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Research Article

Foliar Application of Leaf Extracts of *Glycyrrhiza uralensis* Increases Growth and Nutritional Value of Chinese Flowering Cabbage Plants under Field Conditions

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This study was conducted to explore the effects of ethanolic extracts of leaves of *Glycyrrhiza uralensis* on the growth and nutritional quality of Chinese flowering cabbage (*Brassica rapa* subsp. *parachinensis*) under field conditions. Preliminary greenhouse experiments were carried out with different concentrations of *G. uralensis* extracts to select the suitable dose for field studies. An extract concentration of 12.5 g/L was selected based on relative growth rate analysis and increase in photosynthetic pigment biosynthesis. Shoot length, shoot fresh weight and dry weight, number of leaves, and marketable value of Chinese flowering cabbage plants were significantly increased in the field trials with foliar application of *G. uralensis* leaves extracts at a concentration of 12.5 g/L. This biotic elicitor also enhanced the total phenolic and flavonoid contents, with optimal values increasing by 18.76% and 22.43%, respectively, compared with the control under field conditions. The total glucosinolate content was effectively increased (from 11.21 to 15.37 μmol·g^{-1} DW sinigrin equivalents), particularly 4-methoxyglucobrassicin (from 4.31 to 6.72 μmol·g^{-1} DW sinigrin equivalents) and glucoalyssin (from 0.14 to 0.19 μmol·g^{-1} DW sinigrin equivalents) compared with the control plants in field trials. Overall, foliar application of leaf extracts of *G. uralensis* can markedly increase the growth of Chinese flowering cabbage and enhance its medicinal and nutritional quality in the fields.

1. Introduction

Vegetables belonging to the Brassicaceae family show valuable health benefits owing to the presence of biologically active and strong antioxidant substances. Chinese flowering cabbage (*Brassica rapa* subsp. *parachinensis*) is an annual vegetable crop belonging to the Brassicaceae family. Its growth period is short and multiple cropping index is high, with only 40–56 days from germination to flowering [1]. Chinese flowering cabbage has valuable biological and nutritional properties [2]. Its above ground parts, including leaves, stem, and inflorescence, can be cooked or consumed raw in salads. Its leaves contain adequate amounts of glucosinolates and polyphenolic compounds [3]. This rich chemical composition and scientifically proven biological activity have made Chinese flowering cabbage a famous culinary plant [4].

Glucosinolates (GLS) are primarily found in plants of the genus *Brassica*, which include crops of economic and nutritional importance. GLS are rich in sulfur and anionic secondary metabolites [5]. GLS have been extensively studied for their protective effect against herbivory in plants and chemotherapeutic activity in humans [6]. The consumption of vegetables containing glucosinolates may confer protection against cancer in humans [5]. The hydrolytic breakdown products of glucosinolates have

beneficial effects on human health, including cytotoxic and apoptotic effects in damaged cells and reducing risks of degenerative diseases [5, 6].

Lethal effects of synthetics pharmaceuticals have augmented the discovery and large-scale production of natural bioactive molecules. However, the supply of natural bioactive compounds is limited for various reasons. On the other hand, the consumer demand for these compounds is increasing progressively. Hence, application of novel strategies to meet the current growing demand for natural bioactive compounds is of immense relevance. The use of conventional approaches to accelerate natural biosynthetic pathways in plants is shown to produce high levels of bioactive compounds, without the need of genetic engineering applications [7]. Furthermore, advances in technology have augmented the discovery of new biotic elicitors capable of increasing production of secondary metabolites in plants [8, 9].

Glycyrrhiza uralensis Fisch (Fabaceae), commonly known as licorice, is a traditional plant recognized through the ages for its multiple health benefits and medicinal uses [10, 11]. The roots of this plant are used to treat influenza, coughs, and liver damage in traditional medicinal formulations [10]. However, the roots or rhizomes correspond to merely one fourth of the whole biomass of the plant. The aerial portion of licorice is of lesser importance to cultivators and usually constitutes as agroindustrial waste after the harvest. The purpose of this study was to investigate the effect of using alcoholic extracts of licorice leaves as a foliar spray to improve plant vegetative growth and concentration of specific biologically active substances such as glucosinolates, phenolics, and flavonoids in Chinese flowering cabbage under field conditions.

