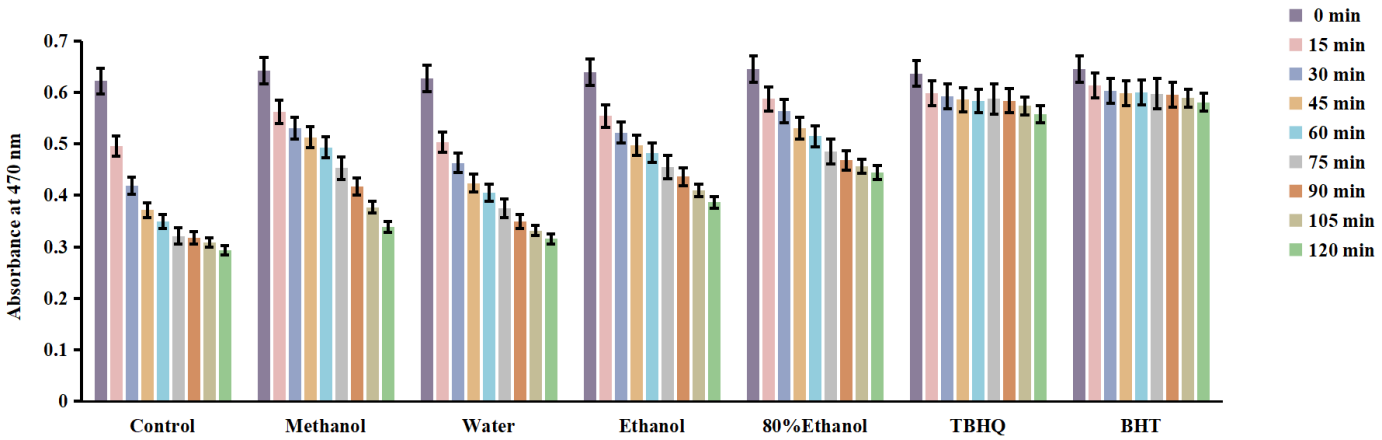



**Figure 3.** Changes in the absorbance of β-carotene were observed when different extracts of ABR were present.

The 80% ethanol and methanol extracts of ABR demonstrate a higher extraction rate and contain elevated levels of TCC, $TP_{he}C$, and $TT_{ri}C$ (Table 1). They both exhibit excellent antioxidant performance in in vitro antioxidant activity experiments. They effectively scavenge DPPH and ABTS free radicals and show strong metal ion chelating ability. These outstanding antioxidant properties may be attributed to the phenolics and triterpenoids present in Anemone baicalensis. These components inhibit oxidative reactions through

various complex biological mechanisms, thereby protecting cells from oxidative stress. Compared to ABR, ABAP has advantages in its content of active ingredients and antioxidant activity [6], highlighting the differences in antioxidant activity within different parts of plants. This suggests that careful selection of plant parts is important when developing plant resources. Additionally, triterpenoid saponins isolated from Anemones raddeanae Rhizoma also demonstrate significant antioxidant effects [7], further confirming the antioxidant potential and substitutability of these two plant rhizomes. A review highlighted that plants of the Anemone genus exhibit significant antioxidant activity due to their high content of triterpenoid saponins [34]. Therefore, although ABAP and ABR extracts do not match the antioxidant activity of standard antioxidants, Anemone baicalensis, as a natural plant resource, still demonstrates notable antioxidant potential in this series of comparative studies. These findings highlight the considerable potential of Anemone plants in the field of natural antioxidants.

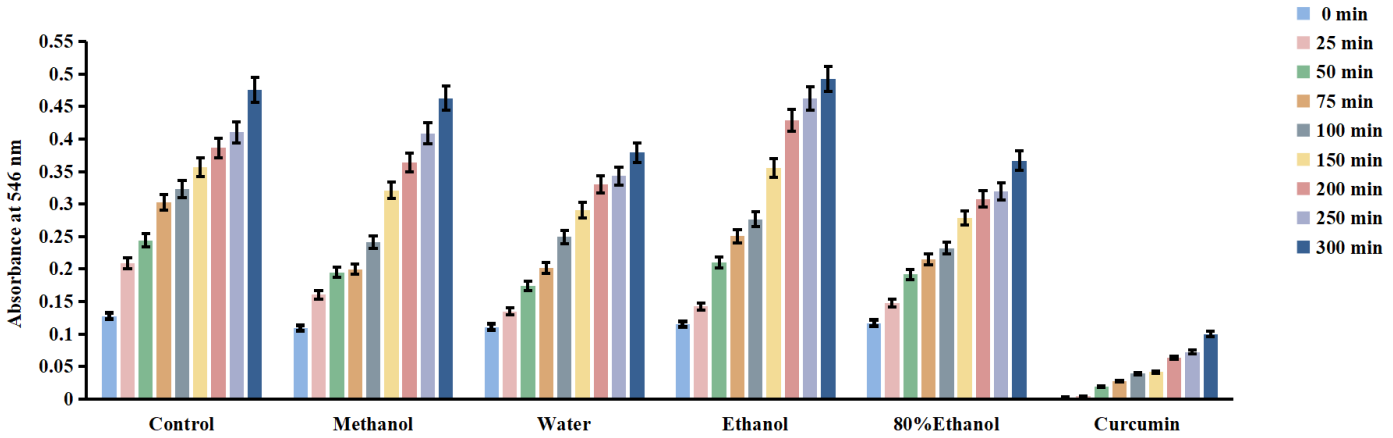



**Figure 4.** The NO scavenging method was used to evaluate how the absorbance of different extracts of ABR changed over time.

### *2.5. Stability Studies of Methanol Extract and 80% Ethanol Extract of ABR*

Previous results exhibited that both 80% ethanol and methanol extracts are rich in active ingredients and possess excellent antioxidant activity. As a result, they were chosen for detailed study, including three stability tests, with results displayed in Figures 5–7.

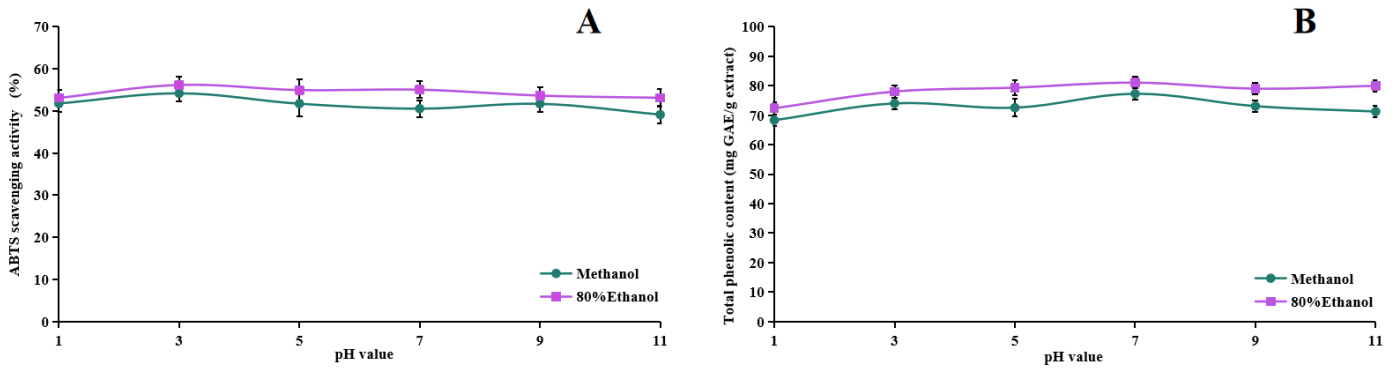



**Figure 5.** The stability of methanol and 80% ethanol extracts was evaluated at various pH levels using ABTS (**A**) and $TP_{he}C$ (**B**) assays.

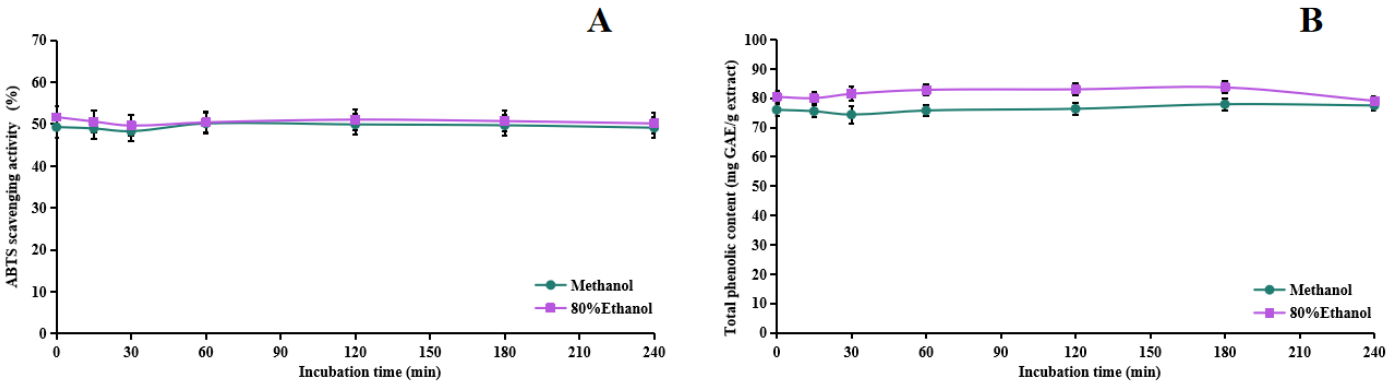



**Figure 6.** The thermal stability of the methanol and 80% ethanol extract was assessed using ABTS (**A**) and $TP_{he}C$ (**B**) assays.

