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Evaluation of different ecological regions for cultivation of best quality *Bupleurum*: a case study from Shanxi, China

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Abstract

Bupleurum, a plant of the genus *Bupleurum* L. in the family Umbelliferae, is prevalent and extensively applied in traditional medicine systems across East and Southeast Asian countries for the treatment of colds, malaria, hepatitis and other diseases. In the current Chinese herbal medicine market, only the dried roots of two species, *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd., are authentic herbs, and their cultivars dominate as the commercial source, contributing to about 80% of the market share. Shanxi Province, known as the suitable habitat for *Bupleurum* in China, has a diverse ecosystem and geographical areas with diverse environmental conditions. These diversity ecosystem and environmental conditions cause prominent variations in the content of active ingredients of *Bupleurum* L. across different sites. Therefore, analyze the ecological, geographical, and soil factors that influence the quality of *Bupleurum* and to recommend the best suitable sites for cultivation of *Bupleurum*. This study demonstrates a close correlation between the quality of Shanxi *Bupleurum* sp. and different ecological factors. A total of 70 sets of *Bupleurum* and soil samples were collected from 25 counties across 6 cities in Shanxi Province. Consequently, the saponin contents of Datong, Shuozhou and Yizhou were generally higher than those of Linfen, Jincheng and Yuncheng. *Bupleurum* from northern Shanxi exhibits higher saikosaponin content compared to that from southern Shanxi; The total content of the five saponins shows a significant positive correlation with longitude ($^{*}P < 0.05$) and a highly significant positive correlation with latitude and altitude ($^{**}P < 0.01$). Saikosaponin levels positively correlate with latitude, longitude, and altitude; Meanwhile, the significance ranking of these ecological factors is: monthly average temperature is equal to monthly average surface temperature is greater than monthly sunshine hours. Low temperatures, arid conditions, and longer sunlight exposure are optimal conditions for the accumulation of saponin components; Besides, high-saponin *Bupleurum* is typically cultivated in low alkaline soils with low nitrogen, while the habitat of Shanxi *Bupleurum* is differentiated into four regions. Overall, the current study presents a foundation for selecting the best cultivation sites for *Bupleurum* and provides a valuable reference for evaluating other medicinal herb production regions.

Keywords *Bupleurum*, Chemical compositions, Ecological factors, Quality, Cultivation division

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Introduction

Bupleurum, a plant of the genus *Bupleurum* L. named by Linnaeus in 1753 in the family Apiaceae, is widely distributed across the northern hemisphere of Eurasia and North Africa and includes 241 species [1–2].There are annuals and perennials in the genus *Bupleurum* L. All plants in the genus *Bupleurum* L. are smooth and glabrous, with mostly narrowly elongated or lanceolate leaves, umbels with yellow flowers that are sometimes purplish, and fruits with double-hanging structures that have ornamented surfaces [3–4].Drude divides the genus *Bupleurum* L. into five groups: the *Perfoliata* group, the *Reticulata* group, the *Rigida* group, the *Coriacea* group, and the *Eubupleura* group [5]. Through numerical classification studies, Chinese researchers have proposed the infrageneric classification system of the genus *Bupleurum* L., which includes 2 subgenera (subgen. *Longifolia*, subgen. *Eubupleura*) and 2 groups (sect. *Ranunculoidea*, sect. *Falcata*) [6].There are 42 species, 17 varieties, and 7 variants of the genus *Bupleurum* L. reported in China, of which 25 species, 15 varieties, and 7 variants are endemic to China according to incomplete statistics [7]. Currently in China, only the dried roots of the two species, *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd., are authentic herbs in the 2020 edition of the Chinese Pharmacopoeia [8]. As a traditional Chinese herb, the root or whole herb of many species of *Bupleurum* have been used for more than 2,000 years in China and other Asian regions, such as the Korean Peninsula and Japan, for the treatment of colds, fevers, hepatitis, malaria, menstrual disorders and other ailments [9].

According to the 2020 edition of the Chinese Pharmacopoeia, it refers to the dried root of *Bupleurum* chinense DC. or *Bupleurum scorzonerifolium* Willd., both belonging to the Umbelliferae family [8]. In traditional Chinese medicine, it is generally employed to treat conditions such as fever, pain, inflammation (including cholecystitis, hepatitis, and pancreatitis), malaria, menstrual disorders, and depression associated with influenza or common cold [10–16]. *Bupleurum*-containing common prescriptions and traditional Chinese patent medicines include Xiaochaihu Decoction, Sini Powder, Xiaoyao Powder, and Shugan Shunqi Tablets. Modern pharmacological studies have evidenced *Bupleurum*'s diverse effects, including its antidepressant, anticonvulsant, antitumor, hepatoprotective, choleretic, antipyretic, and anti-inflammatory properties [17–19]. *Bupleurum* roots contain triterpenoid saponins, volatile oils, flavonoids, lignans, fatty acids, and sterols, with saikosaponins (A, C, D, E, and F) being the primary chemical components [20–21].

Modern pharmacological research has confirmed the antipyretic, anti-inflammatory, antiviral, antitumor, and antidepressant effects of saikosaponin. Specifically, Huo Mengyi et al. [22] employed endotoxin-induced temperature elevation in rabbits as an experimental model. They administered saikosaponin A to febrile rats and discovered that the temperature elevation induced by IL-1β was dependent on the cAMP content in the hypothalamus. Simultaneously, saikosaponin A could inhibit the activation of PKA in the hypothalamus, ultimately decreasing body temperature.The anti-inflammatory mechanism of saikosaponin primarily involves inhibiting the activation of NF-κB and reducing IκB degradation and phosphorylation [23–24]. Puza Li [25] et al. induced ulcerative colitis (UC) in mice via 7-day gavage with 3% DSS. Daily gavage of saikosaponin D (8 mg/kg) significantly suppressed the mRNA levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, while elevating the mRNA level of the anti-inflammatory cytokine IL-10. Zhang Yongbin [26]et al. compared the outcomes of 50 influenza A patients. The control group received oseltamivir monotherapy alone, while the study group was treated with *Bupleurum* decoction combined with oseltamivir. The results revealed a shorter fever reduction time ($^{*}P < 0.05$) and a reduced incidence of adverse reactions ($^{*}P < 0.05$) in the study group. Li Weiping [27] et al.utilized a cell culture-hepatitis C virus (HCV) system for screening and discovered that saikosaponin B2 hindered the entry, replication, and translation of the virus. Saikosaponin A could indeed continuously activate caspase-2 and caspase-8, ultimately triggering caspase-mediated apoptosis in human colon cancer cells [28]. Zhang Yun [29] et al. discovered that saikosaponin D inhibited TLR4 protein expression via the PI3K/AKT/FoxO1 signaling pathway, ameliorated neuroinflammation and neuronal apoptosis in the rat brain, and reversed LPS-induced depression-like symptoms in rats. Generally, the antidepressant mechanism of saikosaponin also encompasses multiple pathways, including the regulation of brain-derived neurotrophic factors, neurotransmitters, and neuroendocrine regulation [30], rendering saikosaponin a critical material basis for *Bupleurum*'s efficacy and a key factor in ensuring its quality.