2. Materials and Methods

2.1. Plant Material and Preparation of the Extracts. Aerial parts of *G. uralensis* were obtained from Qinghaihu Pharmaceutical Co., Ltd. (Qinghai, China). Prof. Dr. Xuebo Hu from College of Plant Sciences and Technology, Huazhong Agricultural University, China, verified the identity of plant material. The plant material was extracted as described in our previous publication [12]. Briefly, the leaves of *G. uralensis* were air-dried and ground to fine powder. This powdered material was extracted twice with ethanol/water solution (70 : 30, v/v) for 2 h at 80°C at 80 revolutions per minute under reflux. The solvent was removed by the rotary evaporator followed by freeze drying under vacuum. The dried extracts were stored in a refrigerator until use.

2.2. Treatment Optimization of Foliar Elicitors under Growth Room Conditions. A preliminary study was preformed to optimize the dose of elicitor for field studies. Plants of Chinese flowering cabbage were raised in plastic pots of 4-inch diameter containing sterilized commercial potting mix. Foliar formulations of the elicitor were prepared in 2.5% dimethyl sulfoxide (DMSO) solution at different concentrations, e.g., 0, 2.5, 5.0, 7.5, 1.0, 1.25, 1.5 g/L. Control plants received 2.5% DMSO solution alone. Application was performed at the trifoliate stage. The relative growth rate (RGR) was calculated over 5 d time spans, after 1 week of elicitor application using the following formula:

$$RGR = \frac{InW2 - InW1}{t2 - t1}, \quad (1)$$

where $W1$ is the initial shoot DW, $W2$ is the final shoot DW, and $t2 - t1$ represents the growth period.

2.3. Determination of Photosynthetic Pigments. Quantification of chlorophyll and carotenoid contents was performed in accordance with the standard method of Arnon [13].

2.4. Field Experiment. Field studies were performed in a high tunnel at the experimental station of the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou city of Guangdong Province, China. Chinese flowering cabbage (*Brassica rapa* subsp. *parachinensis*) were planted in September 2019. The plants were grown on raised beds. The distance between the beds was 0.3 m. Equal amounts of water were supplied, and fertilizers were applied uniformly to all treatments during the trial. Plant protection was carried out by the timely spraying of agricultural pesticides. The trial was carried out in a randomized complete-block design containing three technical replicates of each treatment. Two independent field trials were conducted. No less than 100 plants were included in each treatment.

The elicitor was applied as foliar application starting at the four-leaf stage and repeated on a fortnight basis. The optimized dose of extract (12.5 g L^{-1}) was prepared in 2.5% DMSO solution. Control plants received 2.5% DMSO solution. Plant growth parameters such as shoot length, shoot biomass, and number of leaves were evaluated after 2 months of first foliar spray. The marketable value of plants was calculated using the following formula:

Marketable value (%) = 100 – (100 × percentage of injured or diseased plants/percentage of healthy plants).

A minimum of 20 plants were harvested from each treatment and were used for morphological and metabolomic analyses. Plant samples intended for metabolomics analysis were frozen in liquid nitrogen and stored at −80°C until analysis.

2.5. Analysis of the Nutritional Values of Leaves. Leaf samples for nutritional analysis were prepared as described by Mahmoud et al. [14]. Leaf samples were washed, spread on paper towels, and air dried for 60 minutes at room temperature. Thereafter, leaf samples were oven dried at 70°C to ensure constant weight. These dried samples were ground in a stainless-steel grinder, and the following nutritional analyses were performed.

2.6. Estimation of Total Phenolic, Flavonoid, and Anthocyanin Contents. The total phenolic content was determined using the standard Folin–Ciocalteu method [15] and expressed as

milligrams gallic acid equivalent per gram of dry weight tissue (mg GAE/g DW). The total flavonoid content was estimated using the aluminum chloride colorimetric method of Chang et al. [16] and expressed as mg quercetin equivalent per gram of dry weight tissue (mg QCE/g DW).