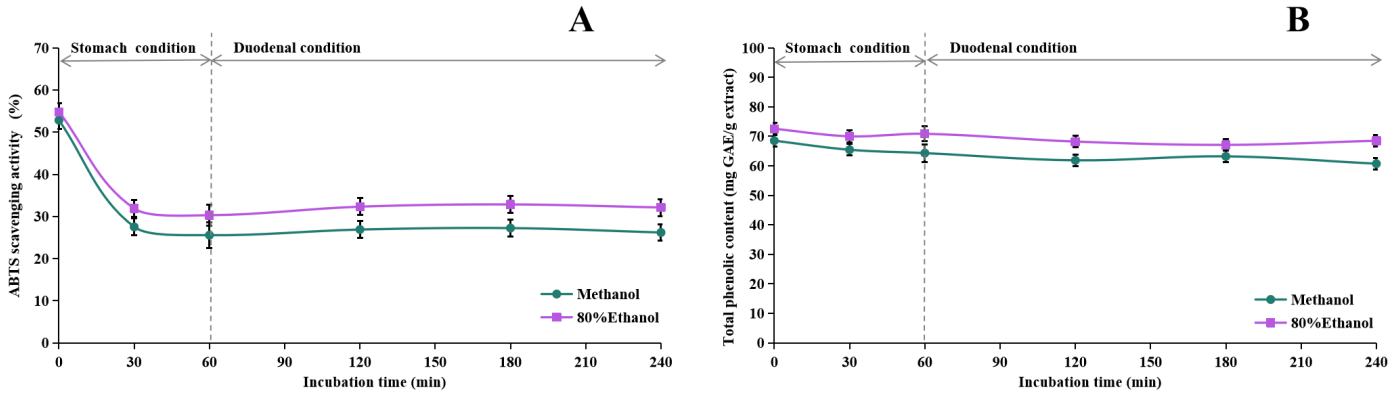



**Figure 7.** The in vitro digestive stability of the methanol and 80% ethanol extract was evaluated using ABTS (**A**) and $TP_{he}C$ (**B**) assays.

The stability study results revealed that both extracts maintain good stability across various pH values. In high-temperature tests, although ABTS values decreased, this is likely due to the adverse effects of temperature on the stability of phenolic components. When simulating the gastrointestinal environment, it was observed that ABTS values initially decreased and then stabilized, likely due to their relative stability under acidic conditions; however, in a strong acidic environment, ABTS values significantly decreased over time. These findings align with the stability test results of ABAP [6]. When comparing the stability of the two extracts, the 80% ethanol extract demonstrated significantly better stability than the methanol extract. This finding provides robust data support for future in-depth research.

### *2.6. UHPLC-MS Analysis*

This study investigates the chemical profile of the 80% ethanol extract from ABR using UHPLC-ESI-Q-TOF-MS analysis. By carefully comparing molecular and fragment ions with reference data sourced from both published literature (the analysis process of each peak for further details) and recognized public databases such as the Human Metabolome Database (https://www.hmdb.ca/, accessed on 27 December 2024) and MassBank (https://www.massbank.jp/, accessed on 27 December 2024), we compiled a list of thirty-eight bioactive compounds (Table 5), with their structural forms depicted in Figure S1. Furthermore, Figure S2 displays the UHPLC-MS results in positive-ion mode, and comprehensive MS and MS/MS spectra can be found in the Supplementary Materials (Figures S3–S84).

**Table 5.** Compounds identified in 80% ethanol extract of ABR.

| No. | RT (min) | Identification | Molecular Formula | Selective Ion | Full Scan MS (*m*/*z*) | | MS/MS Fragments (*m*/*z*) |
|---|---|---|---|---|---|---|---|
| | | | | | Theory | Measured | |
| 1 | 0.84 | Sucrose | $C_{12}H_{22}O_{11}$ | $[M + NH_4]^+$ | 360.1506 | 360.1419 | 342.1313, 325.1067, 312.1210 |
| 2 | 0.93 | Biotin | $C_{10}H_{16}N_2O_3S$ | $[M + NH_4]^+$ | 262.1226 | 262.1221 | 245.1075, 229.1481 |
| 3 | 1.28 | *L*-Pyroglutamic acid | $C_5H_7NO_3$ | $[M + H]^+$ | 130.0504 | 130.0461 | 113.0310, 101.0754 |
| 4 | 1.35 | *L*-Saccharopine | $C_{11}H_{20}N_2O_6$ | $[M]^+$ | 276.1321 | 276.1376 | 294.1475, 132.0980 |
| 5 | 2.43 | *N*-Fructosyl phenylalanine | $C_{15}H_{21}NO_7$ | $[M + H]^+$ | 328.1396 | 328.1311 | 310.1208, 178.1176, 250.1586 |
| 6 | 3.37 | $\gamma$-Glutamyltyrosine | $C_{14}H_{18}N_2O_6$ | $[M + Na]^+$ | 333.1063 | 333.1080 | 166.0819, 145.0028 |
| 7 | 3.79 | Histidine | $C_6H_9N_3O_2$ | $[M]^+$ | 155.0695 | 155.0659 | 137.0558 |
| 8 | 4.37 | Valine | $C_5H_{11}NO_2$ | $[M + H]^+$ | 118.0868 | 118.0828 | 135.0415, 100.0727 |
| 9 | 4.51 | L-Phenylalanine | $C_9H_{11}NO_2$ | $[M + Na]^+$ | 188.0688 | 188.0655 | 150.0534 |
| 10 | 4.53 | Rosmarinic acid | $C_{18}H_{16}O_8$ | $[M + NH_4]^+$ | 378.1189 | 378.1312 | 361.1048, 163.0148, 135.0403 |
| 11 | 5.52 | *L*-Threonyl-*L*-leucine | $C_{10}H_{20}N_2O_4$ | $[M + H]^+$ | 233.1501 | 233.1441 | 216.1182, 169.1294, 118.0827 |
| 12 | 7.10 | Chlorogenic acid | $C_{16}H_{18}O_9$ | $[M + H]^+$ | 355.1029 | 355.0946 | 310.1693, 163.0347, 135.0405 |
| 13 | 7.43 | Naringin | $C_{27}H_{32}O_{14}$ | $[M + H]^+$ | 581.1870 | 581.1954 | 603.1769, 163.0346 |
| 14 | 8.83 | 6-*O*-Feruloylglucose | $C_{16}H_{20}O_9$ | $[M + Na]^+$ | 379.1005 | 379.0940 | 194.1131, 177.0500 |
| 15 | 8.94 | 1-*O*-Feruloyl-$\beta$-D-glucose | $C_{16}H_{20}O_9$ | $[M + NH_4]^+$ | 374.1451 | 374.1361 | 194.1126, 163.0134 |
| 16 | 9.08 | Benzyl $\beta$-primeveroside | $C_{18}H_{26}O_{10}$ | $[M + NH_4]^+$ | 420.1870 | 420.1778 | 313.1698, 149.0566 |
| 17 | 17.68 | Unknown | | | | 205.0557 | 183.0741, 155.0433 |
| 18 | 22.61 | Unknown | | | | 701.4847 | 679.5028, 340.2544 |
| 19 | 24.53 | Polygalasaponin XXIX | $C_{64}H_{102}O_{33}$ | $[M + NH_4]^+$ | 1416.6647 | 1416.6727 | 737.5043, 575.4238 |
| 20 | 26.08 | Unknown | | | | 905.6681 | 569.4659, 453.3367 |
| 21 | 26.77 | Pulsatiloside A | $C_{47}H_{76}O_{18}$ | $[M + H]^+$ | 929.5110 | 929.4915 | 913.5043, 767.4475, 751.4464 |
| 22 | 27.64 | Leonloside D | $C_{59}H_{96}O_{27}$ | $[M + H]^+$ | 1237.6217 | 1237.5965 | 1075.5557, 960.5368, 767.4448 |
| 23 | 31.67 | Hederacolchiside F | $C_{65}H_{106}O_{31}$ | $[M + NH_4]^+$ | 1400.7062 | 1400.6804 | 1335.5651, 340.2525 |
| 24 | 34.42 | Raddeanoside Ra | $C_{41}H_{66}O_{13}$ | $[M + NH_4]^+$ | 784.4847 | 784.4686 | 455.3449, 437.3328 |
| 25 | 36.35 | 3-*O*-$\beta$-D-glucopyranosyl-(1-2)-$\beta$-D-glucopyranosyl-(1-6)-$\beta$-D-galactopyranosyl-hederagenin | $C_{48}H_{78}O_{19}$ | $[M + H]^+$ | 959.5215 | 959.5059 | 797.4584 |
| 26 | 37.12 | Phytosphingosine | $C_{18}H_{39}NO_3$ | $[M + H]^+$ | 318.3008 | 318.2930 | 340.2535, 183.0741, 135.0415 |
| 27 | 37.23 | Leontoside B | $C_{41}H_{66}O_{13}$ | $[M + H]^+$ | 767.4581 | 767.4433 | 587.3847, 455.3447 |
| 28 | 37.78 | Lauryldiethanolamine | $C_{16}H_{35}NO_2$ | $[M + H]^+$ | 274.2746 | 274.2672 | 155.0431, 118.0831, 100.0730 |
| 29 | 38.04 | 27-Hydroxyoleanolic acid 3-*O*-*β*-*D*-glucopyranosyl (1-2)-*α*-*L*-arabinopyranoside | $C_{41}H_{66}O_{13}$ | $[M + H]^+$ | 767.4581 | 767.4410 | 789.4286, 455.3447, 437.3341 |
| 30 | 39.23 | Raddeanoside R22b | $C_{47}H_{76}O_{17}$ | $[M + NH_4]^+$ | 930.5427 | 930.5240 | 589.3988, 571.3921, 439.3496 |
| 31 | 39.79 | Raddeanoside R13 | $C_{47}H_{76}O_{17}$ | $[M + NH_4]^+$ | 930.5427 | 930.5239 | 788.4270, 603.2123 |
| 32 | 40.96 | Dehydrophytosphingosine | $C_{18}H_{37}NO_3$ | $[M + H]^+$ | 316.2851 | 316.2774 | 140.1149, 135.0405, 127.0123 |
| 33 | 41.53 | Gingerglycolipid A | $C_{33}H_{56}O_{14}$ | $[M + NH_4]^+$ | 694.4014 | 694.3871 | 353.2628, 295.2204 |
| 34 | 41.89 | 1-Palmitoylglycerophosphoinositol | $C_{25}H_{49}O_{12}P$ | $[M + H]^+$ | 573.3040 | 573.2914 | 555.2839, 537.2757 |
| 35 | 42.02 | 9-Eicosenedioic acid | $C_{20}H_{36}O_4$ | $[M]^+$ | 340.2614 | 340.2525 | 295.2210, 281.2423 |
| 36 | 42.44 | Sphinganine | $C_{18}H_{39}NO_2$ | $[M + H]^+$ | 302.3059 | 302.2980 | 230.8856, 127.0124, 100.0730 |
| 37 | 45.97 | Linolenic acid | $C_{18}H_{30}O_2$ | $[M + H]^+$ | 279.2324 | 279.2245 | 183.0739, 149.1036 |
| 38 | 47.21 | Stearidonic acid | $C_{18}H_{28}O_2$ | $[M + H]^+$ | 277.2167 | 277.2086 | 155.0428 |
| 39 | 47.60 | Palmitoleic acid | $C_{16}H_{30}O_2$ | $[M + Na]^+$ | 277.2144 | 277.2088 | 196.9613, 140.1150, 130.1555 |
| 40 | 48.10 | 13-HOTrE | $C_{18}H_{30}O_3$ | $[M + H]^+$ | 295.2273 | 295.2190 | 277.2105, 235.1869, 222.1077 |
| 41 | 48.60 | Erucamide | $C_{22}H_{43}NO$ | $[M + H]^+$ | 338.3423 | 338.3330 | 279.1533, 155.0429 |

