Research article

Metabolite and mineral contents in root, seed, testa, stem and leaf of *Peganum harmala* L

Xiaoqing Zhu [a], Munisha Abudouaini [a], Zhufeng Geng [b], Na Liu [c], Ting Peng [c], Qing He [c,**], Yinping Li [a,*]

[a] *College of Chemistry and Chemical Engineering, Xinjiang Normal University, Xinjiang, 830000, China*
[b] *Analytic and Testing Center, Beijing Normal University, Beijing, 100875, China*
[c] *School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300350, China*

A R T I C L E   I N F O

A B S T R A C T

In order to investigate the distribution and accumulation characteristics of metabolites and mineral elements in different parts of *Peganum harmala* L. (*P. harmala*), and the synergistic or antagonistic effects between them. In this study, nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC) and inductively coupled plasma optical emission spectrometer (ICP-OES) were used to determine the contents of metabolites (proline, phosphorylcholine, choline, lysine, 4-hydroxyisoleucine, asparagine, acetic acid, sucrose, harmaline and vasicine) and mineral elements (Ca, Mg, K, P, Na, Cr, Cu, Fe, Zn, Mn, Ni, C, N) in five parts of *P. harmala*, including root, seed, testa, stem and leaf, and to analyze the relationship among the contents of metabolites and mineral elements. The results showed that the contents of acetic acid, proline, lysine, sucrose and Fe in the root were higher than those in other parts, and the contents of harmaline, phosphorylcholine, P, C, N and Zn in the seeds were the highest. The leaves were rich in vasicine, Na, K, Ca, Mg and Mn. The principal component analysis (PCA) showed that the cumulative variance contribution of the first two principal components was 69.00 %, and the loading values of K, Cu and sucrose were higher, which was consistent with the results of biplot and cluster analysis(HCA). Correlation analysis (CA) results showed that there was a strong overall correlation between the different components of seeds and leaves, and the correlation was greater than that of other parts. The results of this study are helpful to understand the correlation of functional traits among different parts of plants, and determine the internal mechanism of controlling functional traits and the proportional relationship between traits, so as to provide a reference for the resource utilization of plants.

1. Introduction

Peganum harmala L. (*P. harmala*), a member of the *Zygophyllaceae* family, is a perennial, glabrous herb which has a prismatic stem, multiple branches, long and deep roots, and good coverage. *P. harmala* as a dominant plant and pioneer species in desert steppe

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* Corresponding author.
** Corresponding author.
 E-mail addresses: qing.he@tju.edu.cn (Q. He), xinjiang20041021@163.com (Y. Li).

because its easy reproduction, rapid growth, adaptability, cold and drought tolerance and competitiveness within arid environments. *P. harmala* is lax with soil requirements and is often used to preserve soil and water and to green the wilderness. *P. harmala* usually grows on arid, arid and salinized grasslands and is widely distributed in North America, North Africa, the Mediterranean, and Asia. *P. harmala* is mainly distributed in the arid and semi-arid areas in northwest China, and it is an important component of desert vegetation. Study shows that *P. harmala* is a plant that can actively adapt to adverse environments by remodeling morphology. Various parts of *P. harmala*, including its roots, seeds, testae, stems, and leaves have long been used in traditional Chinese medicine in China. However, the researches on *P. harmala* mainly focus on the chemical composition and their biological activities, insecticidal and antibacterial effect, allelopathy, and physiological ecology [1–5]. Secondly, there are many related studies on *P. harmala* seed dormancy and germination [6]. Previously, our research group conducted NMR metabolomics studies on stems and leaves of *P. harmala* in different seasons and different parts of *P. harmala* including roots, stems, leaves, flowers, seeds, testa. The results show that *P. harmala* metabolites differ significantly in species and content. Meanwhile, metabolomics and ionomics techniques were used to study metal and nonmetallic elements in *P. harmala* samples from two distinct regions with ecological differences, clarifying the biological effects and mechanism of metallic and nonmetallic elements [7,8].

As is well known that the interaction between mineral elements and metabolites is one of the most important factors determining the absorption, accumulation and distribution of nutrient elements in plant tissues [9]. Studies have demonstrated that minerals, particularly trace elements, are essential components of proteins and enzymes [10]. They play a crucial role in sugar, protein and lipid metabolism, and possess numerous functions including osmotic regulation, immune modulation, anti-oxidation, and anti-aging. Mineral elements and metabolomics techniques have been widely used to elucidate the biological responses of various plants to different biological stresses and to consider tissue specificity within organisms. Potassium, for example, is highly resistant to insect. High levels of potassium enhance secondary compound metabolism, and reduce carbohydrate accumulation and plant damage from insect pests [11]. Although the biosynthesis of plant metabolites is mainly controlled by genetics, environmental factors also affect the production of plant metabolites [12]. For example, macro-volume mineral elements can affect terpene distribution in aromatic plants [13]. Because nitrogen is one of the most important constant mineral elements for the crop growth and development, it lacks the inhibitory chlorophyll content, which disrupts photosynthesis and photorespiration, and ultimately limits crop yield [14–16]. A variety of sugars (including fructose, galactose, glucose, sucrose, and maltose) were significantly increased in the nitrogen-deficient leaves of *Hordeum vulgare* L [17]. Carbohydrate accumulation is thought to be a key signal for reducing plant leaf photosynthesis during the fine-tuning nitrogen limitation [16,18,19]. Phosphorus is a key component of nucleic acids, proteins, and membrane lipids and is essential for many biological processes in plants [20]. Participate in photosynthesis, respiration, and the synthesis of nucleic acids and membranes. Among macroelement, potassium plays a crucial role in plant growth and development as a cofactor for major cations or various enzymes. Unlike nitrogen and phosphorus, phosphorus is not part of an organic compound, but plays important roles in many physiological and biochemical processes, for example, enzyme activation, ion homeostasis, osmoregulation, and protein synthesis [21,22]. In conclusion, mineral element content has a key role in the effects of plant metabolites [23]. The metabolism of plants changes due to various factors such as natural growth environments [24], illumination [25], development stages and different plant parts, thereby providing specific information on the condition of organisms in consistent physiological states.