2.7. Quantification of Glucosinolate Content. We used our recently devised method [17] for the identification and quantification of different types of glucosinolates from the leaves of Chinese flowering cabbage plants. Leaves from 10 plants were taken from each treatment and pooled together. Analysis was performed on an API 4000 QTrap mass spectrometer equipped with a TurboIonSpray probe (AB Sciex; Foster city, CA, USA) connected to a Shimadzu UFLC (Shimadzu, Kyoto, Japan). Chromatographic separations were performed with a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of (A) trifluoroacetic acid (0.1%) and (B) acetonitrile/trifluoroacetic acid (99.9 : 0.1), with a gradient elution program of 2% B (0–5 min), 2–80% B (4–20 min), 80–95% B (20–30 min), and reconditioning with 2% B (30–38 min). The mass spectrometer worked with the triple quadrupole analyzer in the multiple reaction monitoring (MRM) mode in the negative ionisation mode for all analysed compounds. Applied Biosystems Analyst software was used to control the UFLC-MS/MS system and applied for data acquisition and processing. Sinigrin does not exist in *B. rapa* and *B. napus* [18]. In this study, sinigrin was used as an internal standard for quantitative analysis of glucosinolates [19].

2.8. Statistical Analysis. For data interpretation, analysis of variance (ANOVA) was performed using DSAASTAT (Onofri, Italy). Duncan's new multiple range test (DNMRT) was used to find a significant difference among means of different treatments. All experiments were repeated twice, and mean values are presented.

3. Results and Discussion

In the past few years, several studies have shown that exogenous elicitors, including plant extracts, can mediate plant growth and productivity [20–22]. To the best of our knowledge, the present study is the first to report the positive effects of alcoholic extract of nontraditional parts (leaves) of *G. uralensis* on the growth and health-promoting elements of Chinese flowering cabbage.

3.1. Effect of Biotic Elicitors on Growth of Chinese Flowering Cabbage. A preliminary study was conducted to optimize elicitor dose for field application based on the effects on relative growth rates and levels of photosynthetic pigments. The relative growth rate of Chinese flowering cabbage was greatly influenced by exogenous elicitor application (Figure 1). As leaves and shoots of Chinese flowering cabbage are mainly consumed, we focused on the above ground plant biomass. Generally, the relative growth rate (RGR) of Chinese flowering cabbage plants increased in an extract concentration-dependent manner but showed no significant difference when extract concentration was increased from 12.5 to 15 g/L (Figure 1). Plants receiving 12.5 and 15.0 g/L elicitor treatments showed highest RGR with no significant difference between them (Figure 1). An RGR of >0.2 d^{-1} was obtained when plants were sprayed with these two concentrations compared to other treatments. On the basis of these findings, the treatment containing 12.5 g/L of elicitor was selected for field application.

The effect of foliar application of the elicitor (12.5 g/L) was assessed on the growth and nutritional attributes of Chinese flowering cabbage in the experimental station of Guangdong Academy of Agricultural Sciences, Guangzhou, China. We observed that plant height of Chinese flowering cabbage treated with the elicitor was 31.06% higher than that of control plants. In addition, the fresh (39.72%) and dry (28.31%) biomass of Chinese flowering cabbage treated with the elicitor was significantly higher than that of the untreated plants on average in both field trials (Table 1). The number of leaves in the plants treated with the elicitor was 19.28% higher than that of the control plants (Table 1). Overall, the marketable value of the Chinese flowering cabbage plants improved by 18.32% after receiving elicitor treatment (Table 1).

The increased growth rate of Chinese flowering cabbage plants observed under the influence of the foliar elicitor could be attributed to the presence of phytohormones, soluble sugars, amino acids, and mineral elements in the ethanolic extracts of leaves of *G. uralensis* [23]. The constituents of *G. uralensis* leaf extracts may improve the yield and quality of Chinese flowering cabbage by affecting the cellular metabolism [17]. For example, it is known that sugars act as signaling molecules and improve plant growth and development [24]. Amino acids provide improved stress tolerance in plants [25]. Some organic acids present in plant extracts can chelate metal ions to stimulate root growth [26]. All these together could supply nutrition for cell growth, with resulting increase in growth and vigor.

3.2. Analysis of Leaf Pigment Content. Foliar application of *G. uralensis* extracts positively affected total chlorophyll and carotenoids contents in a dose-dependent manner (Table 2). Increasing the elicitor concentration from 2.5 to 15.0 g/L sharply increased the total chlorophyll and carotenoid contents, with no remarkable differences between the elicitor treatments at concentrations of 12.5 and 15.0 g/L (Table 2).