RT: retention time; ABR: *Anemone baicalensis* rhizome.

This comprehensive chemical analysis of the extract provides useful information about the diverse range of compounds present within it. Some of these compounds have also been isolated from the *Anemone* genus in previous studies. These discoveries highlight the role of these compounds in contributing to the therapeutic potential of ABR and provide a solid basis for future research. By zeroing in on specific compounds linked to the extract's antioxidant effects, this study lays the groundwork for targeted therapeutic innovations in medicinal utilizations, paving the way for future developments.

Peak 1 was identified as sucrose by correlating fragment ions ($m/z$ 342.1313 $[M]^+$, 325.1067 $[M - OH]^+$, and 312.1210 $[M - CH_2OH + H]^+$) with reference [35]. Peak 2, with $m/z$ 262.1221, had $MS^2$ ions at $m/z$ 245.1075 $[M + H]^+$ and 229.1481 $[M - O]^+$ and was tentatively identified as biotin [6]. Peak 3 registered an $m/z$ of 130.0461 with fragment ions at $m/z$ 113.0310 $[M - OH + H]^+$ and 101.0754 $[M - CO]^+$, presumptively identified as *L*-pyroglutamic acid [36]. Peak 4 displayed a $[M]^+$ ion at $m/z$ 276.1376 with its main fragment ions at $m/z$ 294.1475 $[M + NH_4]^+$ and 132.0980 $[M - C_6H_{13}N_2O_2 + H]^+$, characteristic of *L*-saccharopine [6]. Peak 5 ($m/z$ 328.1311) was identified as *N*-fructosyl phenylalanine, based on typical fragment ions at $m/z$ 310.1208 $[M - OH]^+$, 178.1176 $[M - C_9H_9O_2]^+$, and 250.1586 $[M - C_6H_5]^+$ [6]. Peak 6, with an $m/z$ of 333.1080, gave fragment ions at $m/z$ 166.0819 and 145.0028, relating to the loss of $-C_5H_9N_2O_3 + H$ and $-C_9H_9O_3$, suggesting it was $\gamma$-glutamyltyrosine [37]. Peak 7 appeared at $m/z$ 155.0659 with an $MS^2$ ion at $m/z$ 137.0558 $[M - H_2O]^+$, identified as histidine [38]. Peak 8 at $m/z$ 118.0828 showed $MS^2$ ions at $m/z$ 135.0415 $[M + NH_4]^+$ and 100.0727 $[M - OH]^+$, identified as valine [39]. The precursor ion $[M + Na]^+$ of peak 9 was observed at $m/z$ 188.0655 with its main fragment ion at $m/z$ 150.0534 $[M - NH_2 + H]^+$, corresponding to *L*-phenylalanine [6]. Peak 10 showed an $[M + NH_4]^+$ ion at $m/z$ 378.1312, yielding significant fragment ions at $m/z$ 361.1048 $[M + H]^+$, 163.0148 $[M - C_9H_9O_5]^+$, and 135.0403 $[M - C_{10}H_9O_6]^+$, indicative of rosmarinic acid [40].

The principal fragment ions for peak 11 ($m/z$ 233.1441) appeared at $m/z$ 216.1182 $[M - OH + H]^+$, 169.1294 $[M - COOH - H_2O]^+$, and 118.0827 $[M - C_6H_{11}O_2 + H]^+$, attributed to *L*-threonyl-*L*-leucine [41]. Peak 12 was identified as chlorogenic acid with an ion at $m/z$ 355.0946. The $MS^2$ spectrum demonstrated losses at $m/z$ 310.1693, 163.0347, and 135.0405, corresponding to the loss of $-COOH + H$, $-C_8H_{11}O_7$, and $-C_7H_{11}O_6$ [6]. Peak 13's mass spectrum showed an ion at $m/z$ 581.1954, and the $MS^2$ spectrum displayed fragments at $m/z$ 603.1769 $[M + Na]^+$ and 163.0346 $[C_6H_{11}O_5]^+$, identified as naringin [42]. Peak 14 had an $[M + Na]^+$ ion at $m/z$ 379.0940, producing primary fragments at $m/z$ 194.1131 $[M - C_6H_{11}O_5 + H]^+$, and 177.0500 $[M - C_6H_{11}O_6]^+$, characteristic of 6-*O*-feruloylglucose [43]. Peak 15 was detected at $m/z$ 374.1361 with fragment ions at $m/z$ 194.1126 $[M - C_6H_{11}O_5 + H]^+$, and 163.0134 $[C_6H_{11}O_5]^+$, identified as 1-*O*-feruloyl-$\beta$-*D*-glucose [6]. Peak 16 ($m/z$ 420.1778) was identified as benzyl $\beta$-primeveroside, based on fragment ions at $m/z$ 313.1698 $[M - C_7H_7O + NH_4]^+$ and 149.0566 $[C_5H_9O_5]^+$ [6]. Peak 17's mass spectrum showed an ion at $m/z$ 205.0557, with $MS^2$ fragments at $m/z$ 183.0741 and 155.0433. The mass spectrum of peak 18 showed an ion at $m/z$ 701.4847, with $MS^2$ fragments at $m/z$ 679.5028 and 340.2544. Peak 19, with $m/z$ 1416.6727, was identified as *polygalasaponin* XXIX, featuring fragments at $m/z$ 737.5043 and 575.4238, linked to the loss of $-C_{36}H_{54}O_{12} + H$ and $-C_{42}H_{64}O_{16} + H$ [44]. Peak 20's spectrum displayed an ion at $m/z$ 905.6681, with $MS^2$ fragments at $m/z$ 569.4659 and 453.3367.