Currently, there are few studies from the perspectives of metalomics and metabolomics on the accumulation differences of elements and chemical components in different parts of *P. harmala*. Therefore, this study aimed at providing a comprehensive understanding of the chemical composition differences in the root, seed, testa, stem, and leaf of *P. harmala* by using the root, seed, testa, stem, and leaf as research materials. The content of metabolites and mineral elements in *P. harmala* from different parts was determined to use proton nuclear magnetic resonance (^{1}H NMR), high performance liquid chromatography (HPLC), inductively coupled plasma optical emission spectrometer (ICP-OES) and elemental analysis (EA). Multiple variable data analysis methods, including principal component analysis (PCA), clustering analysis, Biplot and correlation analysis(CA), were used to study the distribution and accumulation patterns of metabolites and mineral elements in *P. harmala* from different parts. This study provides theoretical support for the rational development and utilization of *P. harmala*.

2. Materials and methods

2.1. Study sites

All samples were collected in Liuhuanggou Town, Changji Prefecture, Xinjiang Uygur Autonomous Region (86°20'–86°48' E, 46°39'–47°24' N). *P. harmala* whole grass with normal growth, basically the same quality and size, the plant height of about 50 cm and no pests and diseases were randomly collected for the experiment.

2.2. Chemical reagents and medicines

The concentrated nitric acid used in the digestion of the sample was purchased by J&K Scientific Company Limited (Beijing, China). The solution was prepared to use high purity water (resistivity 18.2 MΩ/cm) from the Milli-Q purification system (Millipore). The buffer was prepared with NaH_2PO_4 and NaH_2PO_4 (pH = 6), and the sample was digested with concentrated nitric acid. Analytical pure methanol, sodium azide, disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate dihydrate were purchased from Nanjing Chemical Reagent Company Limited (Nanjing, China). HPLC grade methanol and acetonitrile were purchased from Fisher scientific. A standard solution for the preparation of a multi-element calibration standard reserve solution containing Ca, Fe, K, Mg, Na, P, Mn, Zn, Ni, Cr and Cu elements (1 mg/mL in 1% nitric acid) was obtained from the National Nonferrous Metals and

Electronic Materials Analysis and Testing Center provided by the National Standard Testing and Certification Company Limited (Beijing, China). D_2O (99.9 % deuterated), sodium 3-trimethylsilyl [2,2,3,3-2D$_4$] propionate (TSP) was purchased from J&K Scientific Company Limited.

2.2.1. Metabolite extraction and quantitative analysis

The metabolites were analyzed using the ^{1}H NMR method, following the preliminary experimental protocol established by our research group [7]. All spectral data were acquired on a Bruker DRX-500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany).

To obtain the relative content of metabolites, according to the ^{1}H NMR fingerprint of different parts of the *P. harmala* (as shown in Fig. S1), the characteristic peaks (δ_H 4.03、3.23、3.21、3.02、2.22、4.12、1.92、3.69) of eight metabolites (proline, phosphocholine, choline, lysine, 4-hydroxyisoleucine, asparagine, acetic acid and sucrose) in ^{1}H NMR spectroscopy were analyzed [26]. The integrated area of the internal standard peak (d$_4$-TSP) was used as the standard for area integration, and the relative content of the five parts of the metabolites was calculated according to the quantitative formula S1-1 and S1-2.

2.2.2. Quantitative analysis of alkaloids in P. harmala by HPLC

Detection of harmaline and vasicine content in different parts of *P. harmala* was performed using HPLC. Chromatographic conditions: liquid chromatography (LC-16, Shimadzu). The chromatographic column was WondaSil C18 (4.6 mm × 250 mm, 5 μm). The mobile phase A was acetonitrile and the mobile phase B was one thousandth of formic acid water. Gradient elution procedure: equal elution, mobile phase A: mobile phase B 25:75. Detection wavelength: 285 nm, 371 nm; Column temperature: 30 °C; Flow rate: 1.0 ml min^{-1}; Injection volume: 10 μL.

2.3. Determination of mineral content

Determination of mineral content [27]: Mineral element content was determined by ICP-OES spectrometer (SPECTRO ARCOS EOP, SPECTRO Analytical Instruments GmbH, America). The samples were digested using a reaction vessel. The ICP-OES determines the total content of Ca, Mg, K, P, Na, Cr, Cu, Fe, Zn, Mn and Ni in the sample.

The total C and N content was measured by combustion combined with adsorption column separation on the CHN elemental analyzer (VARIO EL, Milan, Italy).

2.4. Data processing and statistical analysis

Quantitative data of metabolite content and mineral elements in different parts of *P. harmala* were analyzed by one-way analysis of variance (ANOVA), multiple comparisons were performed by Student-Newman-Keuls significant difference test. All statistical values were calculated using the SPSS Statisticssoftware (SPSS, Inc, Chicago, IL). The results are expressed as mean ± standard deviation (SD). SIMCA-P (version 11.5, Umetrics AB, Umea, Sweden) software was used for PCA, bitpot, HCA, CA and other multivariate data analysis.

3. Results and discussion

3.1. Metabolite content in various tissues of P. harmala

The ^{1}H NMR and HPLC were used to determine the content of 10 metabolites such as 4-hydroxyisoleucine, lysine, proline, vasicine, sucrose, asparagine, choline, acetic acid, phosphatidylcholine and harmaline in five parts of *P. harmala*, as shown in Table 1.

Our group analyzed NMR quantitative experimental data for differential metabolites in *P. harmala*. We used previous research results and the content of essential elements contained in the plant. We analyzed all data using one-way ANOVA and T-test. We

Table 1
Analysis of metabolites from different parts of *P. harmala*.

