

RESEARCH ARTICLE

Title: The effect of different preparation techniques on the elemental content and hemostatic efficacy of Ophicalcitum

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Abstract: Objective: To investigate the effects of different processing techniques on the chemical element content and hemostatic efficacy of Ophicalcitum, and further explore the interaction between chemical elements and their hemostatic effects. Methods: Ophicalcitum was subjected to four processing techniques (cleaning, calcination, vinegar quenching, water quenching); and X-ray Fluorescence (XRF) was used to analyze the elemental content of the four types of Ophicalcitum powder. Through gavage experiments on KM mice, the hemostatic effect of Ophicalcitum was investigated by measuring clotting time and bleeding time. Correlation analysis between elemental content and hemostatic efficacy was performed to investigate their internal relationship. Result: The contents of Ca, Fe, Si, and Mg in Ophicalcitum varied significantly among different processing techniques, while the contents of other elements (e.g., Sr, Ni, Cu, K) showed no significant changes. Regarding hemostatic effect: vinegar-quenched > calcined > water-quenched > raw. The hemostatic effect is mainly attributed to the contents of Ca, Mg, and their oxides. These components, upon circulation, promote coagulation and inhibit plasma exudation. Conclusion: The hemostatic effect of processed Ophicalcitum (via different techniques) may mainly rely on Ca, Mg and their oxides. Both vinegar-quenched and calcined Ophicalcitum showed superior hemostatic activity, with no statistically significant difference between the two processed products. In clinical practice, the optimal processing technique for Ophicalcitum can be selected based on patients' actual symptoms.

Keywords: Ophicalcitum, Processing Technology, Element content, Hemostatic effect

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Introduction

Ophicalcitum is a calcium-rich mineral medicinal material in traditional Chinese medicine (TCM), classified as a serpentine marble-type metamorphic rock. It mainly consists of calcium carbonate (CaCO_3). First documented in *Jiayou Bencao* (Northern Song Dynasty) and this medicinal material possesses the traditional efficacy of promoting blood stasis resolution and hemostasis. Clinically, it is commonly used for the treatment of hemoptysis, traumatic bleeding, and contusion-induced pain^[1].

Rapid hemostasis is critical in bleeding disorders; delayed control poses substantial risks. Mineral medicinal materials, as an important TCM component, play a

prominent role in the treatment of bleeding-related disorders. Current research on hemostatic pharmacology of mineral TCM drugs focuses on two aspects: elemental composition and the regulatory effects of specific elements on hemostasis. For instance, calcium carbonate (CaCO_3) is the main component, with a minimum content of 40% specified in quality standards^[2]. Gao et al.^[3] investigated the material basis underlying the hemostatic effect of Ophicalcitum via pharmacological experiments. Specifically, they compared the coagulation time and bleeding time of Ophicalcitum with those of the chemical reagent calcium carbonate (CaCO_3). Based on the experimental results, they concluded that the hemostatic effect of Ophicalcitum is not associated with the content of CaCO_3 .

He et al.^[4] employed inductively coupled plasma emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS) to compare the differences in trace element contents between the raw and calcined products of Ophicalcicum from different geographical origins. Among the detected elements, the contents of Ca, Mg, Al, and Fe were relatively high in both raw and calcined Ophicalcicum samples across all origins, with Ca exhibiting the highest content. After calcination, the contents of these four elements in the raw Ophicalcicum were increased to certain extent, while the contents of harmful heavy metals (e.g., Cu, Zn, and Pb) were reduced. Fan et al.^[5] conducted a primary morphological analysis of inorganic elements in Ophicalcicum and its decoction. The content of each detected element in the decoction residue was significantly higher than that in the decoction solution, and the content order of characteristic elements in the raw drug was determined as $Mg > Ca > Fe > K > Zn > Mn > Cu$. Zhao^[6] first screened the active components of Ophicalcicum with anti-hepatocellular carcinoma activity, and simultaneously employed inductively coupled plasma mass spectrometry (ICP-MS) to determine the inorganic elements in the raw and calcined products of Ophicalcicum, so as to investigate its material basis. Both raw and calcined products contain high Mg, Ca, Na, Fe, and Zn, with Mg and Ca predominating. Ophicalcicum is a mineral medicine rich in various elements; some elements may increase in content after processing, while the contents of harmful elements may decrease.