The positive effect of *G. uralensis* leaf extracts on leaf pigment content could be attributed to the delay in leaf senescence or enhancement in leaf pigment biosynthesis [27, 28]. These beneficial effects are possibly due to the presence of natural phytohormones in *G. uralensis* leaf extracts. The physiological parameter of leaf pigment content also acts as indicators of improved quality of Chinese flowering cabbage that can be obtained by the application of exogenous elicitors.

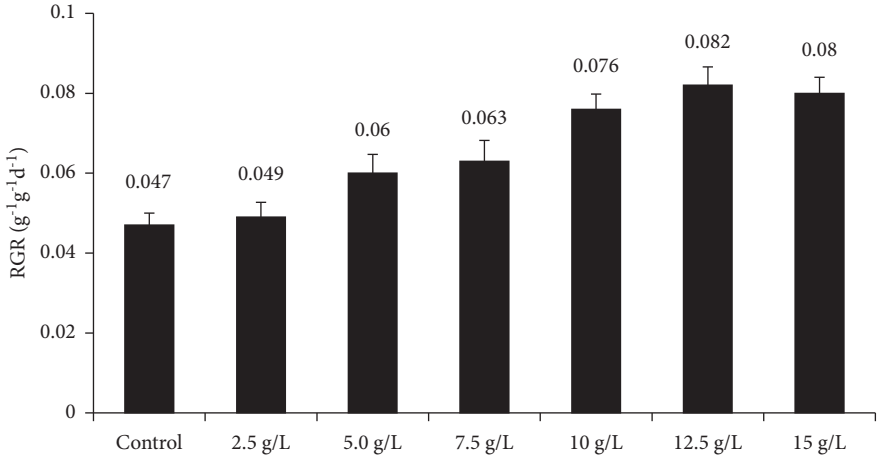


Figure 1: Effect of different concentration of leaf extracts of *Glycyrrhiza uralensis* on the relative growth rate of Chinese flowering cabbage. Vertical bars show standard error, whereas small letters show levels of significance among different treatments as governed by ANOVA and DNMRT at $p = 0.05$.

Table 1: Changes in growth attributes of *Brassica rapa* under field conditions.

Plant traits	Trial 1		Trial 2	
	Control	Treated	Control	Treated
Plant height (cm)	26.32 ± 2.1^{a}	33.57 ± 2.9^{b}	29.07 ± 1.7^{a}	31.18 ± 2.6^{b}
Shoot fresh biomass (g)	88.29 ± 5.3^{a}	141.68 ± 9.1^{b}	91.21 ± 6.2^{a}	123.17 ± 8.7^{b}
Shoot dry biomass (g)	71.91 ± 5.7^{a}	92.27 ± 6.0^{b}	76.93 ± 4.4^{a}	88.43 ± 5.9^{b}
Number of leaves	8.04 ± 0.4^{a}	10.62 ± 0.22^{b}	7.31 ± 0.36^{a}	11.31 ± 0.49^{b}
Marketable value (% age)	91.78 ± 6.24^{a}	83.54 ± 9.37^{b}	88.23 ± 7.31^{a}	81.52 ± 6.27^{b}

Data presented here are mean values of two independent field trials. Letters with ± shows standard error, whereas small letters show levels of significance among different treatments as governed by ANOVA and DNMRT at $p = 0.05$.

Table 2: Effect of *Glycyrrhiza* leaf extracts on leaf photosynthetic pigments.

Concentration (g/L)	Chlorophyll (mg/g F.W)	Carotenoids (mg/g F.W)
Control	08.52 ± 07.31^{de}	3.42 ± 02.74^{ef}
2.5	09.93 ± 05.04^{cd}	3.56 ± 01.91^{ef}
5.0	$10.85 \pm 06.19^{b\text{-}d}$	4.18 ± 02.17^{e}
7.5	12.07 ± 09.54^{bc}	5.02 ± 03.86^{cd}
1.0	14.13 ± 01.27^{ab}	5.84 ± 02.21^{bc}
12.5	14.92 ± 01.09^{a}	6.34 ± 03.87^{ab}
15.0	15.34 ± 01.58^{a}	6.86 ± 04.17^{a}

Data presented here are mean values of triplicates. Letters with ± shows standard error, whereas small letters show levels of significance among different treatments as governed by ANOVA and DNMRT at $p = 0.05$.