Peak 21, observed at $m/z$ 929.4915, was characterized as pulsatiloside A; its $MS^2$ ions at $m/z$ 913.5043, 767.4475, and 751.4464 marked the loss of $-CH_3$, $-C_6H_{10}O_5 + H$, and $-C_6H_{10}O_6 + H$, respectively [6]. Peak 22 had an $m/z$ 1237.5965, with $MS^2$ ions at $m/z$ 1075.5557 $[M - C_6H_{10}O_5 + H]^+$, 960.5368 $[M - C_{11}H_{18}O_9 + NH_4]^+$, and 767.4448 $[M - C_{18}H_{30}O_{14} + H]^+$, tentatively identified as leonloside D from literature [6]. Peak 23,

with $m/z$ 1400.6804, showed $MS^2$ ions at $m/z$ 1335.5651 [M − OH − $CH_2OH$ + H]$^+$ and 340.2525 [M − $C_{52}H_{83}O_{21}$ + H]$^+$, provisionally identified as hederacolchiside F [6]. Peak 24 at $m/z$ 784.4686 was suggested as raddeanoside Ra, with key $MS^2$ ions at $m/z$ 455.3449 and 437.3328, indicating the loss of −$C_{11}H_{19}O_{10}$ and −$C_{11}H_{19}O_{10}$ − $H_2O$ [45]. Peak 25 had an $m/z$ of 959.5059, with an $MS^2$ ion at $m/z$ 797.4584 [M − $C_6H_{10}O_5$ + H]$^+$, identified as 3-*O*-*β*-*D*-glucopyranosyl-(1-2)-*β*-*D*-glucopyranosyl-(1-6)-*β*-*D*-galactopyranosyl-hederagenin [6]. Peak 26 had an $m/z$ of 318.2930, with key fragments at $m/z$ 340.2535 [M + Na]$^+$, 183.0741 [$C_{13}H_{27}$]$^+$, and 135.0415 [M − $C_{13}H_{27}$ + H]$^+$, identified as phytosphingosine [46]. Peak 27's ion [M + H]$^+$ at $m/z$ 767.4433 generated major fragments at $m/z$ 587.3847 [M − $C_6H_{11}O_6$]$^+$ and 455.3447 [M − $C_{11}H_{19}O_{10}$]$^+$, corresponding to leontoside B [47]. Peak 28 at $m/z$ 274.2672 was recognized as lauryldiethanolamine based on the fragment ions at $m/z$ 155.0431 [$C_{11}H_{23}$]$^+$, 118.0831 [M − $C_{11}H_{23}$]$^+$, and 100.0730 [$C_7H_{15}$ + H]$^+$. Peak 29 at $m/z$ 767.4410 with $MS^2$ ions at $m/z$ 789.4286 [M + Na]$^+$, 455.3447 [M − $C_{11}H_{19}O_{10}$]$^+$, and 437.3341 [M − $C_{11}H_{19}O_{10}$ − H2O]$^+$, identified as 27-hydroxyoleanolic acid 3-*O*-*β*-*D*-glucopyranosyl (1-2)-*α*-*L*-arabinopyranoside [48]. Peak 30, at $m/z$ 930.5240, was identified as raddeanoside R22b, indicated by fragment ions at $m/z$ 589.3988 [M − $C_{12}H_{20}O_{10}$ + H]$^+$, 571.3921 [M − $C_{12}H_{21}O_{11}$]$^+$, and 439.3496 [M − $C_{17}H_{29}O_{15}$]$^+$ [45].

Peak 31 exhibited [M + $NH_4$]$^+$ at $m/z$ 930.5239, generating notable fragment ions at $m/z$ 788.4270 [M − $C_6H_{11}O_4$ + Na]$^+$ and 603.2123 [M − $C_{12}H_{21}O_9$]$^+$, characteristic of raddeanoside R13 [6]. Peak 32, with a [M + H]$^+$ ion at $m/z$ 316.2774, was identified as dehydrophytosphingosine, featuring $MS^2$ ions at $m/z$ 140.1149 [$C_{10}H_{20}$]$^+$, 135.0405 [M − $C_{13}H_{25}$ + H]$^+$, and 127.0123 [$C_9H_{19}$]$^+$, corroborated by the literature [49]. Peak 33 showed an $m/z$ of 694.3871, with fragment ions at $m/z$ 353.2628 and 295.2204, resulting from the loss of −$C_{12}H_{20}O_{10}$ + H and −$C_{15}H_{27}O_{12}$ + $NH_4$, leading to the identification of gingerglycolipid A [6]. Peak 34, observed at $m/z$ 573.2914, was identified as 1-palmitoylglycerophosphoinositol, with $MS^2$ ions at $m/z$ 555.2839 and 537.2757, reflecting the loss of −OH and −OH − $H_2O$, respectively [6]. Peak 35, at $m/z$ 340.2525, displayed fragment ions at $m/z$ 295.2210 and 281.2423, indicating the loss of −COOH and −$CH_2COOH$, and was determined to be 9-eicosenedioic acid. Peak 36 was identified as sphinganine at $m/z$ 302.2980, with distinctive fragment ions at $m/z$ 230.8856 [$C_{13}H_{28}NO_2$]$^+$, 127.0124 [$C_9H_{19}$]$^+$, and 100.0730 [$C_7H_{15}$ + H]$^+$ [6]. Peak 37, at $m/z$ 279.2245, with $MS^2$ ions at $m/z$ 183.0739 [$C_{11}H_{19}O_2$]$^+$ and 149.1036 [$C_{11}H_{17}$]$^+$, was tentatively identified as linolenic acid based on the literature [6]. Peak 38 exhibited [M + H]$^+$ at $m/z$ 277.2086, producing a significant fragment ion at $m/z$ 155.0428 [$C_9H_{14}O_2$ + H]$^+$, characteristic of stearidonic acid [6]. Peak 39 had [M + Na]$^+$ at $m/z$ 277.2088, yielding substantial fragment ions at $m/z$ 196.9613 [$C_{14}H_{27}$ + H]$^+$, 140.1150 [$C_{10}H_9$ + H]$^+$ and 130.1555 [$C_7H_{13}O_2$ + H]$^+$, indicative of palmitoleic acid [6]. The precursor ion [M + H]$^+$ of peak 40 was at $m/z$ 295.2190, with primary fragment ions at $m/z$ 277.2105 [M − OH]$^+$, 235.1869 [M − $CH_2COOH$]$^+$, and 222.1077 [M − $CH_2CH_2COOH$]$^+$, corresponding to 13-HOTrE. Peak 41 showed a [M + H]$^+$ peak at $m/z$ 338.3330, producing primary fragment ions at $m/z$ 279.1533 [M − $C_2H_4NO$]$^+$ and 155.0429 [M − $C_{11}H_{21}NO$ + H]$^+$, indicative of erucamide [50].

In the chemical composition analysis, the similarity between ABAP and ABR is remarkably evident (Table 6). Their composition analysis clearly reveals that eighteen components are identical in both [6], including six triterpenoid saponins, five fatty acids, two phenolics, and five amino acids. This undoubtedly highlights the intrinsic relationship between ABAP and ABR in terms of chemical composition. However, upon further exploration of their differences, the unique characteristics of ABR gradually emerge. Compared to ABAP, ABR not only retains the common chemical components but also exhibits a higher abundance of triterpenoid saponins, amino acids, and phenolics. In contrast, the flavonoids present in ABAP are nearly absent in ABR. These compositional similarities and differences not

only endow *Anemone baicalensis* with unique chemical properties but also provide valuable insights into the distribution of chemical components across different parts of the plant.

**Table 6.** Comparison of compounds in ABR, ABAP and *Anemones raddeanae* Rhizoma.

| No. | RT (min) | Ingredient | ABR | ABAP | *Anemones raddeanae* Rhizoma |
|---|---|---|---|---|---|
| 1 | 0.84 | Sucrose | + | − | − |
| 2 | 0.93 | Biotin | + | + | − |
| 3 | 1.28 | *L*-Pyroglutamic acid | + | − | − |
| 4 | 1.35 | *L*-Saccharopine | + | + | − |
| 5 | 2.43 | *N*-Fructosyl phenylalanine | + | + | − |
| 6 | 3.37 | $\gamma$-Glutamyltyrosine | + | − | − |
| 7 | 3.79 | Histidine | + | − | + |
| 8 | 4.37 | Valine | + | − | + |
| 9 | 4.51 | *L*-Phenylalanine | + | + | − |
| 10 | 4.53 | Rosmarinic acid | + | − | − |
| 11 | 5.52 | *L*-Threonyl-*L*-leucine | + | − | − |
| 12 | 6.79 | Thymine | − | + | − |
| 13 | 7.10 | Chlorogenic acid | + | + | − |
| 14 | 7.43 | Naringin | + | − | − |
| 15 | 7.62 | 4-*O*-Caffeoylquinic acid | − | + | − |
| 16 | 8.83 | 6-*O*-Feruloylglucose | + | − | − |
| 17 | 8.94 | 1-*O*-Feruloyl-$\beta$-D-glucose | + | + | − |
| 18 | 9.08 | Benzyl $\beta$-primeveroside | + | + | − |
| 19 | 9.39 | 1-*O*-Caffeoylquinic acid | − | + | − |
| 20 | 9.80 | 4-*p*-Coumaroylquinic acid/3-*p*-Coumaroylquinic acid | − | + | − |
| 21 | 11.55 | 3-*O*-Feruloylquinic acid | − | + | − |
| 22 | 13.05 | Kaempferol 3-*O*-sophoroside 7-*O*-rhamnoside | − | + | − |
| 23 | 14.85 | Rutin | − | + | − |
| 24 | 15.30 | Kaempferol 3-*O*-sophoroside | − | + | − |
| 25 | 15.97 | Kaempferol 3-glucuronide | − | + | − |
| 26 | 18.88 | Apigenin 7-glucuronide | − | + | − |
| 27 | 19.86 | Diosmetin 7-glucuronide | − | + | − |
| 28 | 24.53 | Polygalasaponin XXIX | + | − | − |
| 29 | 25.84 | 1-*O*-{3-[(3-*O*-Hexopyranosylhexopyranosyl)oxy]-28-oxoolean-12-en-28-yl}hexopyranose | − | + | − |
| 30 | 26.37 | Hederagenin 28-*O*-$\beta$-*D*-glucopyranosyl-(1-3)-$\alpha$-*L*-rhamnopyranosyl-(1-4)-$\beta$-*D*-glucopyranosyl-(1-6)-$\beta$-*D*-glucopyranosyl ester | − | + | − |
| 31 | 26.77 | Pulsatiloside A | + | + | + |
| 32 | 26.95 | Oleanolic acid 28-*O*-$\beta$-*D*-glucopyranosyl-(1-3)-$\alpha$-*L*-rhamnopyranosyl-(1-4)-$\beta$-*D*-glucopyranosyl-(1-6)-$\beta$-*D*-glucopyranosyl ester | − | + | − |