Currently, only the dried roots of two species, *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd., are authentic herbs, and their cultivars dominate as the commercial source, contributing about 80% of the market share [31]. Shanxi Province, a primary *Bupleurum* production area, features extensive territory and complex natural geography. Its hilly terrain is higher in the northeast and lower in the southwest, leading to notable regional disparities. Medicinal plants typically contain complex chemical components, with production and accumulation closely tied to plant germplasm and growth environment. This study collected 70 batches of *Bupleurum* and soil samples from 25 counties (districts) in 6 cities of Shanxi Province to assess *Bupleurum* quality across different production areas and identify key influencing

ecological factors. Ecological and geographical factors were gathered, and soil factor data was tested. Through the identification of *Bupleurum* germplasm from different production areas, quality evaluation, and correlation analysis with ecological, geographical, and soil factors, primary factors influencing *Bupleurum* quality were hereby determined, and different production areas were delineated by taking Shanxi as a case study, forging a basis for selecting cultivation sites for high-quality Bupleurum. Meanwhile, this study provides a scientific basis for the protection of wild *Bupleurum* resources and realizes sustainable development. It also provides basic data for international scientific cooperation and promotes the clinical research and application of *Bupleurum* worldwide. It also provides ideas for the origin, quality, wild resources and clinical studies of other herbs.

Materials

Materials

During 2020, from October to November, a total of 70 samples of *Bupleurum* roots were collected, spanning from the north to the south, in 25 counties (districts) across 6 cities in Shanxi Province, including Datong, Shuozhou, Yizhou, Linfen, Jincheng, and Yuncheng. Among these samples, only one batch was wild *Bupleurum*, while the rest were cultivated. All samples were identified and verified through flora of China at Beijing University of Chinese Medicine and are stored in the Center for Identification of Traditional Chinese Medicines within the School of Traditional Chinese Medicine. Detailed sample numbers and related information can be found in Table 1. During collection of *Bupleurum* samples, soils surrounding the roots at a depth of 30 cm below the soil surface were collected. Following natural air drying, the soil was preserved for subsequent use. Information regarding altitude, latitude, and longitude was sourced from various collection sites, while meteorological data was obtained from the Spatial Information Grid Database of Traditional Chinese Medicine Resources (http://www.tcm-resources.com/) and the meteorological station at the sampling location.

Instruments and reagents

The instruments used in the current research work were, MM400 cryogenic mixing ball mill (RETSCH, Germany); High speed centrifuge (Sigma, Germany); PCR machine (Bio RadT100TMThermal Cycler); BioRad electrophoresis instrument; JY-SPDT type horizontal electrophoresis tank (Beijing Junyi Electrophoresis Co., Ltd.); CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); e2695 Waters ultra-high performance liquid chromatograph.

The following reagents were used for amplification of the target DNA fragment: Broad spectrum plant genome DNA rapid extraction kit (TIANGEN); 2 x Taq plus per mastermix (including dye) (Beijing Biomed Development Co., Ltd.); Agarose G-10 (Saibaisheng Company); ITS primers [32](F-5'-GAAGTAAAAGTCGTAACAAGG-3', R-5'-TCCCCCGCTTATTGATGC-3'), synthesized by Shanghai Sangong Bioengineering Co., Ltd; The purity of saikosaponin A (batch number: 20736-09-8), saikosaponin E (batch number: 58316-41-9), saikosaponin C (batch number: 20736-08-7), saikosaponin D (batch number: 20874-52-6), and saikosaponin F (batch number: 62687-63-2) are all 98%, purchased from Shanghai Yuanye Biotechnology Co., Ltd; Acetonitrile (Fisher, chromatographically pure), and methanol (Fisher, chromatographically pure).

Methods

Identification of *Bupleurum*

For authentication and recognition of the target genus/species, approximately 20 mg of the sample was used for DNA extraction, and the quality of DNA was assessed using a micro nucleic acid quantification instrument. The PCR reaction (30 μL) comprised 2 μL DNA, 1 μL each of ITS 4/5 forward and reverse primers, 15 μL of 2× Mix polymerase, and the remaining volume made up with ddH_2O.

The PCR reaction consist of a pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, with 30 cycles, followed by a final extension at 72 °C for 7 min. PCR products were detected using 1% agarose gel electrophoresis at 130 V for 20 min. The gel imag detected and sent a single, bright, and clear amplification band to Shanghai Sangong Bioengineering Co., Ltd. for bidirectional sequencing. The sequencing results were aligned with BLAST sequences in the NCBI gene database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the sequence with the highest similarity was downloaded. Additionally, DNAMAN and ContigExpress software were employed to align and edit all sequences. ITS sequences of outgroups were downloaded from NCBI, and MEGA software was used to perform NJ clustering tree analysis on all sequences [33].

UPLC of saikosaponin

Preparation of solution

0.5 g of dried *Bupleurum* root powder was accurately weighed and transferred into a conical flask with a stopper. Then, 25 mL of methanol solution containing 5% concentrated ammonia was added, the flask was sealed tightly, and it was subjected to ultrasonic treatment at 30℃ (200 W, 40 kHz) for 30 min. The mixture was subsequently filtered, and the container and drug residue were washed twice with 20 mL of methanol each time. The washing solution and filtrate were combined, and the

Table 1 Differential loci of different genotypes

Genotype	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5
	0	3	3	4	4	6	8	8	8	9	9	2	9	2	2	1	1	3	3	8	8	9	0	0	2	4	5	6	7	7
	3	5	7	6	7	8	3	7	9	8	9	4	1	3	7	8	9	3	5	4	5	2	3	8	5	0	2	8	2	9
Case	C	A	T	A	G	T	G	C	A	-	-	T	A	T	T	C	C	-	G	·	T	C	-	G	G	G	A	-	T	A
1	T	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	T	·	·	·	·	·	·	A	T
2	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	G	·	·	T
3	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	·	·	·	·
4	·	C	G	G	·	G	·	T	C	·	·	A	T	A	A	-	-	·	A	G	·	·	·	A	·	A	·	·	·	T
5	·	·	·	·	·	·	A	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	·	·	·	·
6	·	·	·	·	·	·	A	·	·	C	A	·	C	·	·	-	-	·	·	G	·	·	C	·	·	·	·	·	·	·
7	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	G	·	·	T
8	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	·	A	·	·
9	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	G	·	·	·	·	·	·	G	·	·	T
10	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	A	·	·	G	·	·	·	·	·	G	·	·	T
11	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	A	·	G	·	·	T
12	·	·	·	·	C	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	·	·	·	·
13	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	·	·	·	T
14	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	G	·	·	·	·	·	·	G	·	·	T
15	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	-	-	·	·	·	·	·	·	·	·	·	G	·	·	T

solvent was evaporated to dryness. The residue was dissolved in methanol and transferred to a 5 mL volumetric flask. Methanol was added to the mark, the solution was shaken well and filtered, and the filtrate was collected.

Preparation of standard solution

Appropriate amounts of saikosaponin A, C, D, E, and F reference solutions were weighed, accurately transferred into a 5 mL volumetric flask, dissolved in methanol, and diluted to a constant volume. This process yielded reference standard solutions of saikosaponin A, C, D, E, and F with concentrations of 1.220 mg/mL, 0.636 mg/mL, 1.906 mg/mL, 0.346 mg/mL, and 0.296 mg/mL, respectively, for future use.