Metabolite	Root	Seed	Testa	Stem	Leaf
4-Hydroxyisoleucine	184.60 ± 2.28bcBC	–	353.86 ± 28.40 aA	204.45 ± 5.80bB	160.88 ± 7.56 cC
Lysine	176.08 ± 4.91 aA	–	–	154.00 ± 2.41bB	68.77 ± 3.49 cC
Proline	84.52 ± 0.83 aA	–	27.05 ± 0.95dD	48.30 ± 2.43 cC	56.44 ± 1.17bB
Vasicine	15.64 ± 0.75bB	6.35 ± 0.28dD	0.12 ± 0.01eE	13.23 ± 0.56 cC	42.78 ± 0.96 aA
Sucrose	329.96 ± 3.26 aA	62.79 ± 1.07 cC	13.55 ± 3.20eE	115.94 ± 2.85bB	40.42 ± 0.72dD
Asparagine	18.22 ± 2.33bB	18.69 ± 6.89bB	124.50 ± 17.75 aA	17.42 ± 1.07bB	30.33 ± 0.96bB
Choline	2.69 ± 0.13 cB	2.45 ± 1.47 cB	8.52 ± 0.57 aA	4.28 ± 0.10bB	3.31 ± 0.17bcB
Acetic acid	7.56 ± 0.19 aA	1.02 ± 0.36eE	4.42 ± 0.55bB	1.83 ± 0.09dD	2.69 ± 0.09 cC
Phosphorylcholine	2.98 ± 0.06 cB	5.20 ± 0.47 aA	3.58 ± 0.20bB	0.53 ± 0.13eD	2.01 ± 0.04 dC
Harmaline	0.80 ± 0.11 cC	18.33 ± 0.21 aA	2.71 ± 0.04bB	0.11 ± 0.01dD	0.19 ± 0.01dD

Note: The unit is mg/g, and different lowercase letters in the same column indicate significant differences ($P < 0.05$), with different capital letters in the same column indicating extremely significant differences ($P < 0.01$), "-" is not detected.

evaluated *P. harmala* using one-way ANOVA, in order to measure the significance of differences in levels of metabolites and mineral nutrients in different parts. The analysis of variance shown that lysine, proline, sucrose, vasicine and acetic acid had significant differences in different parts. The mean concentrations and standard deviations of the analyzed metabolites are shown in Table 1.

The metabolite contents in the five parts of *P. harmala* varied significantly. The contents of acetic acid, proline, lysine, and sucrose in the root were significantly higher than in other parts. Sucrose content was 329.96 ± 3.26 mg g^{-1}, whereas harmaline had minimum measurement (0.80 ± 0.11 mg g^{-1}). The seed with the highest metabolite content was sucrose (62.79 ± 1.07 mg g^{-1}), followed by asparagine (18.69 ± 6.89 mg g^{-1}), and the secondary metabolite harmaline (18.33 ± 0.21 mg g^{-1}). In testae, the content of 4-hydroxyisoleucine was 353.86 ± 28.4 mg g^{-1} which was significantly higher than that of other metabolites. Asparagine and choline were also abundant. In Fig. 1, it is known that in the leaves, apart from the primary metabolite products of sucrose, lysine, and proline, vasicine content was significantly higher (42.78 ± 0.96 mg g^{-1}) than that of the other metabolites. In *P. harmala*, the sucrose content in the root was approximately 24 times higher than that in the testa and 5 times higher than that in the seed. The harmaline content in seeds was 160 times greater than in stems, 90 times greater than in leaves, and 22 times greater than in roots. Vasicine was detected in the leaf at a concentration of approximately 350 times higher than in the testa and around six times higher than in the seed. The testa contained approximately seven times more asparagine than other parts.

The plant tissues with the highest content of 4-hydroxyisoleucine are the leaves, stems, and testae, whereas sucrose dominates in the seeds and roots. Among the root metabolites, sucrose content is significantly higher than any other compounds, with the order of concentration being sucrose > 4-hydroxyisoleucine > lysine > proline > asparagine > vasicine > acetic acid > phosphorylcholine > choline > harmaline. In the seed, the order of concentration is sucrose > harmaline > asparagine > vasicine > phosphorylcholine > choline > acetic acid. In the testa, the concentrations rank as follows: 4-hydroxyisoleucine, asparagine, proline, sucrose, choline, acetic acid, phosphorylcholine, harmaline, and vasicine. In the leaf, the concentrations rank as 4-hydroxyisoleucine > lysine > proline > vasicine > sucrose > asparagine > choline > acetic acid > phosphorylcholine > harmaline. The order of concentration in stems is 4-hydroxyisoleucine > lysine > sucrose > proline > asparagine > vasicine > choline > acetic acid > phosphorylcholine > harmaline.

3.2. Mineral nutrient, total C, and N contents of P. harmala

The results of the determination of mineral elements, total carbon and nitrogen contents in different parts of *P. harmala* are presented in Table 2. The total contents of C, N and K were highest in all parts of the plant. Among them, the roots had the highest content of carbon (464.17 ± 1.58 mg g^{-1}), while the leaves had the lowest content of Ni (1.98 ± 0.34 µg g^{-1}).

The highest C content in seeds was 226 times that of the lowest Ni content. The differences in the mineral content of the different parts can be seen in Fig. 2. In the root, the order is C > N > Ca > Na > K > Mg > P > Fe > Mn > Zn > Cr > Cu > Ni. In the seed, the order is C > N > K > Na > P > Ca > Mg > Fe > Mn > Zn > Cr > Cu > Ni. In the testa, the order is C > K > N > Na > Ca > Mg > P > Fe > Zn > Cr > Cu > Ni. The order of content is Ca > Mg > P > Fe > Mn > Cr > Zn > Cu > Ni. Whereas in leaves, the order of the content of mineral element is C > Na > N > K > Ca > Mg > P > Fe > Mn > Zn > Cr > Cu > Ni. In the stem, the order is C > K > N > Na > Ca > Mg > P > Fe > Mn > Zn > Cr > Cu > Ni.

The roots contained higher levels of Fe, Zn, Cr, Cu, and Ni compared to other plant parts. In seeds, C, N, P, Zn, and Cu were found to be predominant. There were no significant differences in K content among the outer testae, stems, and leaves.

However, there were significant differences and higher than in K content in the roots and seeds. The content of Na, Ca, Mg and Mn
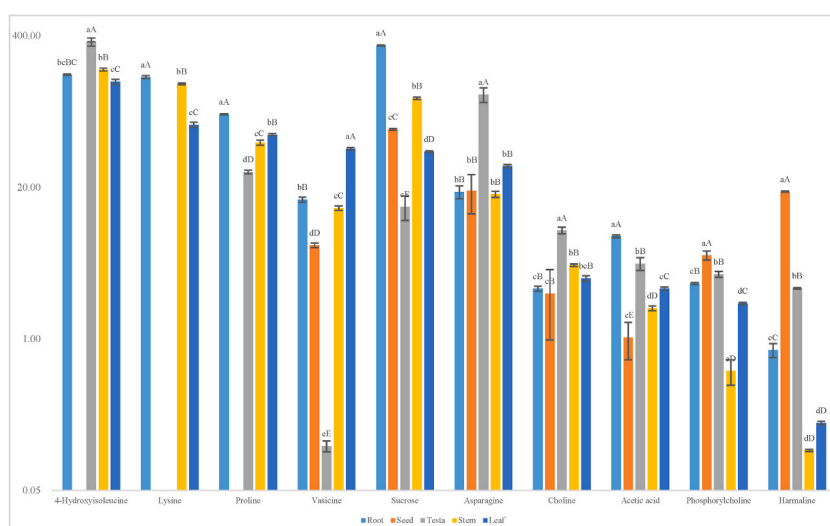

Fig. 1. Histogram of metabolite content in different parts of *P. harmala*.
Note: in mg/g, blank area is not detected, different lowercase letters indicate significant differences (P < 0.05), with different capital letters indicating extremely significant differences (P < 0.01).