**Table 6.** *Cont.*

| No. | RT (min) | Ingredient | ABR | ABAP | *Anemones raddeanae* Rhizoma |
|---|---|---|---|---|---|
| 33 | 27.64 | Leonloside D | + | + | + |
| 34 | 28.41 | Raddeanoside R18 | − | + | + |
| 35 | 29.98 | Cussonoside B | − | + | + |
| 36 | 31.67 | Hederacolchiside F | + | + | + |
| 37 | 32.37 | Anhuienoside E | − | + | − |
| 38 | 32.52 | Hederacolchiside E | − | + | + |
| 39 | 32.94 | Hederacoside C | − | + | − |
| 40 | 33.17 | Raddeanoside R14 | − | + | + |
| 41 | 34.42 | Raddeanoside Ra | + | − | + |
| 42 | 36.35 | 3-*O*-*β*-D-glucopyranosyl-(1-2)-*β*-D-glucopyranosyl-(1-6)-*β*-D-galactopyranosyl-hederagenin | + | + | + |
| 43 | 37.12 | Phytosphingosine | + | − | − |
| 44 | 37.23 | Leontoside B | + | − | + |
| 45 | 37.78 | Lauryldiethanolamine | + | − | − |
| 46 | 38.04 | 27-Hydroxyoleanolic acid 3-*O*-*β*-*D*-glucopyranosyl (1-2)-*α*-*L*-arabinopyranoside | + | − | + |
| 47 | 39.23 | Raddeanoside R22b | + | − | + |
| 48 | 39.79 | Raddeanoside R13 | + | + | + |
| 49 | 40.96 | Dehydrophytosphingosine | + | − | − |
| 50 | 41.53 | Gingerglycolipid A | + | + | − |
| 51 | 41.89 | 1-Palmitoylglycerophosphoinositol | + | + | − |
| 52 | 42.02 | 9-Eicosenedioic acid | + | − | − |
| 53 | 42.44 | Sphinganine | + | + | − |
| 54 | 45.97 | Linolenic acid | + | + | + |
| 55 | 47.21 | Stearidonic acid | + | + | − |
| 56 | 47.60 | Palmitoleic acid | + | + | − |
| 57 | 48.10 | 13-HOTrE | + | − | − |
| 59 | 48.60 | Erucamide | + | − | − |

(+) indicates presence; (−) indicates absence; RT: retention time; ABR: *Anemone baicalensis* rhizome; ABAP: *Anemone baicalensis* aerial part.

According to a detailed comparison of the composition analysis reports of ABR and *Anemones raddeanae* Rhizoma (Table 6), twelve components are consistent between them, including nine triterpenoid saponins [4]. When considering ABAP and ABR as a whole and comparing their components with those of *Anemones raddeanae* Rhizoma, the similarity in composition is further enhanced, with sixteen shared components (Table 6), including thirteen triterpenoid saponins. Specifically, the high degree of overlap in chemical composition between ABR and *Anemones raddeanae* Rhizoma offers a new perspective and framework for exploring their potential substitutability. However, despite the significant similarities in chemical composition, notable differences remain between them. This preliminary comparison, focusing on composition and pharmacology, suggests that further in-depth research

is necessary to determine whether true substitutability can be achieved. These findings serve as a bridge connecting ABR and *Anemones raddeanae* Rhizoma in terms of chemical composition, providing a fresh perspective for evaluating their substitutability.

Given the limited existing research on the chemical constituents of *Anemone baicalensis*, the information revealed by UHPLC-MS remains incomplete. Therefore, future research should focus on two key areas: First, advanced extraction and separation techniques should be utilized to isolate more chemical components from ABR, thereby enhancing our comprehensive understanding of its composition and deepening our knowledge of both ABR and *Anemones raddeanae* Rhizoma. Second, thorough studies should be conducted on the pharmacological similarities and differences of the shared triterpenoid saponins or extracts between ABR and *Anemones raddeanae* Rhizoma to provide a scientific basis for evaluating their substitutability.

### 2.7. Oral Acute Toxicity Study

In the oral acute toxicity experiment in mice, a notable phenomenon was observed: when twenty experimental mice did not show any adverse reactions or death during the 24 h monitoring period, it preliminarily suggested that the 80% ethanol extract of ABR may have relatively low acute toxicity. Additionally, it was reported in detail that mice orally administered the extract from *Anemones raddeanae* Rhizoma, extracted by petroleum ether, chloroform, and *N*-butanol at doses as high as 1000 mg/g, exhibited no obvious signs of poisoning during a 14-day observation period [51]. Another study found that administering 2.1 g of raw medicinal material per kilogram of body weight via gavage of *Anemones raddeanae* Rhizoma showed no acute toxicity in mice, with the minimum lethal dose determined to be 151.14 g of raw medicinal material per kilogram [52]. Additionally, both ABAP and ABR demonstrated relatively low acute toxicity [6]. This discovery not only broadens the understanding of the biological activity of *Anemone baicalensis* but also provides a more comprehensive safety reference for the development and application of this plant.

### 2.8. Hepatoprotective Activity

The health of the liver is directly linked to overall vitality and the body's ability to defend against diseases. Recent studies have demonstrated that triterpenoids play a crucial role in liver-protective agents due to their significant liver-protective effects [53]. This study found that the 80% ethanol extract of ABR was rich in triterpenoids and exhibited strong antioxidant activity in vitro. Consequently, a liver injury model experiment was designed to verify its antioxidant and hepatoprotective potential in vivo. D-galactosamine, widely used as an inducer of liver injury, effectively simulates liver damage [54]. Silymarin, a well-known natural antioxidant and liver-protecting agent, is favored for treating liver diseases due to its protective properties and therapeutic potential [55]. Thus, silymarin was selected as the positive control drug, and D-galactosamine as the liver injury inducer in this experiment to evaluate the hepatoprotective effect of the 80% ethanol extract of ABR.

The human equivalent dose (HED) can be estimated using body surface area normalization methods, as outlined in FDA guidelines. Based on the formula, the HED for a 150 mg/kg dose in rats would be approximately 24.3 mg/kg, while for a 300 mg/kg dose, it would be approximately 48.6 mg/kg. These estimates indicate that the doses used in the study fall within a reasonable range for potential human applications. Therefore, doses of 150 mg/kg and 300 mg/kg were selected for this study in rats.

Forty rats were randomly assigned to five different groups in total, with eight rats in each group, which received different oral treatment regimens. The control group (GI) received only 0.5% sodium carboxymethyl cellulose. The low-dose group (GII) and high-dose

group (GIII) were administered 150 mg/kg and 300 mg/kg of ABR 80% ethanol extract according to body weight, respectively. The positive control group (GIV) received 100 mg/kg of silymarin, while the model group (GV) was given 700 mg/kg of D-galactosamine to assess the damage degree. After 7 d of pretreatment, rats in the GII to GV groups received an intraperitoneal injection of D-galactosamine to induce liver injury. The findings indicated that, compared to GIV, the high-dose 80% ethanol extract substantially decreased the hepatic viscera index in rats (Figure 8). This finding indicated that the high-dose 80% ethanol extract of ABR had a pronounced effect in reducing liver injury and exhibited the same protective effect as silymarin in preventing liver function damage.

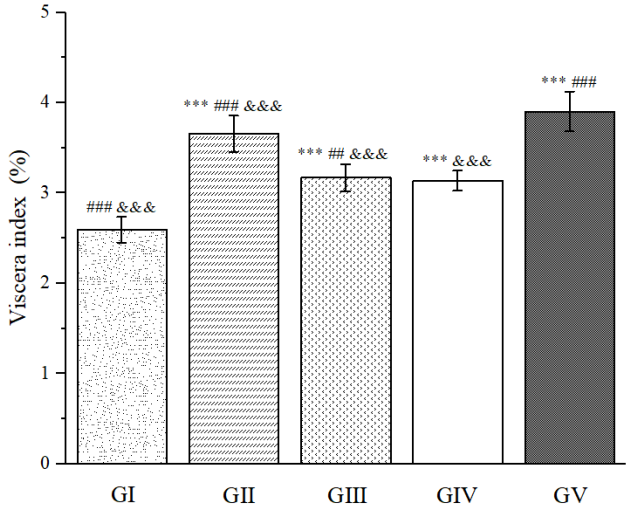



**Figure 8.** The effects of treating rats with liver injury with the 80% ethanol extract of ABR on their hepatic viscera index were investigated. The results are presented as the mean, with the standard error of the mean indicating variability ($n = 8$). Viscera index as follows: viscera index = viscera weight (g)/body weight (g) × 100%. GI: Control group; GII: D-GalN + $ABR_{150}$ group; GIII: D-GalN + $ABR_{300}$ group; GIV: D-GalN + SMN group; GV: D-GalN group. ABR: *Anemone baicalensis* rhizome; D-GalN: D-Galactosamine; SMN: Silymarin; significantly different from the control group at *** $p < 0.001$. Significantly different from the D-GalN + SMN group at ## $p < 0.01$ and ### $p < 0.001$. Significantly different from the D-GalN group at $^{\&\&\&}$ $p < 0.001$.