UPLC test conditions

The UPLC test conditions included: Chromatographic column: ACQUITY UPLC BEHC18 (2.1 mm×100 mm, 1.7 μm); Chromatographic conditions: Column temperature: 30℃; Flow rate: 0.4 mL/min; Mobile phase: Acetonitrile (A) - water (B); Gradient elution: 0–4 min, 73% B; 4–11 min, 73% B-71% B; 11–15 min, 71% B-70% B; 15–28 min, 70% B-50% B; 28–38 min, 50% B-10% B. Detection wavelength: 210 nm, injection volume: 3 μL.

Methods

Linear relationship

Specifically, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the mixed reference solutions were accurately aspirated and placed into 5 mL volumetric flasks, then diluted to volume. 3 μL of each reference solution was accurately injected and measured to construct a standard curve correlating concentration with peak area. The results indicated a good linear relationship among saikosaponin A, C, D, E, and F.

Precision investigation

A mixed reference solution was taken and injected consecutively six times. The peak area was recorded, and the RSD value was calculated. The RSD values for saikosaponin A, C, D, E, and F were 0.55%, 0.47%, 0.23%, 1.46%, and 1.08%, respectively, demonstrating good instrument precision.

Stability investigation

The test solution was prepared according to the method outlined in the Chinese Pharmacopoeia (2020 edition). After allowing the test solution to stand at room temperature for 0, 2, 4, 8, 12, and 24 h, the sample was measured and the peak area was recorded to calculate the RSD value. The RSD values for saikosaponin A, C, D, E, and F were 2.31%, 1.94%, 1.36%, 2.85%, and 2.43%, respectively. The results indicated good stability of the test solution within 24 h.

Repeatability investigation

One batch of samples was selected and extracted in parallel six times. The solutions were injected separately, and the peak area was recorded to calculate the RSD value. The RSD values for saikosaponin A, C, D, E, and F were 2.50%, 2.04%, 2.80%, 2.70%, and 2.51%, respectively, demonstrating good repeatability of the method.

Investigation of recovery rate of adding samples

A batch of samples with known content was used for recovery tests. According to pharmacopoeia regulations, recovery tests were conducted at 80%, 100%, and 120% of the original content. The recovery rates for saikosaponin A, C, D, E, and F were 100.68%, 98.31%, 102.89%, 101.06%, and 96.54%, respectively, with RSD values of 1.72%, 1.45%, 1.08%, 1.59%, and 1.22%, respectively. The recovery rates and corresponding relative standard deviations of each component fulfilled the required standards.

Soil factor data determination

Thirty-four representative batches of soil samples were selected for data screening, and fresh soil collected at the sampling points was analyzed for nutrient composition, trace elements, pH, mechanical composition, soil type, and other parameters, referencing "Soil Agricultural Chemistry Analysis".

Construction of quality evaluation model of Shanxi *Bupleurum*

The variance contribution of principal components was further utilized as weights to calculate the indicator weights through principal component analysis, by normalizing the weighted average of the coefficients in each linear combination of principal components.

The scores for each principal component could be calculated as:

$F_i=w_{1i}X_1+w_{2i}X_2+\ldots+w_{ni}Xn$ (1).

where w_i represents the weight of each variable in the principal component, which could be calculated by dividing the coefficient corresponding to each variable in the component matrix by the arithmetic square root of the eigenvalue corresponding to the i-th principal component.

The comprehensive score could be calculated as:

$F=\alpha_1F_1+\alpha_2F_2+\ldots+\alpha nFn$ (2).

where α_i represents the percentage variance of the i-th principal component.

The comprehensive scoring model could be obtained as:

$F=0.534\ {}^*F_1+0.190\ {}^*F_2+0.074\ {}^*F_3+0.049\ {}^*F_4+0.040\ {}^*F_5+0.031\ {}^*F_6$. (3)

Data analysis

Correlation and stepwise regression analyses on geographical, meteorological, and soil factors, as well as saikosaponin content were hereby conducted using SPSS 24.0 statistical software, facilitating to establish a correlation coefficient table and formulate a multiple regression equation, so as to identify the primary factors influencing the accumulation of effective ingredients.

Results and analysis

Identification of *Bupleurum* through DNA barcode

The target fragment of DNA was successfully amplified through PCR (Fig. 1), and the gel electrophores is showed quality PCR products. All samples exhibited a single, bright, and clear band on the gel, showing the target region of DNA. After the successful sequencing of the target fragment DNA, which has a sequence length of 607 bp, it was BLAST searched. The BLAST search result showed that the query sequences exhibiting high similarity, broad coverage, and the highest scores, were selected for next step analysis. Following a comparative analysis, the query sequence possessed abundant and relatively stable mutation sites [34]. After analysis through DNAMAN Software, 70 sets of samples were hereby categorized into 15 haplotypes, and the detailed mutation sites of different haplotypes are presented in Table 1. Figure 1 shows the NJ dendrogram of *Bupleurum* and adulterants based on ITS sequences. In Fig. 2, the NJ dendrogram in *Bupleurum* and its adulterants was constructed based on ITS sequences。 Among the 15 haplotypes, only type 4 was grouped with *Bupleurum* stenophyllum LC489151.1, showing a 99.83% consistency between the two. The remaining haplotypes were grouped with *Bupleurum* chinense. Notably, the two batches of samples from Qinshui County, Jincheng City, are *Bupleurum marginatum* var. stenophyllum, while the rest are *Bupleurum chinense*.

Saikosaponin analysis

The Chinese Pharmacopoeia (2020 edition) stipulates that the content of saikosaponin A + D in *Bupleurum* medicinal materials should be ≥ 0.3% [8]. As depicted in Figs. 3 and 4, there are notable variations in the content of saikosaponin components across different regions of Shanxi Province. Referring to Fig. 3, on a city-by-city basis analysis, the ranking for saikosaponin A ratio is higher in Shuozhou as compared to Datong, Yizhou, Jincheng, Linfen, and Yuncheng. While the rate of saikosaponin C is higher in Shuozhou as compared to Datong, Yizhou, Linfen, Jincheng, and Yuncheng. similarly, the rate of saikosaponin D is higher in Shuozhou as compared to Datong, Yizhou, Linfen, Jincheng, and Yuncheng; While the rate of saikosaponin E is higher in Shuozhou as compared to Jincheng, Datong, Yuncheng, Linfen, and Yizhou, similarly, the rate of saikosaponin F content is higher in Yizhou as compared to Shuozhou, Linfen, Datong, Yuncheng and Jincheng; Eventually, the ranking for the total content of the five saponins ratio is higher in Shuozhou as compared to Datong, Yizhou, Linfen, Jincheng, and Yuncheng. As illustrated in Fig. 4, the saponin content in the northern Shanxi region (Datong, Shuozhou, and Yizhou) is generally higher than that in the southern Shanxi region (Linfen, Jincheng, and Yuncheng) upon comprehensive analysis.