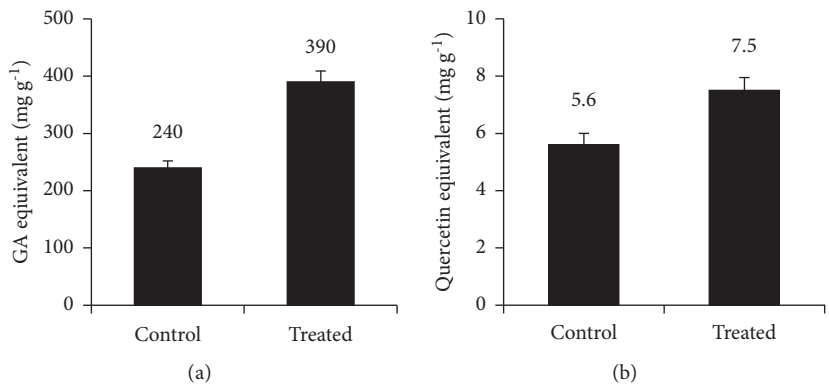


Figure 2: Effect of leaf extracts of *Glycyrrhiza uralensis* on total phenolic (a) and flavonoid (b) contents of Chinese flowering cabbage under field conditions. Data presented here are mean values of two independent field trials. Vertical bars show standard error, whereas small letters show levels of significance among different treatments as governed by ANOVA and DNMRT at $p = 0.05$.