Alanine aminotransferase (ALT) is typically regarded as a liver-specific enzyme, which is released into the bloodstream in large quantities when hepatocytes are damaged, resulting in a notable elevation of ALT levels in serum. Therefore, fluctuations in ALT levels are widely used to assess the degree of liver injury [56]. The experimental results showed that, compared to GV, the ALT activity in rat serum decreased by 25.48% in GII and by 38.13% in GIII. In liver tissue, ALT activity decreased by 9.54% in GII and by 50.43% in GIII compared to GV (Figure 9). The ALT activity levels in GIII were very similar to those in GIV, further indicating that the degree of liver injury was comparable between the two groups.

In addition to ALT, aspartate aminotransferase (AST) is also significant in the auxiliary diagnosis of liver injury. When AST and ALT levels rise simultaneously, it further confirms the occurrence of liver injury. As marker enzymes of liver injury, ALT and AST are essential biomarkers used for assessing liver damage in rats [57]. The experimental results showed that AST activity in serum and liver tissue of injured rats was significantly increased. The trend in AST results was consistent with ALT results.

In rat serum, the AST activity of the 80% ethanol administration groups (GII and GIII) decreased compared to other injured groups, indicating that the 80% ethanol extract could inhibit the increase in AST activity. In liver tissue, compared to GV, AST activity decreased by 45.19% in GII and by 59.23% in GIII (Figure 9). This result indicates that the protective effect of the 80% ethanol extract on the liver exhibits a significant dose-dependent manner.

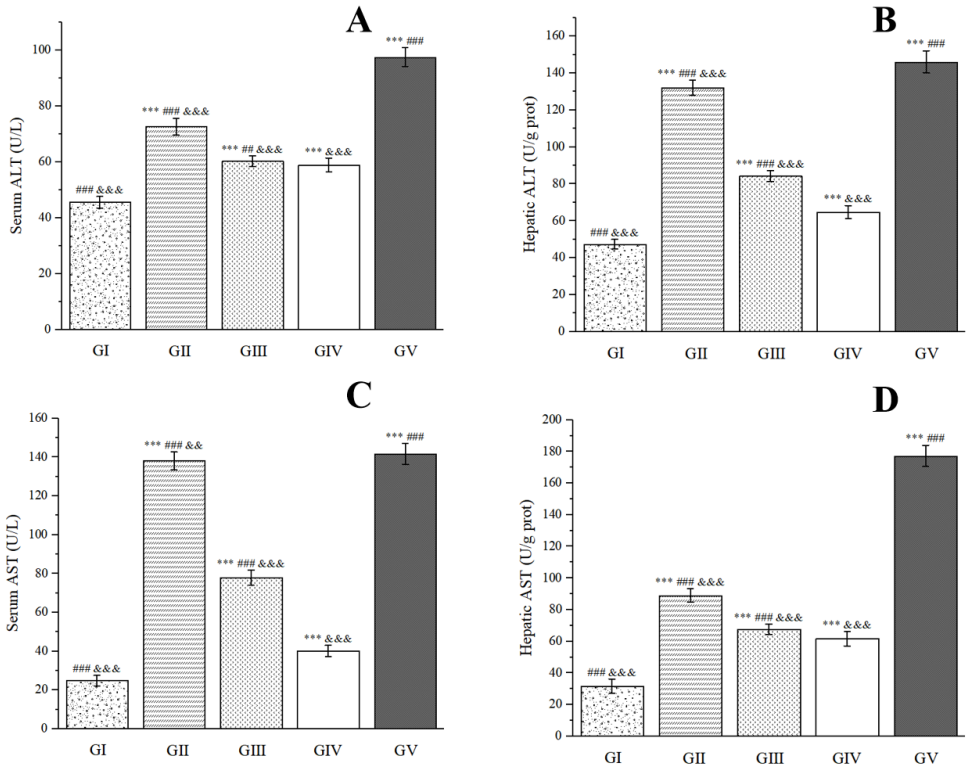



**Figure 9.** The study examines the impact of the 80% ethanol extract of ABR on serum ALT (**A**), hepatic ALT (**B**), serum AST (**C**), and hepatic AST (**D**) in rats exhibiting liver injury. Values are expressed as the mean ± standard error of the mean ($n$ = 8). GI: Control group; GII: D-GalN + $ABR_{150}$ group; GIII: D-GalN + $ABR_{300}$ group; GIV: D-GalN + SMN group; GV: D-GalN group. ABR: *Anemone baicalensis* rhizome; D-GalN: D-Galactosamine; SMN: Silymarin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase. Significantly different from the control group at *** $p < 0.001$. Significantly different from the D-GalN + SMN group at ## $p < 0.01$ and ### $p < 0.001$. Significantly different from the D-GalN group at && $p < 0.01$ and &&& $p < 0.001$.

To thoroughly assess the protective actions of an 80% ethanol extract on the liver, experiments were designed to measure various key biochemical markers in rat serum and liver tissue, including $\gamma$-glutamyl transpeptidase ($\gamma$-GT) and albumin (ALB), which are essential for evaluating liver health and function [58]. The experimental results indicated that, compared to GV, GIII exhibited positive changes: ALB levels increased, while $\gamma$-GT activity decreased (Figure 10). This trend was similar to that observed in the GIV. Although the degree of improvement was slightly less than that seen with silymarin, it still demonstrated that a high dose of 80% ethanol extract has potential in protecting liver function.

As a substance capable of affecting the body's redox balance, D-galactosamine causes significant liver injury. When rats are treated with D-galactosamine, it leads to the accumulation of lipid peroxidation products in their livers, characterized by a significant increase in malondialdehyde (MDA) content and accompanied by a significant decrease in CAT activity [59]. The continuous accumulation of these peroxidation products further exacerbates damage to hepatocytes and impairs their functions. Therefore, monitoring these two indicators is particularly important. Experimental results indicated that, compared to GI, the CAT activity in the liver and serum of rats administered D-galactosamine was markedly reduced, whereas the MDA content was noticeably increased ($p < 0.001$, Figure 11). Additionally, increased MDA levels in liver tissue and serum due to liver injury were significantly inhibited in rats given an 80% ethanol extract, and the reduced CAT activity was effectively alleviated, with GIII and GIV having very similar effects.

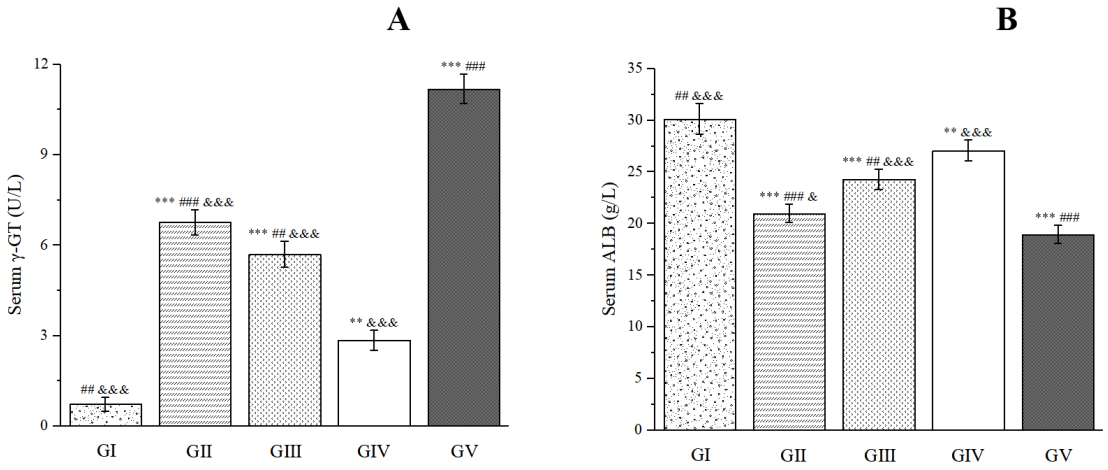



**Figure 10.** The impact of the 80% ethanol extract of ABR on serum $\gamma$-GT (**A**) and ALB (**B**) levels in rats with liver injury was assessed. The results are presented as the average, with the standard error of the mean indicating the variability ($n$ = 8). GI: Control group; GII: D-GalN + $ABR_{150}$ group; GIII: D-GalN + $ABR_{300}$ group; GIV: D-GalN + SMN group; GV: D-GalN group. ABR: *Anemone baicalensis* rhizome; D-GalN: D-Galactosamine; SMN: Silymarin; $\gamma$-GT: $\gamma$-Glutamyl transpeptidase; ALB: Albumin. Significantly different from the control group at ** $p < 0.01$ and *** $p < 0.001$. Significantly different from the D-GalN + SMN group at ## $p < 0.01$ and ### $p < 0.001$. Significantly different from the D-GalN group at $^{\&}$ $p < 0.05$ and $^{\&\&\&}$ $p < 0.001$.