Correlation between Saikosaponin content and ecological factors

Herein, the primary active ingredients of *Bupleurum*, namely saikosaponins A, C, D, E, F, and the

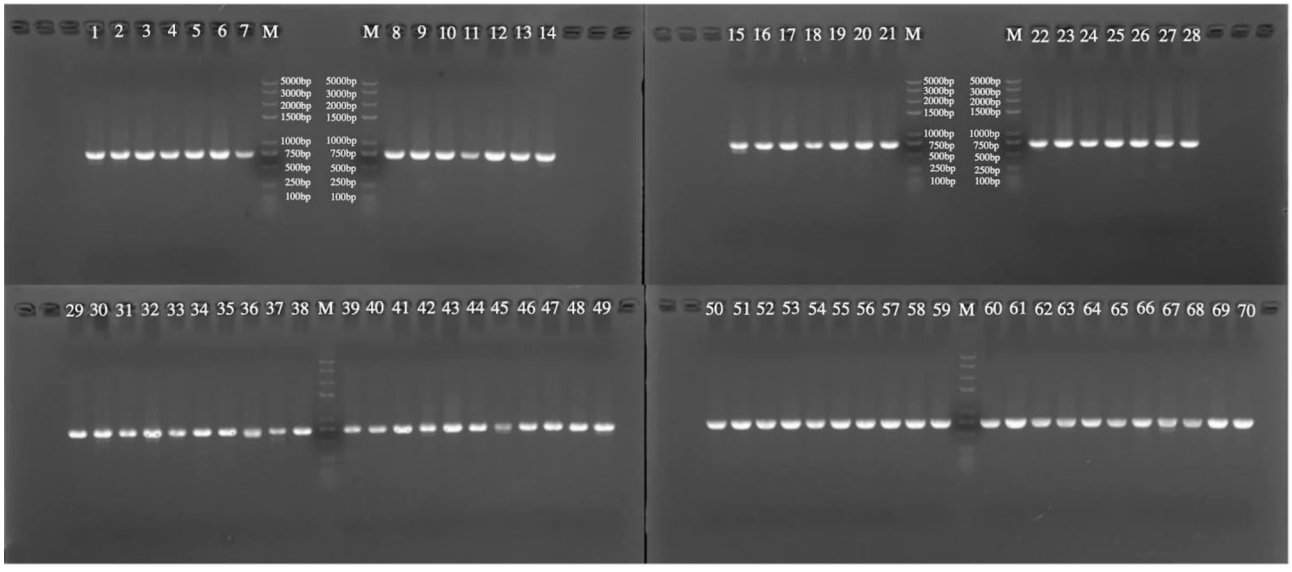

Fig. 1 Gel electrophoresis detection diagram of PCR Products

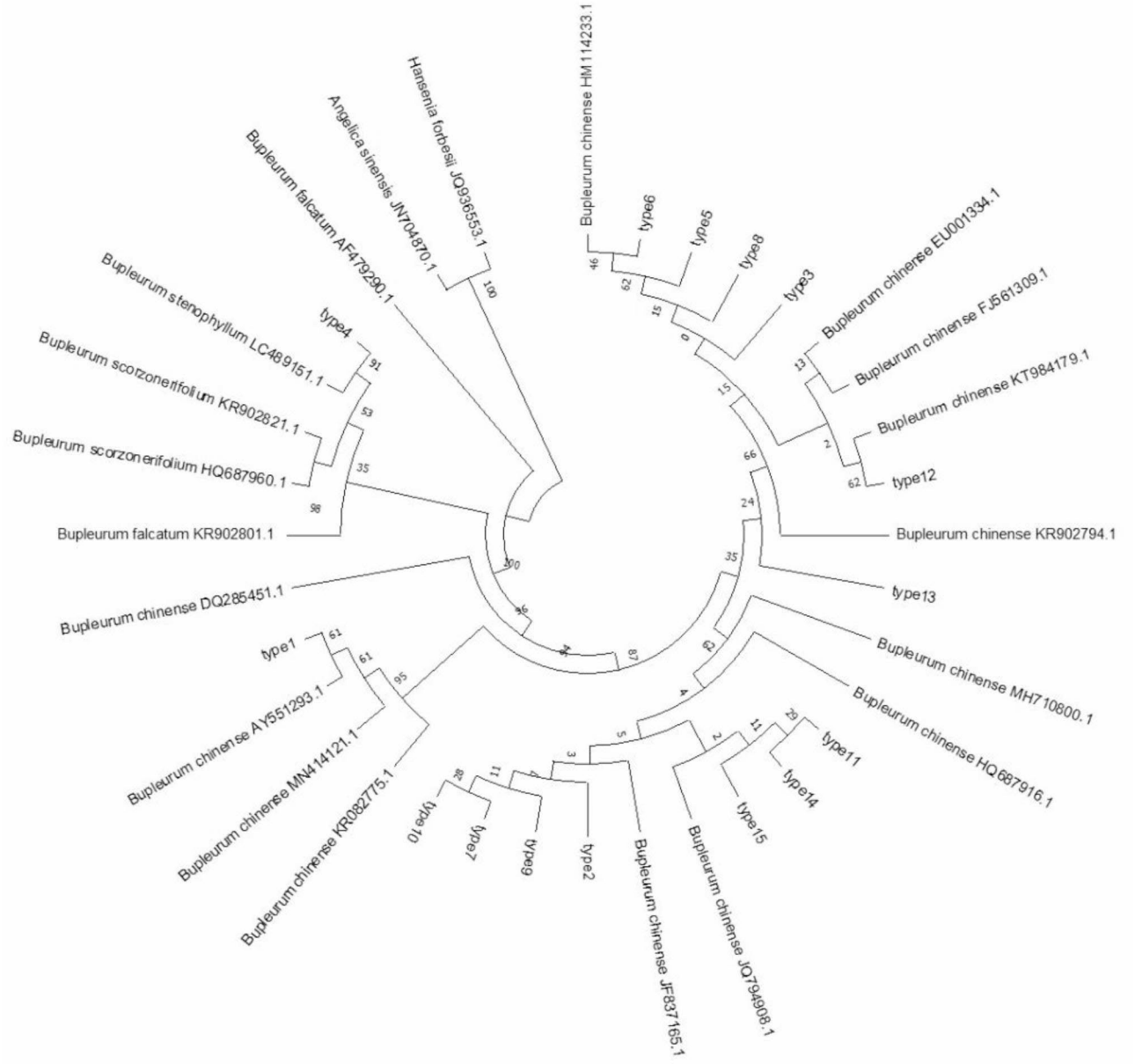

Fig. 2 NJ cluster analysis of ITS Sequences of 70 copies of *Bupleurum*

overall content of these five saponins, were measured, as depicted in Fig. 5. Subsequently, Pearson correlation analyses were conducted with climate, geographical, and soil factors.

Regarding the correlation analysis with climate factors, on the whole, the results for the correlation between monthly average temperature (T) and monthly average surface temperature (OT) with the saikosaponin content are comparable (Fig. 5A and B), both indicating a negative correlation. Apart from saikosaponin E, lower temperatures favor the accumulation of saikosaponins A, C, D, and F, as well as the overall content within *Bupleurum* medicinal herbs. Furthermore, the saikosaponin content positively correlates with monthly sunshine hours (S) (Fig. 5E). Longer sunshine durations correspond to higher levels of saikosaponin A and D, the main active ingredients of *Bupleurum*, and an increased overall content. Conversely, the saikosaponin content negatively correlates with monthly cumulative rainfall (W) and monthly average relative humidity (H) (Fig. 5C and F).