Studies on the hemostatic effects of inorganic elements have shown that magnesium ions (Mg^{2+}) exert regulatory effects on blood coagulation by competing with calcium ions (Ca^{2+}). Specifically, Mg^{2+} prolongs the clotting time of plasma and whole blood in a concentration-dependent manner; conversely, as the Mg^{2+} concentration increase, the dissolution time of whole blood clots gradually shortens^[7]. In a study investigating the potential changes in Mg^{2+} and Ca^{2+} levels in wound fluid collected during the early stages of skin wound repair, it was observed that fluctuations in Mg^{2+} and Ca^{2+} concentrations in the wound fluid were consistent with the migration of inflammatory cells, keratinocytes, fibroblasts, and the formation of new vasculature during *in vivo* wound healing. The Mg^{2+} and Ca^{2+} levels in the wound fluid were approximately twice as high as the normal plasma concentrations of these two ions, which may potentially contribute to wound healing^[8]. Other studies have demonstrated that calcium magnesium phosphate /

hydrogel composites can significantly accelerate nerve regeneration. Ultimately, this composite facilitates wound healing through the sustained and moderate release of magnesium and calcium ions^[9]. Collectively, the aforementioned evidence indicates that calcium (Ca) and magnesium (Mg) elements play a crucial role in blood coagulation and wound healing.

Despite extensive research on processing, elemental content, pharmacology, and clinical use of Ophicalcicum, studies on the correlation between elemental content and hemostatic efficacy remain limited. Therefore, this paper aims to explore the influence of different processing techniques on the chemical element content and hemostatic efficacy of Ophicalcicum. Through gavage experiments in mice, we investigated the effects of Ophicalcicum processed by different techniques on hemostatic efficacy in mice. Furthermore, we explored the interaction relationship between its chemical elements and hemostatic efficacy through X-ray fluorescence spectroscopy technology, in order to clarify the hemostatic effectiveness and material basis of Ophicalcitu, and provide reliable data for further application research of Ophicalcicum.

1. Experimental material

1.1 Experimental drug

Samples were purchased from Taisheng Chinese Medicinal Materials Market (Guizhou Province, China) and originated from Henan Province, China. Samples were identified as authentic Ophicalcicum by morphology and complexometric titration ($CaCO_3$ content). All reagents used in the experiment, including calcein and disodium EDTA, were of analytical reagent (AR) grade and complied with the standards specified in the Pharmacopoeia of the People's Republic of China (2025 Edition).

1.2 Experimental animal

This experiment used KM species of mice, purchased from SPF (Beijing) Biotechnology Co. Ltd, SPF grade. License number: SCXK (Jing) 2024-0001, quality testing unit: Suzhou Xishan Biotechnology Co. Ltd. Weighting 18.00g to 22.00g.

2. Experimental methods

2.1 Different processing techniques of ophicalcicum[10, 11]

The different processing techniques are detailed in Table 1.

Table 1. Different Processing Techniques of Ophicalcitum

processing technique	processing method	mesh	Dosage of auxiliary materials
purifying processing	Take the raw material of the Ophicalcitum, and pick up impurities, wash it with water, smash it into small pieces, grind it into fine powder, and sieve it		—
calcining	Take the crushed pieces of clean Ophicalcitum, place them in a refractory container at 800°C, and calcine for 1.5 hours (90 minutes) until they turn completely red. Then take them out, let them cool down, grind them into fine powder, and sieve it	100 mesh (No. 6)	—
Quenched in vinegar	Calcined by the calcination method until thoroughly red, then promptly take it out while still hot and quench it in vinegar. After calcination and quenching until fully absorbed, take it out, let it cool, grind it into fine powder, and sieve it	200 mesh (No. 9)	For every 10.00kg of Ophicalcitum, there is 2.50 to 3.00kg of vinegar
water quenching	Calcined by the calcination method until thoroughly red, then immediately take it out while still hot and quench it in water. Take it out immediately, let it cool down, grind it into fine powder, and sieve it		For every 10.00kg of Ophicalcitum, there is 2.50 to 3.00kg of water

Note: 100-mesh powder is used for XRF detection, and 200-mesh powder is used for hemostatic pharmacological experiments.