Table 2
Analysis of essential elements in different parts of *P. Harmala*.

Element	Root	Seed	Testa	Stem	Leaf
C	464.17 ± 1.58bB	511.43 ± 3.73 aA	396.87 ± 1.88 cC	391.27 ± 1.61 dC	337.3 ± 2.95eD
N	32.57 ± 0.91 cC	44.8 ± 0.62 aA	21.83 ± 0.45dD	21.97 ± 0.42dD	37.5 ± 0.61bB
Na	6.19 ± 0.40dD	6.70 ± 1.22dD	10.46 ± 0.35 cC	18.60 ± 0.24bB	39.27 ± 0.83 aA
K	4.70 ± 0.22 cC	13.97 ± 1.78bB	30.28 ± 0.20 aA	31.65 ± 0.04 aA	30.14 ± 0.86 aA
Ca	13.79 ± 0.62bB	1.74 ± 0.22eE	8.44 ± 0.06dD	9.6 ± 0.11 cC	25.82 ± 0.43 aA
Mg	1.08 ± 0.07 dC	1.43 ± 0.18 cC	3.18 ± 0.04bB	3.23 ± 0.05bB	10.93 ± 0.23 aA
P	0.812 ± 0.04cBC	3.33 ± 0.40 aA	0.66 ± 0.02 cC	0.75 ± 0.01cBC	1.21 ± 0.029bB
Fe	1.12 ± 0.12 aA	0.12 ± 0.007 cC	0.1 ± 0.02 cC	0.14 ± 0.007cBC	0.3 ± 0.054bB
Mn	46.27 ± 3.22bB	14.81 ± 1.74cD	11.45 ± 0.93cD	22.52 ± 0.67 cC	63.91 ± 2.04 aA
Zn	26.44 ± 6.20 aA	28.15 ± 2.92 aA	5.59 ± 0.21 cC	29.72 ± 0.54 aA	15.73 ± 0.98bB
Cr	17.27 ± 0.38 aA	7.95 ± 0.95bcB	6.96 ± 0.96 cB	7.72 ± 1.37bcB	9.54 ± 1.93bB
Cu	8.29 ± 0.28 aA	7.23 ± 1.08bA	3.65 ± 0.06 cB	3.18 ± 0.10 cB	3.23 ± 0.13 cB
Ni	4.96 ± 0.23 aA	2.26 ± 0.19bB	2.1 ± 0.24bB	2.26 ± 0.30bB	1.98 ± 0.34bB

Note: The unit is mg/g, where Mn Zn Ni Cr Cu is μg/g, and different lowercase letters in the same column indicate significant differences ($P < 0.05$), with different capital letters in the same column indicating extremely significant differences $P < 0.01$.

in leaves was significantly higher than in other parts. In *P. harmala*, the content of element C was much higher than in all other fractions. There were significant differences in the five different fractions for C and extremely significant differences for Ca. There were no significant differences in the levels of N between N in the stems and testae of *P. harmala*. Seeds contain high amounts of P, C, N elements compared to other parts. The leaves contained a significant amount of Mg, Zn, Na, Ca, Cr, Cu, Ni. The Fe content of the roots was significantly higher than that of the other parts. Additionally, the Mg content in the leaves was much higher than that of the other parts, ranging 3–10 times higher. Zn was similarly present in the roots, stems, and seeds, and was nearly 6 times higher than that of the testa; and the content of Mn in the leaves was about 6 times higher than that in the testa, and about 4 times higher than that in the seeds.

3.3. Discriminant analysis of the metabolites and mineral elements from different tissues of P. harmala

3.3.1. Principal component analysis

The impact of different components of *P. harmala* combined with metabolites and mineral elements was determined by constructing a PCA model, which generated a score matrix plot and a load matrix plot used to obtain the grouping information for each [28]. The load matrix plot indicates the impact of individual variables on the sample grouping. The PCA pattern recognition technique was utilized to differentiate the disparities in metabolites and mineral elements among various sections of *P. harmala*. By implementing PCA, a clear differentiation of five segments of *P. harmala* was achieved (Fig. 3). The combined influence of Principal Component 1 and Principal Component 2 (PC1 and PC2) accounts for 69 %. This implies that these two principal components can explain more than 69 % of the underlying variables, making them ideal for analyzing the score and loading plots. Based on the spatial projection distance and the degree of mixing among the five sample groups illustrated in the graph, significant disparities were observed in metabolites and mineral elements across different parts of *P. harmala*. A distinct separation pattern was noted, while clustering occurred within each
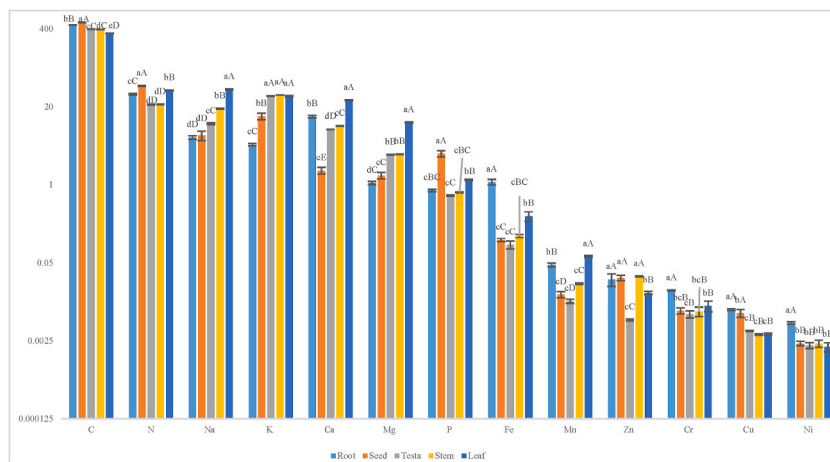

Fig. 2. Histogram of metabolite content in different parts of *P. harmal*a.
Note: in mg/g, different lowercase letters in the same column indicate significant differences ($P < 0.05$), with different capital letters in the same column indicating extremely significant differences ($P < 0.01$).

individual part group. According to the analysis of the score plot of PCA, the chemical composition of roots and seeds was significantly different from that of stems, leaves and seed coats in the PC1 direction. In the PC2 direction, the chemical composition of seeds and seed coats was significantly different from that of roots, stems and leaves.