Geographical factor correlation analysis, as depicted in Fig. 5D, reveals a highly significant positive correlation (**$P < 0.01$) between saikosaponin A and latitude, longitude, and altitude; Saikosaponin C exhibits a significant positive correlation with longitude (*$P < 0.05$) and a highly significant positive correlation with latitude (**

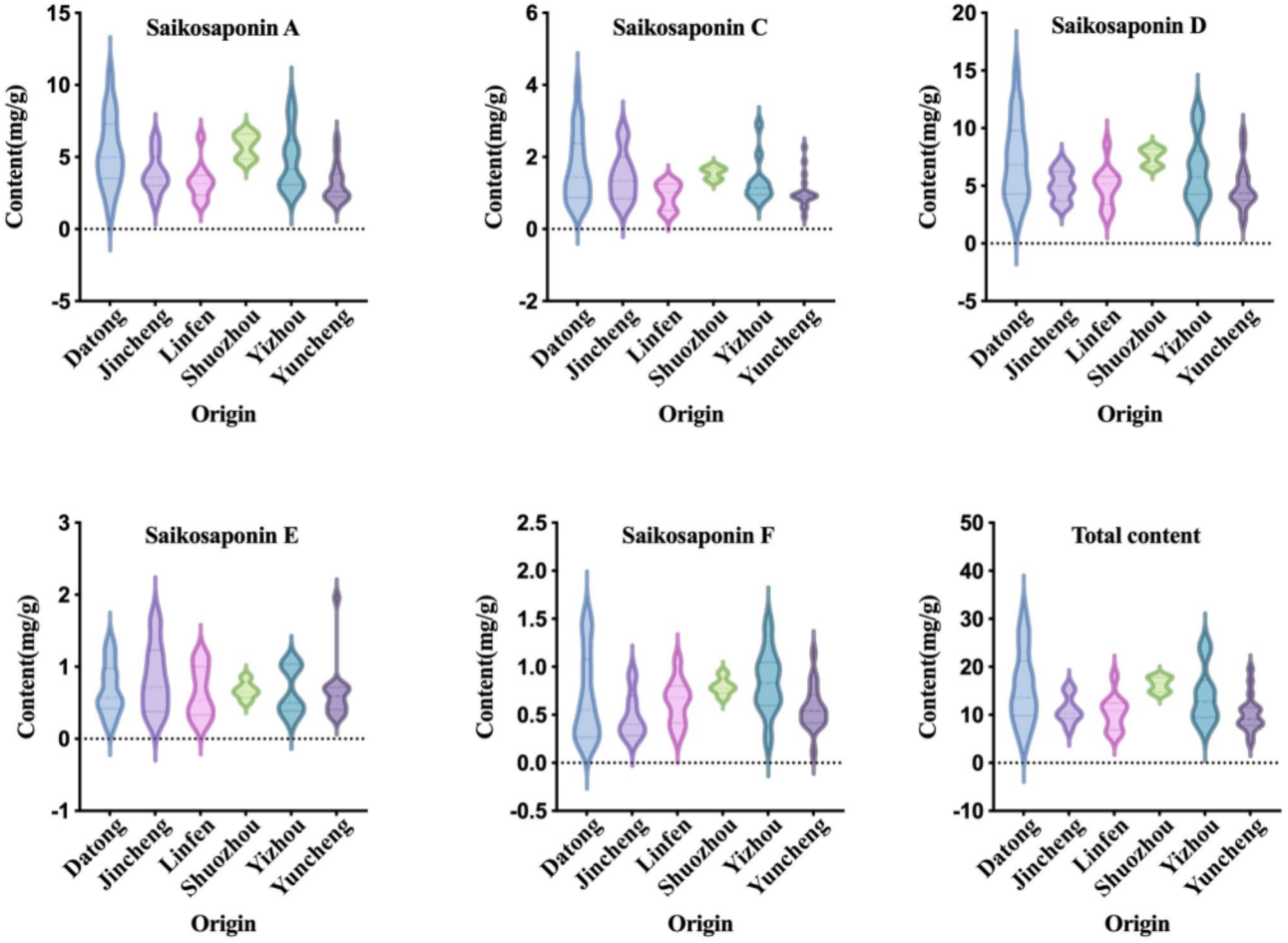

Fig. 3 Comparison of saikosaponin content in different geographical areas (cities) of Shanxi Province

$P < 0.01$), while saikosaponin D demonstrates a highly significant positive correlation with latitude ($^{**}P < 0.01$) and a significant positive correlation with altitude ($^{*}P < 0.05$); The correlation coefficients for saikosaponin E with latitude, longitude, and altitude do not reach the 5% significance level, indicating insignificant correlations; Saikosaponin F demonstrates a highly significant positive correlation with latitude ($^{**}P < 0.01$); The total content of the five saponins shows a significant positive correlation with longitude ($^{*}P < 0.05$) and a highly significant positive correlation with latitude and altitude ($^{**}P < 0.01$). Overall, the saikosaponin content is positively correlated with latitude, longitude, and altitude, indicating higher saponin levels in *Bupleurum* grown at elevated sites, such as the Taihang Mountains in northeastern Shanxi Province.

The correlation analysis of soil factors, as illustrated in Fig. 5G, indicates that only saikosaponin A, C, and E have significant correlations with the measured soil factors. Among them, saikosaponin A, the main active ingredient of *Bupleurum*, exhibits a significant negative correlation with alkaline hydrolyzed nitrogen, available potassium, and available Cu ($^{*}P<0.05$). Meanwhile, saikosaponin C demonstrates a notable negative correlation with total nitrogen ($^{*}P < 0.05$), an extremely significant negative correlation with alkaline hydrolyzed nitrogen ($^{**}P < 0.01$), and a significant positive correlation with pH ($^{*}P < 0.05$). Furthermore, saikosaponin E shows a significant positive correlation with available phosphorus and exchangeable Mg ($^{*}P < 0.05$) and is also significantly correlated with soil texture. Notably, the content of saikosaponin E is relatively higher in *Bupleurum* cultivated in sandy soil. In summary, *Bupleurum* with high saponin content is typically cultivated in weakly alkaline soils low in alkaline nitrogen.

Correlation analysis reveals a significant or extremely significant correlation between saikosaponin components and most ecological factors. However, ecological factors do not affect plants but result from multiple factor interactions. In this study, multiple stepwise regression was employed based on correlation analysis to identify the primary ecological factors influencing saikosaponin components. This method excluded variables not significantly correlated with the dependent variable, thereby screening the ecological factors. Subsequently,