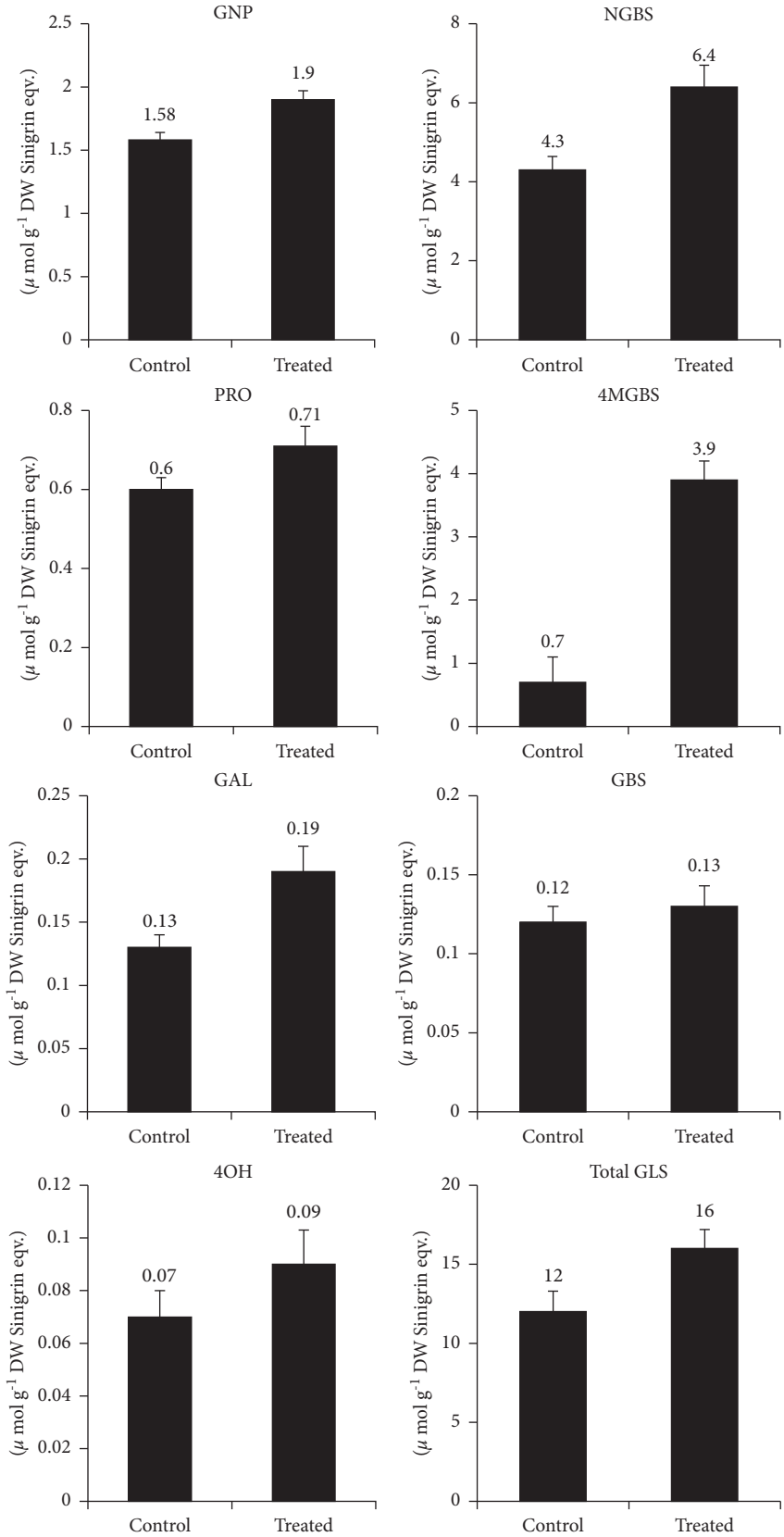


Figure 3: Effect of leaf extracts of *Glycyrrhiza uralensis* on glucosinolate content of Chinese flowering cabbage under field conditions. Data presented here are mean values of two independent field trials. Vertical bars show standard error, whereas small letters show levels of significance among different treatments as governed by ANOVA and DNMRT at $p = 0.05$.

Table 3: Details of glucosinolates quantified in of Chinese flowering cabbage.

ID	Compound	Type	Q1	Q2
Sin	Sinigrin	Aliphatic	358	195
PRO	Progoitrin	Aliphatic	388	259
GAL	Glucoalyssin	Aliphatic	450	259
GNP	Gluconapin	Aliphatic	372	371
GBS	Glucobrassicin	Aliphatic	446	259
NGBS	Neoglucobrassicin	Indolic	477	466
4OH	4-Hydroxyglucobrassicin	Indolic	463	267
4MGBS	4-Methoxyglucobrassicin	Indolic	477	259

Sinigrin was used as an internal standard for quantitative analysis of glucosinolates.

3.3. Increase in Nutritional and Medicinal Quality of Chinese Flowering Cabbage under Field Conditions. Natural bioactive compounds are isolated from a wide variety of plants. Their demand is increasing due to high therapeutic potentials and nutritional values. Data shown in Figures 2 and 3 indicate that the elicitor treatment significantly improved the production of total phenolic, flavonoid, and GLS contents in leaves of Chinese flowering cabbage plants in field trials. The foliar elicitor increased the total phenolic and flavonoid contents by 18.76% and 22.43%, respectively, compared to the control plants on average across both the field trials (Figure 2).

GLSs in Chinese flowering cabbage plants was analysed using the LC–MS/MS in MRM mode. A total of seven different GLS were quantified (Table 3). Same type of GLS has been reported in Chinese flowering cabbage in previous studies [29, 30]. Statistical analysis showed that the levels of total GLS in Chinese flowering cabbage plants was increased up to 32.62% under the influence of the foliar elicitor (Figure 3). Among different GLSs quantified, 4-methoxyglucobrassicin and glucoalyssin were sharply increased in the leaves of the Chinese flowering cabbage plants (Figure 3). Similar significant increases were also seen for other GLS such as gluconapin, neoglucobrassin and 4-hydroxyglucobrassicin (Figure 3).

The results presented in this study are consistent with the findings of Ashraf et al. [31] who reported that foliar application of plant extracts increased total phenolic and flavonoid contents of *Raphanus sativus* plants. Baenas et al. [32] showed that the nutritional quality of sprouts of *Brassica* vegetables was improved by foliar application of biotic elicitors.

In this study, application of *G. uralensis* leaf extracts caused an improvement in nutritional and medicinal values of Chinese flowering cabbage plants. This could be attributed to the presence of various nutrients and phytohormones in the leaf extracts of *G. uralensis*, which increase the production of precursor/intermediate compounds employed in biosynthetic pathways of secondary metabolites [33].

4. Conclusion

This study demonstrates that leaf extracts of *G. uralensis* could be used as an effective plant growth biostimulant. Our findings indicate that the nutritional and medicinal contents in leaves of Chinese flowering cabbage plants can be increased by foliar application of leaf extracts of *G. uralensis* at a rate of 12.5 g/L. Further study is required to understand the mechanism underlying the bioeffect on crop growth, which will be helpful in promoting the use of this biotic elicitor in organic agriculture.

Data Availability

The data used to support the study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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