The Biplot plot serves to combine PCA scores and loadings for simultaneous depiction and interpretation, thereby illustrating both the similarities and differences among the observations and presenting them in light of the variables. Observations positioned close to the variables have higher scores for those variables and lower scores for opposite variables. Observations situated near the plot origin have an intermediate nature. Variables near the plot origin do not contribute significantly to the formation of the discussed scores; in other words, these model components inadequately account for them. To establish the correlation between metabolites and mineral elements in the various tissues of *P. harmala*, the data underwent load vector analysis. The sample data were subjected to PCA to obtain the scores and load plots (Fig. 4). Based on the scores and loadings plots of the five different parts of *P. harmala*, it was found that 4-hydroxyisoleucine, vasicine, and the minerals Mg, Na, and Ca were present in both the leaves and stems of *P. harmala*. Sucrose was identified as the characteristic metabolite in the roots, while Ni, Cr, and Fe were determined to be the characteristic elements. Characteristic metabolites in the seeds included phosphorylcholine, harmaline, and essential minerals such as P, Cu, Zn, N, and C. The testa was found to contain choline, asparagine and K.

3.3.2. Hierarchical Clustering analysis

The HCA is used to visualize Metalomics and metabolomics. In order to assess the discriminatory power of identified chemical markers with significant differences, the heatmap based on the 23 tested biomarkers was used to visualize the variations in all samples at the molecular level. In this plot, the color shading shows the different levels of chemical composition: green to red corresponds to higher or lower concentrations, respectively. Positive values indicate levels above the average concentration and negative values below zero indicate levels below the average concentration. Therefore, data visualization was performed to get a full picture of the distribution of significantly different chemical compositions. Thus, the relative trend of chemical composition of all the tested samples is shown in Fig. 5. The heatmap clearly shows a high level of chemical heterogeneity, and there is better clustering with five different fractions grouping together. It is noteworthy that among the identified labelled compounds, Na, Mg, Mn, and vasicine were found at higher levels in leaves; N, C, P, phosphorylcholine were found at higher levels in seeds; sucrose, acetic acid, proline, lysine, Fe, Cr, Cu, and Ni were found at higher levels in roots; choline, asparagine, and 4-hydroxyisoleucine were found at testa levels were higher in stems. Except for phosphorylcholine, acetic acid and N were significantly lower than the average concentration, the rest of the mineral elements and metabolites exhibited concentrations no significant difference.

3.4. Analysis of correlation between metabolites and mineral elements

The metabolites and mineral elements of different parts of *P. harmala* were analyzed according to Pearson's correlation, as shown in Fig. 6. The 5 groups of metabolites and mineral elements showed significant negative correlations ($P < 0.05$) at the root (Fig. 6A); lysine and Cu content, proline and Cr, proline and C content, asparagine and phosphorylcholine content, choline and Zn content. Significant positive correlations were found for lysine and 4-hydroxyisoleucine, K and vasicine, acetic acid and Fe, Mn and Fe. These data suggest complex antagonistic and synergistic interactions between metabolites and mineral elements in roots.

Correlation between metabolites and mineral elements in seeds (Fig. 6B) of *P. harmala*. In seeds, 21 groups of metabolites and mineral elements were significantly correlated, with significant negative correlation were C with vasicine and phosphorylcholine, Cr with phosphorylcholine, Cu with N; with significant positive correlation were sucrose and Mn, Na, choline and Zn, Mg, Ca, acetic acid, acetic acid and Zn, Mg, Ca, K, harmaline, harmaline and K, C and Cr, Zn and Ca, Mg, Mg and K, Ca.

Correlation between metabolites and mineral elements in testa (Fig. 6C) parts of *P. harmala*. In testa, 9 groups of metabolites and phytonutrient elements had a significant correlation. There was a significant negative correlation between phosphorylcholine and acetic acid, phosphorylcholine and Cu, 4-hydroxyisoleucine and Cr, vasicine and Fe. The correlation between asparagine and Zn,
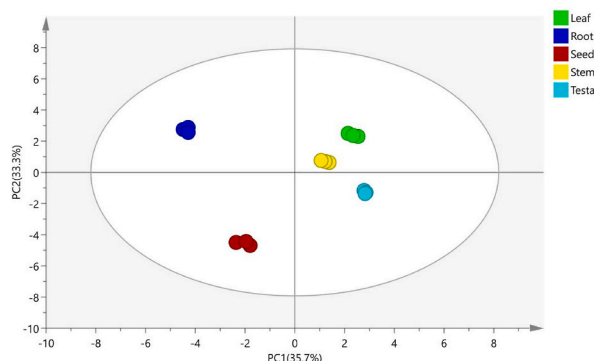