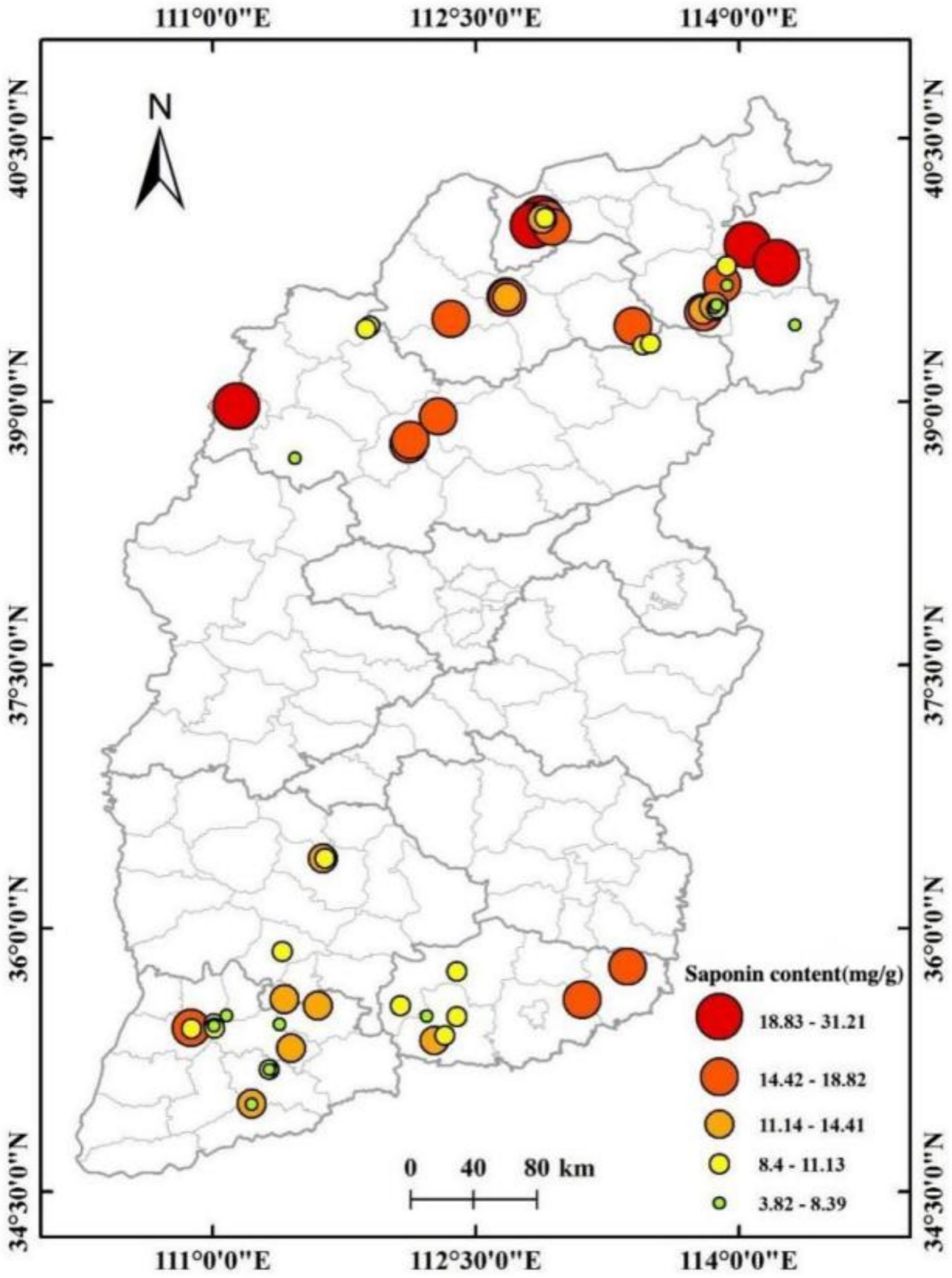

Fig. 4 Heat map of saikosaponin distribution

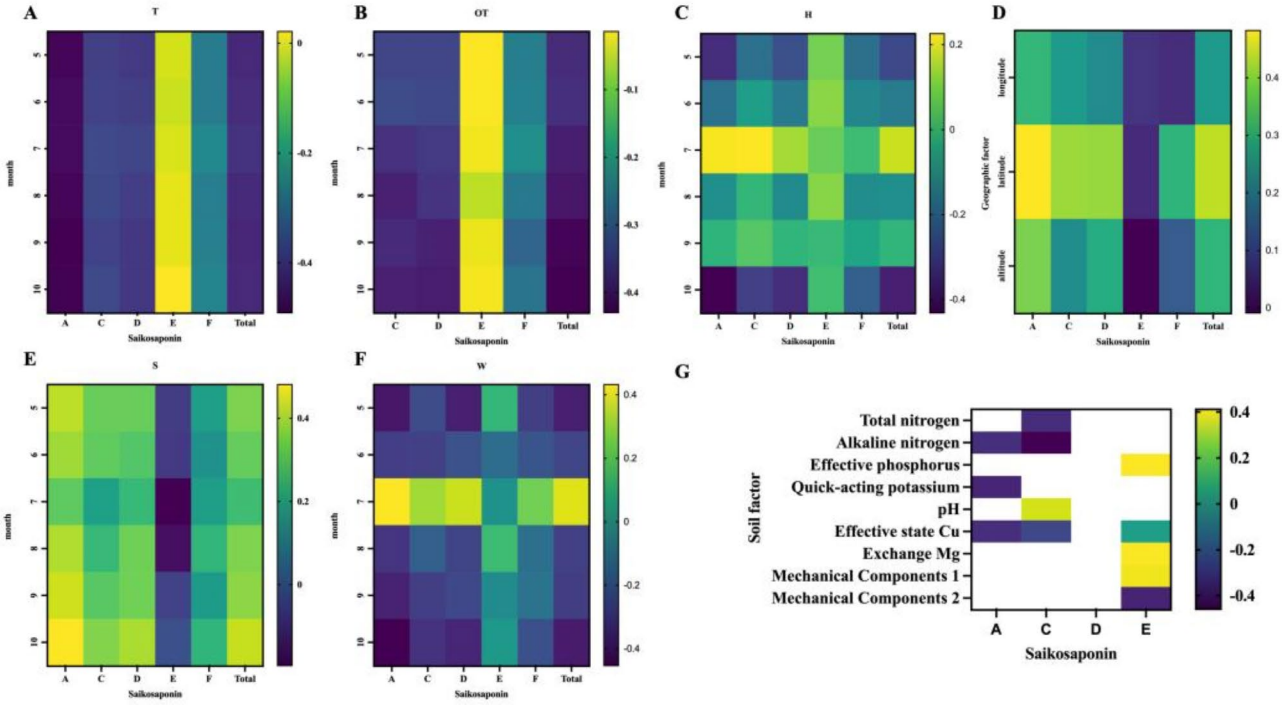

Fig. 5 Correlation analysis heatmap between saikosaponin content and ecological factors、soil factors.(**A**) Monthly average temperature T of climate factors.(**B**) Monthly average surface temperature OT. (**C**) Monthly average relative humidity H.(**D**) Geographical factors. (**E**) Monthly sunshine hours S of climate factors.(**F**) Monthly cumulative rainfall W of climate factors. (**G**) Soil factors

Table 2 Regression equation of saikosaponins and dominant ecological factors

Components	Regression equation	F	*P*
A (mg/g)	$Y = 10.487\text{-}0.035X_{23}$	21.623	0.000
C (mg/g)	$Y = 2.565\text{-}0.010X_{26}$	12.152	0.001
D (mg/g)	$Y = 9.567\text{-}0.035X_{28}$	13.724	0.000
E (mg/g)	——	——	——
F (mg/g)	$Y = 3.239\text{-}0.026X_{21}\text{-}0.036X_{24}+0.020X_{6}$	6.012	0.001
Total content (mg/g)	$Y = 28.309\text{-}0.086X_{23}$	16.001	0.000

a regression equation table was established to correlate saikosaponin content with the primary ecological factors.