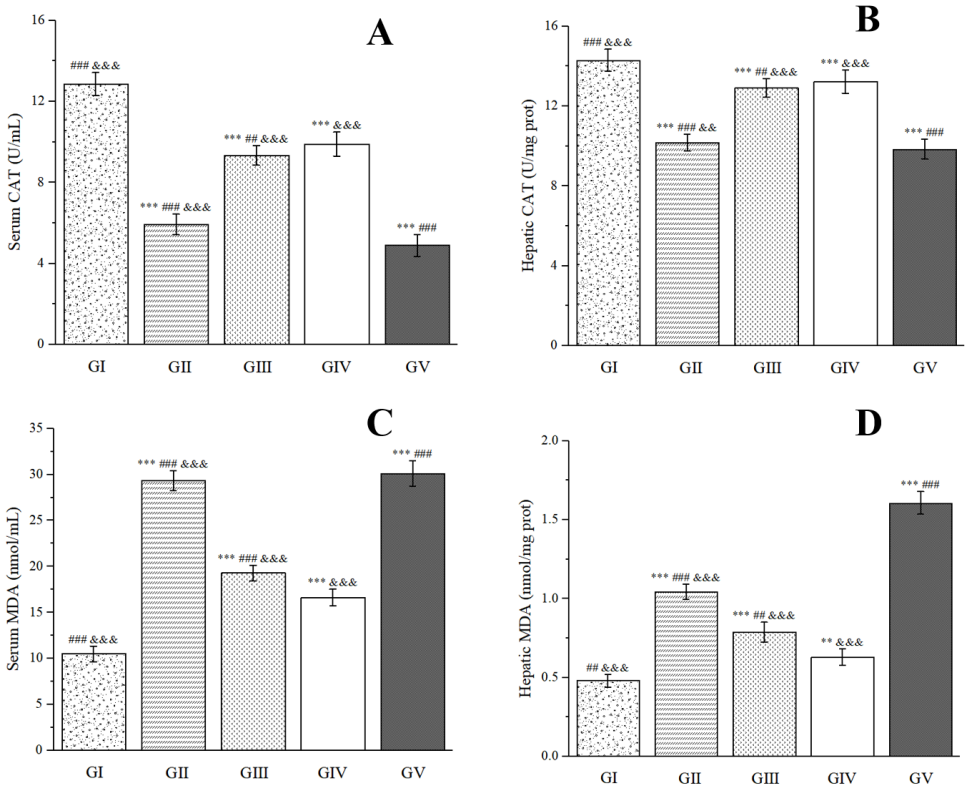



**Figure 11.** The study investigated the impact of the 80% ethanol extract of ABR on the levels of serum CAT (**A**), hepatic CAT (**B**), serum MDA (**C**), and hepatic MDA (**D**) in rats with liver injury. Values are expressed as the mean ± standard error of the mean ($n$ = 8). GI: Control group; GII: D-GalN + $ABR_{150}$ group; GIII: D-GalN + $ABR_{300}$ group; GIV: D-GalN + SMN group; GV: D-GalN group. ABR: *Anemone baicalensis* rhizome; D-GalN: D-Galactosamine; SMN: Silymarin; CAT: Catalase; MDA: Malondialdehyde. Significantly different from the control group at ** $p < 0.01$ and *** $p < 0.001$. Significantly different from the D-GalN + SMN group at ## $p < 0.01$ and ### $p < 0.001$. Significantly different from the D-GalN group at $^{\&\&}$ $p < 0.01$ and $^{\&\&\&}$ $p < 0.001$.

The histopathological examination results are shown in Figure 12, providing an intuitive and detailed depiction of hepatocyte damage and repair. In Figure 12A, the liver tissue structure of GI is clearly visible, with hepatocytes arranged orderly and no infiltration of inflammatory cells observed around the portal vein, indicating a healthy liver state. In GII and GIII, clear improvements in hepatocyte injury were evident. In these groups, the number of inflammatory and necrotic cells was relatively small, and the overall state of hepatocytes was inferior to the positive control group, yet a clear recovery trend was observed (Figure 12B,C). The treatment effect in GIV was most significant; the number of inflammatory and necrotic cells significantly reduced, and the hepatocyte morphology was relatively intact, indicating a better recovery state (Figure 12D). Conversely, more severe hepatocyte injury was observed in GV. Under a 200× microscope, this group's liver tissue structure appeared destroyed, with clear pathological changes (Figure 12E). Examining under a 400× microscope, the loss of hepatic cord and presence of hepatocyte necrosis (green tail-less arrows) were evident, accompanied by significant inflammatory cell infiltration (black tail-less arrows) (Figure 12F). These results suggest that without effective treatment, hepatocyte injury will continue to worsen, potentially leading to serious liver disease.

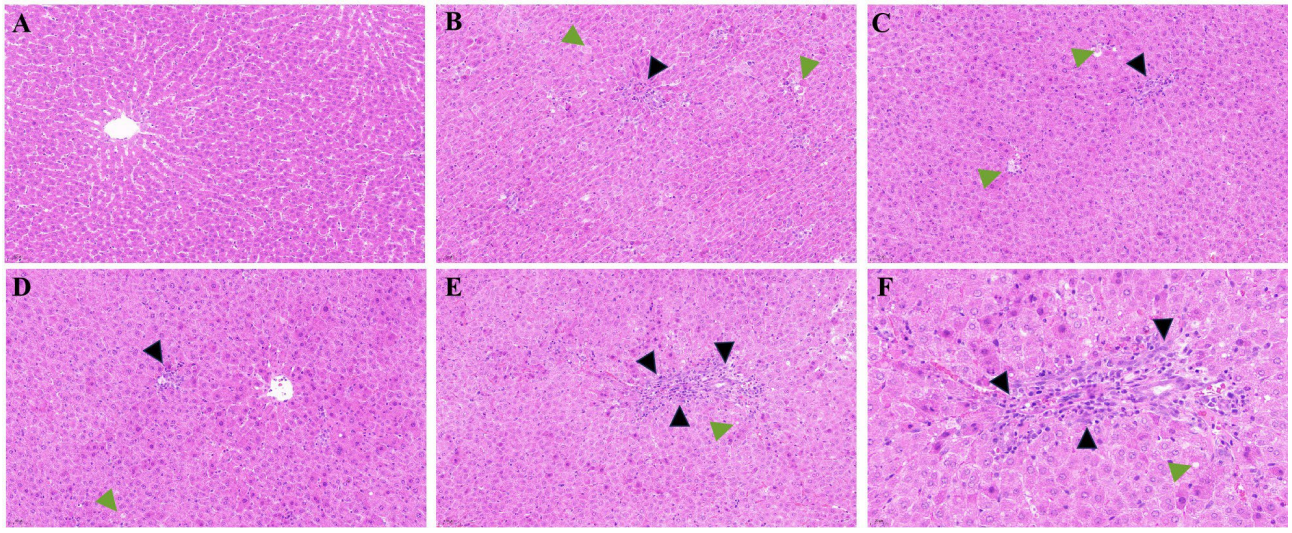



**Figure 12.** Histological examination of liver sections in different groups. (**A**): Control group (200× magnification); (**B**): D-GalN + $ABR_{150}$ group (200× magnification); (**C**): D-GalN + $ABR_{300}$ group (200× magnification); (**D**): D-GalN + SMN group (200× magnification); (**E**): D-GalN group (200× magnification); (**F**): D-GalN group (400× magnification). ABR: *Anemone baicalensis* rhizome; D-GalN: D-Galactosamine; SMN: Silymarin. Green tail-less arrow: single-cell necrosis; black tail-less arrow: inflammatory cells.

Triterpenoid saponins have demonstrated significant liver-protective activity by improving and correcting markers associated with hepatocyte integrity loss (such as serum ALT, AST, $\gamma$-GT, and alkaline phosphatase), oxidative stress (liver MDA and GSH), dyslipidemia (serum total cholesterol and triglycerides), and hepatocyte function (serum bilirubin and ALB) [60,61]. Naringin can protect the liver by enhancing the functioning of the hepatic antioxidant system as well as the metabolism of hepatotoxic substances [62]. Research has indicated that chlorogenic acid plays a crucial role in improving different types of liver diseases. Chlorogenic acid displays remarkable antioxidant and anti-inflammatory effects by activating Nrf2 and inhibiting TLR4/NF-$\kappa$B signaling pathways. Some important molecules such as AMPK and ERK1/2, and other key physiological processes like those of the intestinal barrier and gut microbiota, have also been discovered to participate in chlorogenic acid-provided amelioration on various liver diseases [63]. Rosmarinic acid exerts its effects through various mechanisms. These include scavenging or reducing superoxide ac-

tivity, mitigating indicators of hepatic toxicity (such as ALT, AST, and GSH), and enhancing the activity of antioxidant enzymes such as CAT, and glutathione peroxidase. Additionally, rosmarinic acid inhibited the proliferation of hepatic stellate cells and suppressed the expression of TGF-$\beta$1, CTGF, and $\alpha$-SMA in cultured hematopoietic stem cells. It also reduced fibrosis grade, improved biochemical indicators, and ameliorated histopathological morphology. Furthermore, rosmarinic acid demonstrated significant anti-inflammatory effects by modulating several inflammatory mediators [64]. The anti-inflammatory effect of hederacolchiside F was investigated in rats with carrageenan-induced acute foot swelling. The results indicated that hederacolchiside F exhibited a mild anti-inflammatory effect during the initial stage of acute inflammation. However, in the second stage of acute inflammation, hederacolchiside F was found to be highly effective. Its anti-inflammatory mechanism may involve the inhibition of bradykinin or other inflammatory mediators [65].