Fig. 3. Plot of PCA scores for the major metabolites of five distinct plant organs. Leaf (green), root (indigo blue), seed (reddish brown), stem (bright yellow), and testa (sky blue). The first and second principal component score points are represented by PC1 and PC2, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
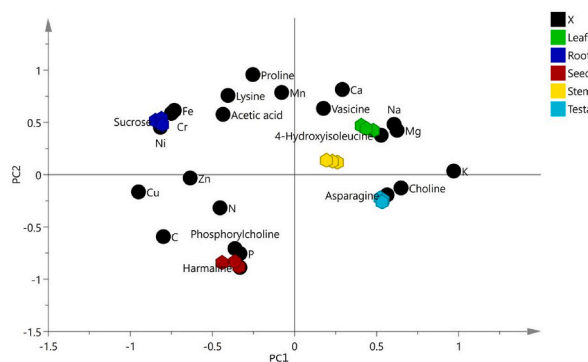
Fig. 4. Biplot of mineral elements and metabolites of five different parts of *P. harmala*. leaf (green), root (indigo blue), seed (reddish brown), stem (bright yellow), and testa (sky blue). The first and second principal component score points are represented by PC1 and PC2, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
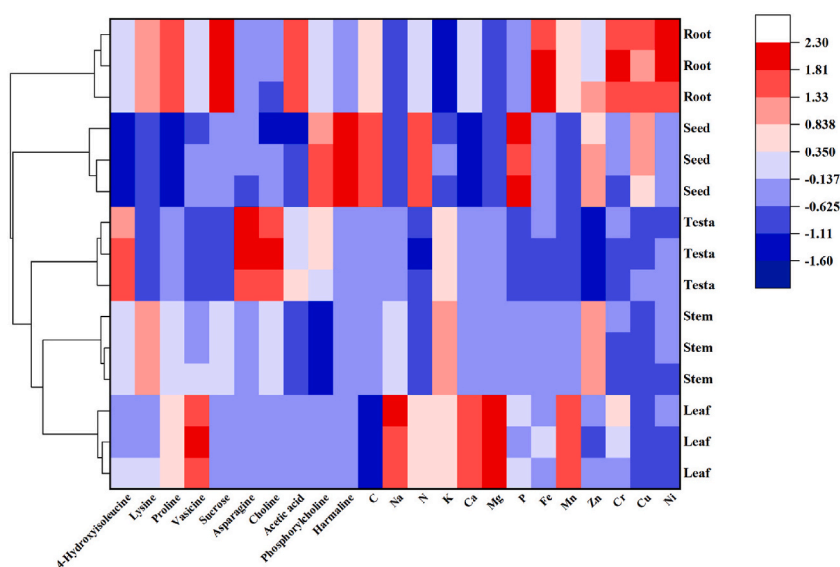

Fig. 5. Hierarchical Clustering plot. Heat map of clustering of metabolites and phytonutrients from five different organs (positive (green) and negative (red) correlations). Hierarchical trees were generated by applying the Euclidean distance metric and averaging links. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

asparagine and Cr, Cu and acetic acid, and Mg and K was significantly positive. There was no significant correlation between other metabolites and phytonutrients in testa. Thus, there is a relatively complex synergy between the nine nutrient elements in testa.

Correlation between metabolites and mineral elements in stem (Fig. 6D) parts of *P. harmala*. In stems, there was no significant correlation between most metabolites and elements, only 8 groups of substances had significant correlation. The significant negative correlation between acetic acid and lysine, sucrose and Cr, sucrose and Zn, sucrose and P. The significant positive correlation between P and Cr, P and Zn, Mg and vasicine, Ca and proline.

Correlation between mineral elements or metabolites in plants.Correlation between metabolites and mineral elements in leaf (Fig. 6E) parts of *P. harmala*. In leaves, 17 groups of metabolites and mineral elements were significantly correlated, those with significant negative correlation were Ni with acetic acid, Cu with Zn, Ca; those with significant positive correlation were Cu with vasicine, harmaline, Zn with Ca, Mn with K, Mg with lysine, sucrose, phosphorylcholine, phosphorylcholine with lysine, sucrose, sucrose and lysine, proline and 4-hydroxyisoleucine and other correlations were not significant. These data suggest that there are also complex synergistic effects between metabolites and mineral content in leaves. The pearson correlations between metabolites, mineral nutrients in different parts of the plant are given in Fig. 6. Among them, in roots and seeds, choline and Zn are negatively correlated in the former and positively correlated in the latter; Ca and Mg are both positively correlated; and Mg and K are positively correlated in both seeds and testae. In conclusion, mineral elements and metabolites interact with one another, either inhibiting or promoting the concentration of associated.
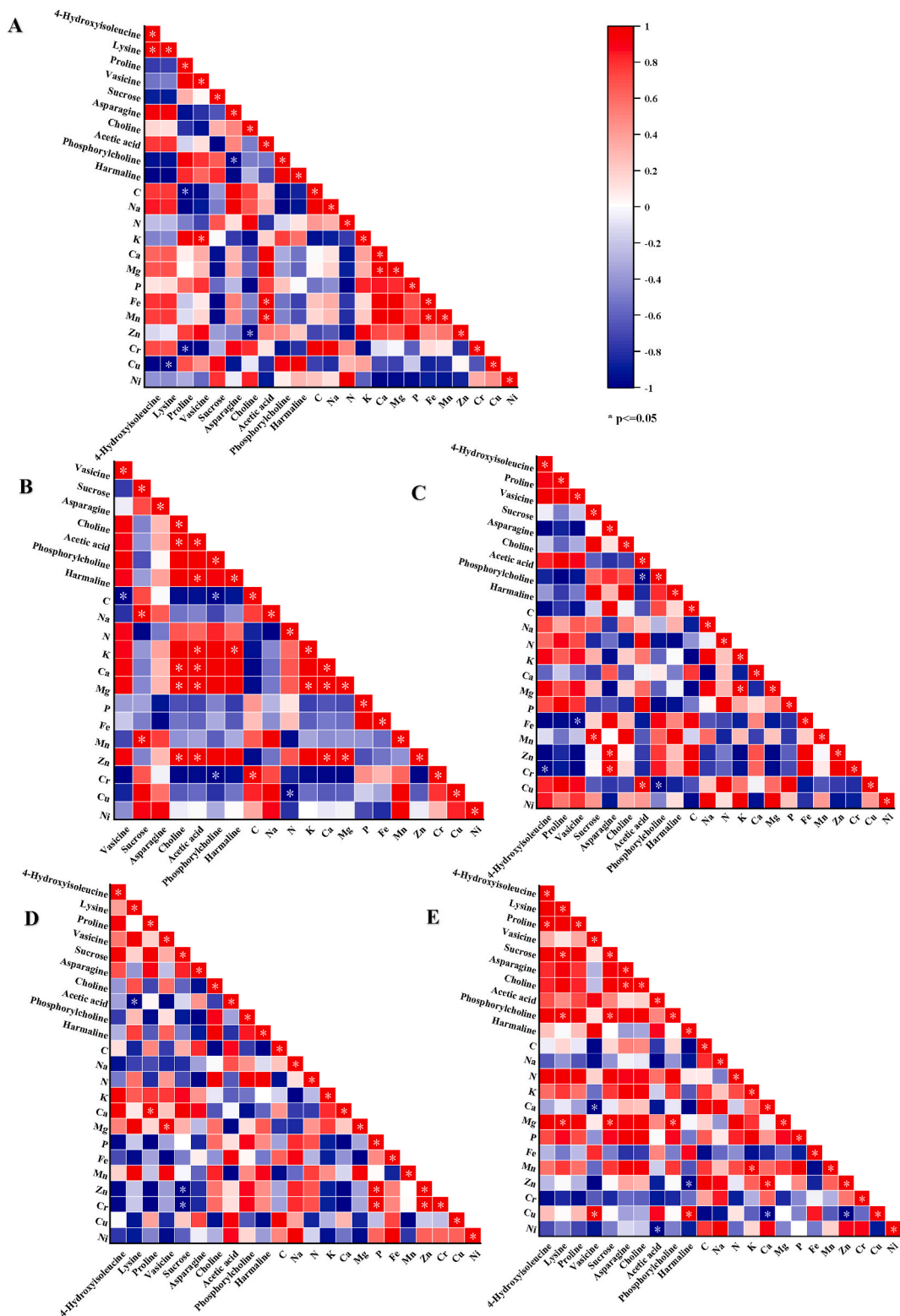
Fig. 6. Correlation analysis showing the correlation of these chemical components. This is a pseudo-color plot with the colors symbolizing the Pearson's correlation coefficient values (positive correlation (red) and negative correlation (blue)) where * represents P < 0.05. (A: root; B: seed; C: testa; D: stem; E: leaf). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Researches show that *P. harmala* is a perennial herb with long and deep roots and good coverage. It has the functions of soil conservation and sand fixation, strong adaptability, cold and drought tolerance. Due lax soil requirements, it can grow in all kinds of soils in the arid areas of Northwest China [29]. It is widely acknowledged that *P. harmala*'s roots, functioning as the nutritive organs of the plant, primarily absorb water from the soil alongside dissolved inorganic salts for the plant growth and development, vegetation fixation, organic nutrient storage, and organic matter synthesis and secretion [30]. The present study showed that the Fe content in the root of *P. harmala* was significantly higher than that in the other four parts. From the results of CA, acetic acid content in roots was significantly positively correlated with Fe and Mn content. This is consistent with the results of previous studies, and further proves that Fe and acetic acid in the roots occur chelation reaction, which promotes the desorption of metal from the soil, and at the same time enhances its absorption by plant roots [4,31,32]. Because Mn has the characteristics of changes in valence number ($Mn2+\rightleftarrows Mn4+$) in plants, it plays an important role in the redox process in plants [33]. The deficiency of Mn could lead to an increase in the concentration of hydrogen peroxide in plants, thereby affecting the balance of hydrogen ion concentration in plants and thus affecting the ability of plant roots to absorb and transport water and nutrients [34]. Weak acid conditions are more favorable for redox production. Our results showed that increasing the content of Cu and Cr in roots was detrimental to the accumulation of proline and lysine. Proline not only acts as an osmotic regulator in plant cytoplasm, but also plays an important role in stabilizing biomacromolecule structure, reducing cellular acidity, detoxizing ammonia toxicity, and acting as an energy store to regulate cellular redox potential [35]. Proline, in addition to its aforementioned functions, also serves as a scavenger of free radicals, stabilizer of membranes, and protector of cytoplasmic enzymes, safeguarding plant cells against the oxidative stress caused by heavy metal stress [36]. Excessive Cu can lead to lipid peroxidation in the membrane system of various organelles, affect the activity of enzymes in plants, inhibit photosynthesis and interfere with the normal physiological metabolic process of plants, leading to malabsorption of nutrients and reduced stress resistance of plants [37–39].