Table 2 presents regression equation results. Evidently, except for saikosaponin E, all other components have established regression equations with ecological factors, and all P-values are below 0.05, indicating statistical significance of the regression equations.

The primary ecological factor influencing the saikosaponins are: X23 for saikosaponin A and total content, X26 for saikosaponin C, X28 for saikosaponin D, and X21, X24, and X6 for saikosaponin F. Among these, three components are primarily influenced by monthly average temperature, average surface temperature, and one by monthly sunshine hours. The significance ranking of these ecological factors is: monthly average temperature is equal to monthly average surface temperature is grater than monthly sunshine hours.

For studying the influence of primary soil factors on saikosaponin, a stepwise regression analysis was conducted between 34 soil measurement results and their corresponding saponin contents. A regression equation table correlating saikosaponin components with the dominant soil factors was established.

Table 3 presents the regression equation results. Regression equations were established between saikosaponin A, C, and E and soil factors, with all P-values being less than 0.05, indicating statistical significance of the regression equations. For saikosaponin A, the primary soil factor is M4 (available potassium). Reducing the application of potassium fertilizer appropriately is beneficial for increasing the content of saikosaponin A. For saikosaponin C, the primary soil factor is M2 (alkaline hydrolyzable nitrogen), presenting a negative regression coefficient. Appropriately reducing the content of alkaline hydrolyzable nitrogen in the soil is conducive to increasing the content of saikosaponin C. For saikosaponin E, the primary soil factors are M3 (available phosphorus) and M16 (mechanical composition 1). Higher levels

Table 3 Regression equation of saikosaponins and soil factors

Components	Regression equation	F	*P*
A (mg/g)	$Y = 5.584\text{-}0.008M_4$	5.046	0.032
C (mg/g)	$Y = 2.351\text{-}0.014M_2$	8.538	0.006
D (mg/g)	——	——	——
E (mg/g)	$Y = -0.246 + 0.018M_3 + 0.011M_{16}$	6.412	0.005
F (mg/g)	——	——	——
Total content (mg/g)	——	——	——

of available phosphorus are more beneficial for increasing the content of saikosaponin E. Based on various regression equations, the contributions of the dominant ecological factors are ranked equally: available potassium is equal to alkaline hydrolyzable nitrogen is equal to available phosphorus is equal to mechanical composition 1.

Regionalization of suitable cultivation of Shanxi *Bupleurum*

Based on the quality of *Bupleurum* samples and the influencing factors of related production areas, a quality evaluation model was hereby constructed to assess the quality level of *Bupleurum* in various regions of Shanxi and investigate the suitable cultivation areas for high-quality *Bupleurum*. According to the correlation results, the total content is significantly positively correlated with geographical and climatic factors, yet exhibits weak correlation with soil factors, necessitating the construction of an indicator system for evaluating the quality level of *Bupleurum* through geographical and climatic factors. Furthermore, using SPSS 24.0 statistical analysis software, factor analysis was conducted on 33 ecological factors to eliminate potential adverse effects caused by dimensional differences in the data. Standardization was applied to the data. According to the principle of eigenvalues exceeding 1, principal component analysis was utilized to extract 6 common factors, involving a cumulative variance contribution rate of 91.816%. Therefore, extracting 6 common factors could reflect the variance of the original variable at 91.816%. The total variance explained is shown in Exhibit 10.

Using the total content of five saikosaponins of *Bupleurum* in currently known counties and cities as the dependent variable Y, and the comprehensive score calculated in Sect. Identification of *Bupleurum* as the independent variable X, an appropriate model was fitted to estimate the total content at unsampled points.

$Y = -0.5686X + 12.5580$ (4).

Based on the calculated comprehensive scores for each county and city in Shanxi Province, the total content estimated by the models for each county and city was obtained by substituting the above formula. A heatmap was drawn using ArcGIS 10.7 to reflect the overall trend, as shown in Fig. 6. According to the predicted total content of saikosaponin, the cultivation areas of *Bupleurum* in Shanxi Province were hereby divided into four zones.

1) Optimal cultivation area This area includes most of Datong City, Shuozhou City, and Yizhou City. Located in the northern region of Shanxi Province, this area boasts complex terrain, with the Taihang Mountains to the east and the Lvliang Mountains to the west. It features the highest latitude and high altitude in Shanxi Province, making it ideal for the growth of *Bupleurum*.

2) Better cultivation area This district includes Yuanping City, Yifu District, Jingle County, Yangquan City, Taiyuan City except for Qingxu County, most areas of Lyuliang City except for Shilou County, Jiaokou County, Xiaoyi City, Fenyang County, and Wenshui County, Shouyang County, Yuci District, Xiyang County, Yushe County, and Zuoquan County in Jinzhong City, and Wuxiang County in Changzhi City. Most areas in this suitable habitat are located in the central northern part of Shanxi Province.

3) Second-best cultivation area This area includes Shilou County, Jiaokou County, Xiaoyi City, Fenyang County, Wenshui County in the southern part of Lvliang, Qingxu County in Taiyuan City, Heshun County, Taigu District, Qi County, Pingyao County, Jiexiu City, Lingshi County in Jinzhong City, most of Linfen City except for Xiangfen County, Quwo County, and Yicheng County, most of Changzhi City except for Wuxiang County, Gaoping City and Lingch3an County in Jincheng City, and Hejin City in Yuncheng City. Most of this area is located in the central southern part of Shanxi Province.

4) General cultivation area This area includes most of Yuncheng City except for Hejin City, most of Jincheng City except for Gaoping City and Lingchuan County, as well as Xiangfen County, Quwo County, and Yicheng CouGty in Linfen City. Located in southern Shanxi Province, it represents the region with the lowest latitude in the province.