In conducting a comprehensive and in-depth evaluation of liver injury in rats, this study goes beyond using the key marker enzyme ALT as the sole evaluation standard. Instead, it employs a more holistic approach by combining a series of critical biochemical indicators, including AST, MDA, CAT, and ALB, to achieve more thorough evaluation results [66]. The experiment revealed that 80% ethanol extract of ABR, at both high and low doses, exhibited hepatoprotective effects by significantly lowering the activities of MDA, ALT, AST, and $\gamma$-GT, while increasing the levels of CAT and ALB. This further confirms its protective effect on the liver and its role in aiding the repair and regeneration of liver cells. Moreover, Li et al. [67] studied the effects of *Anemones raddeanae* Rhizoma saponins on liver fibrosis in sixty-seven chronic hepatitis B patients. Over 12 weeks, thirty-four patients received saponins plus standard therapy, while thirty-three received only diammonium glycyrrhizinate. The treatment group showed an 82.35% effective rate, significantly higher than the control group's 69.70% ($p < 0.05$), with notable improvements in liver histopathology, function, and fibrosis markers ($p < 0.05$). The study confirmed the anti-fibrotic and liver-protective effects of *Anemones raddeanae* Rhizoma saponins. Recent studies further demonstrate that *Anemones raddeanae* Rhizoma exhibits significant anti-fibrotic and hepatoprotective effects both in vivo and in vitro. in vivo, it effectively alleviates carbon tetrachloride-induced liver injury by reducing serum levels of AST, ALT, and $\gamma$-GT, lowering MDA content, and increasing SOD activity in rats. Additionally, it improves carbon tetrachloride-induced liver fibrosis, with its molecular mechanism likely involving the inhibition of the PI3K/Akt and TGF-$\beta$/Smad signaling pathways. in vitro, it inhibits the proliferation of HSC-T6 rat hepatic stellate cells, arrests the cell cycle at the G0/G1 phase, and induces apoptosis. Furthermore, over twenty triterpenoid saponins were identified from the blood components of *Anemones raddeanae* Rhizoma decoction, providing robust data to support its therapeutic potential for liver fibrosis [68]. These findings not only highlight the pharmacological similarities between ABR and *Anemones raddeanae* Rhizoma but also suggest that ABR has significant potential as a candidate drug and an effective alternative to *Anemones raddeanae* Rhizoma in liver protection therapy.

Although this study demonstrates that the chemical composition and pharmacological effects of ABR and *Anemones raddeanae* Rhizoma are similar, this does not provide conclusive evidence that ABR can fully replace *Anemones raddeanae* Rhizoma. The pharmacokinetics, efficacy, and safety of *Anemones raddeanae* Rhizoma have been well documented [4,51,69], but ABR has not yet been studied in these areas. These aspects will be a critical focus of future research on ABR.

## 3. Material and Methods

### 3.1. Materials

In May 2021, the plant species *Anemone baicalensis* was collected from Tonghua, located in Jilin Province, China, to ensure uniformity by sourcing plants from the same region on the same day. The specific geographical coordinates of the collection site were recorded as latitude N 42°0′50.80″ and longitude E 126°12′40.11″, at an elevation of 683.4 m. A voucher specimen, numbered 2021-05-29-001, was authenticated by Professor Junlin Yu and is now preserved in the Herbarium of Tonghua Normal University. Prior to processing, the rhizomes were minimally prepared through cleaning and washing, followed by slicing and air-drying in a cool, ventilated environment for subsequent use.

### 3.2. Methods

#### 3.2.1. Qualitative Phytochemical Analysis

A qualitative analysis of phytochemicals was performed on fifteen components, adhering strictly to a previously validated methodology [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.2. Preparation of Four Extracts of ABR

To examine how different solvents affect the extraction efficiency of active constituents from ABR, the extraction followed our previously established methodology [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.3. Quantitative Phytochemical Analysis

A comprehensive quantitative phytochemical analysis was carried out to determine the levels of various compounds, using a well-established methodology as outlined in reference [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.4. Antioxidant Activity Assays

A wide range of antioxidant activity assays were performed using various methods, all in accordance with the protocols specified in reference [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.5. Stability Studies of Methanol and 80% Ethanol Extract

The stability of the methanol extract and 80% ethanol extract of ABR was evaluated for pH, thermal stability, and within a gastrointestinal tract model system, strictly following the protocols outlined in our previously established methods [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.6. UHPLC-MS Analysis

The experimental conditions for UHPLC-MS were meticulously followed as described in our previously established method [6]. The detailed procedures refer to the Supplementary Materials.

#### 3.2.7. Oral Acute Toxicity Study

An acute oral toxicity assessment was performed following standardized procedures as described in our prior methodology [6]. The study aimed to determine the potential harmful effects of the 80% ethanol extract when given orally in a single dose. This evaluation serves to preliminarily assess the safety profile of the plant extract and establish a basis for selecting appropriate doses in subsequent experiments. The detailed procedures refer to the Supplementary Materials.

#### 3.2.8. Hepatoprotective Experiments

The reference [70] describes a methodology aimed at investigating the potential of an 80% ethanol extract to alleviate liver injury caused by D-galactosamine in rats. The detailed procedures refer to the Supplementary Materials.

#### 3.2.9. Statistical Analysis

A comprehensive statistical analysis was performed to determine the significance of the collected data using SPSS software (version 22.0, IBM, Armonk, NY, USA) and Origin software (version 8.0, OriginLab, Northampton, MA, USA). A one-way ANOVA followed by post hoc LSD tests and DUNCAN tests were employed to identify significant differences among the groups. The *p*-values of 0.05, 0.01, and 0.001 were taken to represent significance, a higher level of significance, and a very high level of significance, respectively.

## 4. Conclusions

This study provides a comprehensive evaluation of the chemical composition and bioactivity of ABR, comparing it with ABAP and exploring its potential as a substitute for *Anemones raddeanae* Rhizoma. The strengths of the study lie in its systematic approach, combining phytochemical analysis, antioxidant activity assays, stability studies, and in vivo hepatoprotective evaluations. Using UHPLC-ESI-Q-TOF-MS technology, thirty-eight compounds, primarily phenolics and triterpenoid saponins, were identified in ABR, many of which are also present in *Anemones raddeanae* Rhizoma. This chemical similarity, along with ABR's demonstrated hepatoprotective effects and low acute toxicity, underscores its potential as a safe and viable alternative. However, the study has limitations, including the need to further elucidate the pharmacological mechanisms of its active components, explore additional extraction methods, and validate findings in human clinical trials. The novelty of this research lies in its comparative analysis of ABR and ABAP, revealing distinct differences in chemical composition and bioactivity. While ABAP exhibits stronger antioxidant properties, ABR's rich triterpenoid content and exceptional hepatoprotective capabilities highlight its unique value. By bridging the gap between ABR and *Anemones raddeanae* Rhizoma, this study provides a scientific basis for the sustainable use of ABR as an alternative medicinal resource. Future research should focus on isolating additional bioactive compounds, clarifying their mechanisms of action, and conducting clinical trials to further establish ABR as a promising candidate for drug development.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr13030844/s1. Table S1: Phytochemical analysis of ABR; Figure S1: Chemical structures of the compounds identified in 80% ethanol extract of ABR; Figure S2: Positive-ion mode UHPLC-MS findings of 80% ethanol extract of ABR; Figures S3–S84: Presentation of MS and MS/MS spectra for peaks 1 through 41.

**Author Contributions:** L.L. and H.Z. conceived, designed, and supervised the research project, as well as preparing and editing the manuscript. S.S. and G.X. collected plant material and prepared different solvent extracts and accomplished the biological evaluation experiments. H.P. accomplished the statistical analysis. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki. The animal study protocol was approved by the Institutional Animal Care and Use Committee of Tonghua Normal University approved the experimental protocol (Ethic approval code: 20240036, 29 April 2024).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data presented in this study are available in the article.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| ABR | *Anemone baicalensis* rhizome |
| ABAP | *Anemone baicalensis* aerial parts |
| ABTS | 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt |
| ALB | Albumin |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| BHT | Butylated hydroxytoluene |
| BSAE | Bovine serum albumin equivalents |
| BHE | Berberine hydrochloride equivalents |
| CAT | Catalase |
| CAE | Caffeic acid equivalents |
| CTC | Condensed tannin content |
| CUPRAC | Cupric ion reducing antioxidant capacity |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| D-GalN | D-Galactosamine |
| $EDTANa_2$ | Ethylenediaminetetraacetic acid disodium salt |
| FRAP | Ferric-reducing antioxidant power |
| GE | Glucose equivalents |
| GAE | Gallic acid equivalents |
| GRE | Ginsenoside Re equivalents |
| GSH | Glutathione |
| GC | Gallotannin content |
| GI | Control group |
| GII | D-GalN + $ABR_{150}$ group |
| GIII | D-GalN + $ABR_{300}$ group |
| GIV | D-GalN + SMN group |
| GV | D-GalN group |
| $H_2O_2$ | Hydrogen peroxide |
| HClO | Hypochlorous acid |
| $IC_{50}$ | Half maximal inhibitory concentration |
| MDA | Malondialdehyde |
| QE | Quercetin equivalents |
| SMN | Silymarin |
| Trolox | 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid |
| TBHQ | Tertiary butylhydroquinone |
| TCC | Total carbohydrate content |
| $TP_{ro}C$ | Total protein content |
| TAC | Total alkaloid content |
| $TP_{he}C$ | Total phenolic content |
| TPAC | Total phenolic acid content |
| TFC | Total flavonoid content |
| $TT_{an}C$ | Total tannin content |
| $TT_{ri}C$ | Total triterpenoid content |

| | |
|---|---|
| TAE | Tannic acid equivalents |
| UHPLC-ESI-Q-TOF-MS | Ultra-high-performance liquid chromatography–electrospray ionization–quadrupole–time of flight–mass spectrometry |
| UHPLC–MS | Ultra-high-performance liquid chromatography–mass spectrometry |
| $\gamma$-GT | $\gamma$-Glutamyl transpeptidase |

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