In our experiment, we found that the content of harmaline, phosphorylcholine, P, C, N and Zn elements in seeds of P. harmala was generally higher than in other parts of the plant, making a significant contribution. As an important reproductive organ of plants, seeds accumulate high content of harmaline, which can not only inhibit the growth and development of herbivores and the reproduction of offspring, but also reduce the invasion of bacteria or fungi [40,41]. CA results showed that 19 pairs of metabolites and mineral elements were significantly correlated in the seeds of harmaline. The high levels of C were detrimental to phosphorylcholine and vasicine production, and the heavy metals Cr and Cu were detrimental to phosphorylcholine and N accumulation. Phosphorylcholine is a phosphorus carrier, accounting for 5–20 % of total phosphorus in plants, and is a major component of plant cell membranes. In addition, phosphates in the plant cytosol are mainly in the form of phosphorylcholine [42]. N is one of the most important nutrients that determine the yield of plant seeds, and nitrogen plays an important role in promoting seed yield [43]. Chen Zhihong [44] pointed out that N application can significantly increase the activity of seed phosphodiesterase and dehydrogenase, resulting in an increase in seed vitality and germination rate. Many studies have shown that N fertilisation can significantly increase the transfer and accumulation of plant metabolites to the reproductive organs, and this process can increase plant vigour and thousand kernel weight [45]. At the same time, N is also an important component of protein in the plant body. Protein is the fundamental substance that constitutes protoplasm. Seed germination requires a significant amount of energy; therefore, seeds contain a substantial amount of protein. In our study, *P. harmala* seeds had a high content of Zn. According to the CA of elements and metabolites in *P. harmala* seeds, Zn content was positively correlated with organic acids and choline metabolites. The physiological role of Zn in plants is related to the synthesis of chlorophyll, growth hormone (indoleacetic acid) and it is a component of many enzymes, so the appropriate concentration of Zn can promote seed germination, improve the photosynthetic metabolism of the plant seedling and protect its antioxidant system [46], which is also a sufficient preparation for the germination of *P. harmala* seeds.