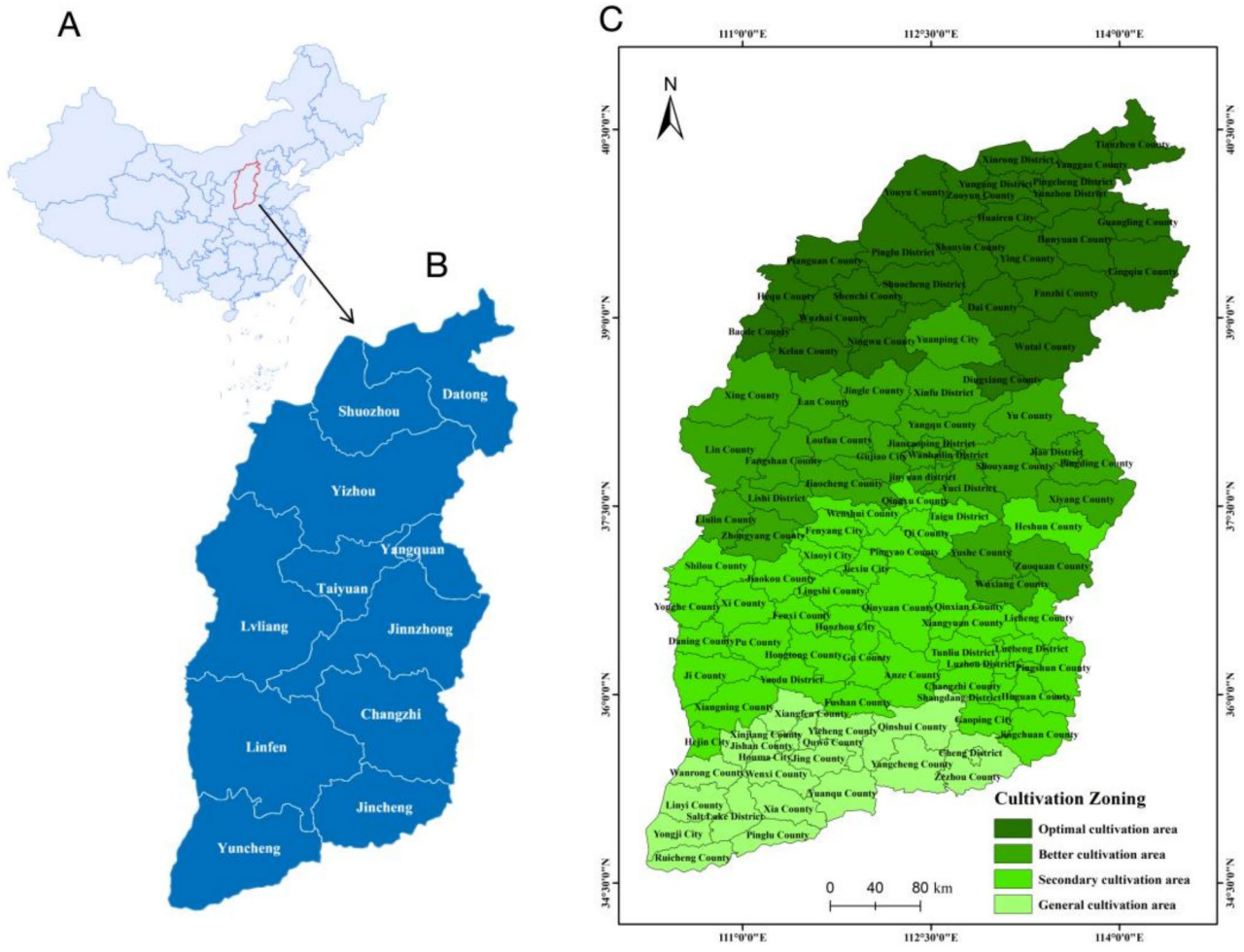

Fig. 6 Distribution map of cultivation zones. (**A**) Map of China. (**B**) Map of Shanxi Province. (**C**) Distribution map of *Bupleurum* cultivation zones in Shanxi Province

Discussion

Bupleurum, termed "Chaihu" in traditional Chinese medicine, has an extensive history and broad application [35]. Market research indicates that Shanxi *Bupleurum* production in 2024 was approximately 12,000 tons, a 15% increase from the previous year. Approximately 70% of this production is consumed within the domestic pharmaceutical market, with the remaining 30% exported primarily to Southeast Asia, Europe, and America [36]. Shanxi Province stands as one of the primary production areas for cultivating *Bupleurum* chinense in China [37–38]. Currently, there is limited investigation and analysis of the resources of authentic medicinal herb *Bupleurum* in Shanxi Province. The findings of this study align with previous research [39] on the correlation between saikosaponin content and geographical and ecological factors: Saikosaponin levels show positive correlation with latitude and longitude, and negative correlation with the frost-free period and annual average temperature. However, this article supplements the effects of ecological and soil factors on the saikosaponin content: drought and prolonged sunlight are more conducive to the accumulation of saponin components, while weakly alkaline and low alkaline nitrogen content soils are more favorable for the accumulation of saponin components. This further confirms the pivotal roles of humidity, sunlight, and soil in accumulating saponin components.

Additionally, contrasting with other studies, a quality evaluation model was hereby further constructed for *Bupleurum* chinense, with the appropriate splitting of areas of Shanxi for cultivation *Bupleurum* divided. Compared with the traditional quality evaluation model, the maximum entropy model MaxEnt was used, such as the zoning study of *Codonopsis Radix* in Shanxi Province [40], *Salvia miltiorrhiza* in Shandong Province [41], *Adenophorae Radix* [42], and Wild *Ziziphus jujubain* in Shanxi Province [43]. The model of this study is easy to operate and fast to analyze with the variance contribution of principal components as the weights for the zoning study of Shanxi *Bupleurum*, which has some novelty.

The same point of the model is that the analysis results not only ensure the quality of herbs, but also lay the foundation for the sustainable utilization of resources. The quality model construction can quickly and accurately analyze the growth suitability area of herbs, and provide new ideas and methods for the planting zoning of Chinese herbs.

Bupleurum chinense, as a plant of the genus *Bupleurum L.* in the family Umbelliferae, is well documented in Volume 1 of the Flora of China, where its taxonomic status is clear and has received widespread attention [7]. whereas, *Bupleurum marginatum* var. stenophyllum has not been reported in this botanical journal. This omission, to a certain extent, reflects the uncertainty regarding the safety and efficacy of the medicinal herb.The DNA barcode identification results reveal the presence of 2 sets of *Bupleurum marginatum* var. stenophyllum among the 70 samples collected in this study. *Bupleurum marginatum* var. stenophyllum exhibits significant differences in morphology and chemical composition compared to *Bupleurum chinense* [44]. Its lower price and higher yield and saikosaponin content compared to *Bupleurum chinense* make it a common adulterant, which can compromise the safety and effectiveness of *Bupleurum* medicinal materials. Therefore, precise and authenticated identification of *Bupleurum* is crucial during market circulation to ensure safe clinical use.

This study, while yielding significant insights, is still subjected to certain limitations. Firstly, since April marks the germination period of *Bupleurum*, May to November represent the growth phase, December to March of the following year its dormancy period, and October to November generally mark the harvesting period, this study focused on climate factors from May to October for correlation analysis. Future research should explore more comprehensive climate factors to further validate these findings. Secondly, while the saikosaponin content is negatively correlated with cumulative precipitation (W) and average relative humidity (H) in each month, the correlation analysis indicates a positive correlation between saikosaponin content in July and both W and H. This anomalous phenomenon in July may be attributed to a rapid increase in temperature and intensified transpiration, leading to a series of physiological changes within the plant, which could be a protective mechanism for the plant [45–46]. Further experiments are warranted for verification.

In summary, the findings of this study forge a theoretical foundation for selecting optimal cultivation sites for high-quality *Bupleurum* and present more effective approaches for evaluating and cultivating other medicinal herb production areas. Meanwhile, this study provides a scientific basis for the protection of wild *Bupleurum* resources and realizes sustainable development. It also provides basic data for international scientific cooperation and promotes the clinical research and application of *Bupleurum* worldwide.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06479-w.

Supplementary Material 1

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Author contributions
CSL and GXR conceptualized、directed and supervised the research. DJ provided conceptual advice and contributed to the analysis of the data. FYX designed the study and conducted the experiments, and WLZ analyzed the data and wrote the manuscript. JYZ was involved in creating the chart.QYX assisted with the experiments and with manuscript revision.

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Data availability
Data is provided within the supplementary information files.

Declarations

Ethics approval and consent to participate
The authors declare that they have obtained the necessary permissions to collect plant materials.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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