The testa serves as a safeguard that shields the seed embryo from potentially harmful external influences, as well as impedes the loss of moisture. Moreover, it acts as a barrier to pests and diseases [47]. Experimental data shows that the testa of *P. harmala* possesses elevated concentrations of K, asparagine, choline, and 4-hydroxyisoleucine in comparison to other plant components. K is exceedingly resistant to pests, and elevated levels of K advance the metabolism of secondary compounds, which reduces the buildup of carbohydrates and curtails insects' injury to plants [48,49]. The concentration of 4-hydroxyisoleucine exceeds that found in other parts of the testa in *P. harmala*. 4-hydroxyisoleucine is a non-protein amino acid, which has been deemed a potential drug for treating diabetes, owing to its bioactivities, which include induction of insulin secretion that is dependent on glucose concentration [50]. The biosynthesis of 4-hydroxyisoleucine mainly occurs through the synthesis of isoleucine from glucose using the Embden-Meyerhof-Parnas pathway, the Hexose monophosphate pathway, and the aspartic acid family amino acid reaction pathway. Isoleucine is then catalyze by enzymes to produce 4-hydroxyisoleucine. Asparagine displays a substantial positive correlation with Zn and Cr. Certain studies have also demonstrated that sufficient Zn can significantly enhance the activity of aspartic acid synthase in winter wheat [51]. Zn serves not only as a structural component of enzymes in plants but also as an activating factor for enzymes to carry out their biological functions. Additionally, the current investigation indirectly indicates that Zn aids the synthesis of 4-hydroxyisoleucine.

In our study, only K and Zn content in the stem of *P. harmala* was advantageous, while the metabolite content in the stem did not show any particular advantage over other parts. The K content in the stem can resist diseases and pests and ensure the healthy growth of the plant trunk [48,49]. In our research, there was a notable inverse relationship between sucrose in stems Cr levels, whereas P exhibited a significant direct correlation with Cr content. The phytotoxic effects of excess Cr lead to decreased growth, photosynthesis, mineral nutrients, and crop productivity, resulting in decreased sucrose content [52,53]. This indicates that the stem and the testa have similar stress resistance. The main role of the stem is to transport water, inorganic salts and organic nutrients to all parts of the plant body, and to store nutrients. The correlation study indicated [54] that the exogenous application of Zn may enhance the synthesis of

photosynthetic products and their transportation to reservoir organs by regulating carbon metabolism-related enzyme activities and boosting the synthesis capacity of photosynthetic products. This suggests that Zn contributes to the production of carbohydrates and their rapid transfer to the root [55]. Therefore, Sucrose and Zn display a noteworthy negative correlation in the stem.

In our study, the contents of vasicine, Na, K, Ca, Mg and Mn in leaves were higher than the other parts, except for the content of K in the stem and the testa, the other five substances were not significantly different than those in other parts. Analysis of data from CA revealed a significant correlation between 17 pairs of metabolites and mineral elements in *P. harmala* leaves. Mg was significantly positively associated with lysine, sucrose, and acetylcholine. It is well known leaves have a variety of important physiological and ecological functions, mainly including photosynthesis, transpiration, respiration, and nutrient transformation effects. Mg is mainly involved in photosynthetic carbon fixation and as a cofactor for a range of enzymes for metabolic activities in leaves, and plays a crucial role in the metabolism of carbohydrates, proteins, and fats as well as energy conversion in plants [56,57]. Ca can helps to balance organic acids in plants and activate some plant enzymes. It also helps to form compounds that form part of the cell wall, thus strengthening the plant's cellular structure and making it healthier and stronger [58]. Cu is an indispensable element for plants, even though it is required in very small quantities. From our experimental data, Cu was low in *P. harmala* leaves and stems. Based on the CA between mineral elements and metabolites in *P. harmala* leaves, it was found that Cu content was significantly and positively correlated with secondary plant metabolites such as vasicine and harmaline. It has been reported [59,60] that Cu stress can significantly increase the accumulation of alkaloids which may be related to the fact that the accumulation of alkaloids in plants is induced by a variety of external environmental stressors. Our results further prove that the increase of Cu facilitates the synthesis of vasicine and harmaline. Plastocyanin in chloroplasts is part of the photosynthetic electron transport chain and is involved in the nitrate reduction process [61]. A significant negative correlation between Cu and Zn, so Cu and Zn also interact antagonistically. Mn content in leaves is much higher than in other sites, and further prove that Mn participates in the photosynthetic reaction of higher plants and plays an important role in the redox process [62]. In our experimental data, there is a positive correlation between Na content and leaf, and the results of relevant literature prove that the presence of an appropriate amount of Na will promote leaf growth. Increased concentration of Na can promote carbohydrate consumption and amino acid synthesis, thereby sustaining the growth of new leaves [63].

5. Conclusion

In this study, the concentration of 10 metabolites and 13 mineral elements in 5 different parts of *P. harmala* were determined using different analytical detection techniques such as ICP-OES, EA, NMR and HPLC. Multivariate data analysis such as One-Way ANOVA, PCA, CA and HCA were used to analyzes the distribution and accumulation patterns of the metabolites and mineral elements with significant differences in roots, seeds, testae, stems and leaves of *P. harmala* and the relationships among them. The results showed that the characteristic metabolite in roots of *P. harmala* is sucrose, and the characteristic elements are Ni, Cr, and Fe. These mineral elements in the root parts are more favorable for cell division and expansion, as well as carbohydrate transfer and metabolism, and maintenance of root growth. Characteristic metabolites in seeds are harmaline, phosphorylcholine, P, Cu, Zn, N and C. The physiological function of the metabolites contained in the seed is self-protection to avoid herbivore nibbling and insecticidal activity, and it contains the mineral element Zn to make adequate preparations for the germination of the seeds. In testa, choline asparagine and K, which play a key role in controlling seed dormancy and protection from biotic and abiotic stresses of the habitat as a protective layer on the outside of the seed. Whereas, the characteristic metabolites 4-hydroxyisoleucine, vasicine and the mineral elements Mg, Na and Ca in the stem and leaf of *P. harmala* are basically the same, suggesting that the differences in their physiological functions are not significant. In the present study, the physiological responses to each other are more comprehensively inferred from the correlation analyses based on the differences in the metabolome and mineral elements in the contents of different parts of *P. harmala*. The results of this study are more useful in controlling the physiological functions of the plant. The results of the study are more helpful in determining the intrinsic mechanisms controlling the functional traits as well as the proportionality among the traits, providing important data for plant ecological physiology studies and references for the development of medicinal resources of *P. harmala*.

CRediT authorship contribution statement

Xiaoqing Zhu: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Munisha Abudouaini:** Writing – original draft, Methodology. **Zhufeng Geng:** Supervision, Resources, Methodology. **Na Liu:** Supervision, Resources, Methodology. **Ting Peng:** Supervision, Resources, Methodology. **Qing He:** Writing – review & editing, Resources, Investigation. **Yinping Li:** Writing – review & editing, Resources, Investigation.

Data availability

The data that has been used is confidential.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40009.

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