2.2 Xrf analysis

X-ray fluorescence (XRF) spectrometry (Zetium Mineralsedition, Malvern) was used. The raw, calcined, vinegar-processed, and water-processed samples of Ophicalcitum were finely ground in a mortar to ensure uniformity, dried, sieved through a No.6 sieve (100 mesh), and packaged into 0.20000g/bag. These samples of Ophicalcitum processed by different techniques were placed in the sample chamber of an X-ray fluorescence spectrometer in batches. Characteristic X-ray fluorescence emitted by excited atoms (Ca, Mg, Si, Fe, Al, K, Mn) was detected and quantified by the instrument, obtaining the content of four types of elements and their oxides in Ophicalcitum processed by different technique.

2.3 Hemostatic efficacy assessment

2.3.1 Sample preparation

According to the Pharmacopoeia of the People's Republic of China (2025 Edition), the oral dose of Ophicalcitum is 4.5–9.0 g as powder. Four processed powders (raw, calcined, vinegar-quenched, water-quenched) were sieved (200 mesh) and suspended in distilled water (0.4–0.8 mL) for gavage.

2.3.2 Method of administration

These 30 mice were divided into five groups: A (blank control group), B (raw product group), C (calcined product group), D (vinegar-processed product group), and E (water-processed product group), with 6 mice in each group. For group A, normal saline was administered by gavage at a dosage of 40.00ml/kg/d. For group B, C, D and E, which received Ophicalcitum treatment, these

suspensions of each Ophicalcitum samples were administered by gavage at a dosage of 0.00117mg/kg/d, continuous administration lasted for 7 days.

2.3.3 Determination of clotting time and bleeding time[12]

Capillary glass tube method: Insert the capillary glass tube into the posterior venous plexus of the mouse's eye socket for 4 to 5mm. Start timing when blood begins to injected into the capillary glass tube. After filling the capillary glass tube, take it out and place it flat on the table. Every 20 seconds, break both ends by about 0.5cm and slowly pull them apart left and right. When blood streaks appear, it indicates the clotting time.

Slide method: Quickly remove one eyeball of a mouse with medical forceps to allow blood to flow out. Drop a drop of blood onto a slide with a diameter of 5mm. Start timing immediately with a stopwatch. Every 20 seconds, gently lift the edge of the blood drop with a cleaning pin

once to observe if there are any blood streaks. When there are blood streaks, it indicates the clotting time.

Tail-cutting method: Cut the tail tip of the mouse by 1cm and start timing immediately. When blood drops form, use a quantitative filter paper to absorb it until the blood stops flowing out. Start timing until the blood stops flowing out, which is the bleeding time.

3 Experimental results and analysis

3.1 Detection result for xrf

The elemental content of Ophicalcitum obtained by different processing techniques (raw, calcined, vinegar-processed, and water-processed products) were detected and analyzed by X-Ray fluorescence spectroscopy. The detection results of Ophicalcitum from different processing techniques were shown in Table 2.

Table 2. Results of XRF element (elemental) determination of Ophicalcitum by different

Elements	Purification (%)	Calcination (%)	Vinegar (%)	Water (%)
Ca	94.465	96.565	94.930	94.872
Mg	2.672	2.066	2.531	2.723
Si	1.787	0.677	1.055	1.672
Fe	0.29	0.241	0.290	0.281
K	0.257	0.252	0.254	0.058
Sr	0.048	0.047	0.047	0.045
Cu	0.048	0.046	0.042	0.021
Mn	0.042	0.042	0.043	0.038
Ni	0.040	0.064	0.060	0.048
S	0.011	—	—	—
Al	0.231	—	0.164	0.242
Cl	0.109	—	0.584	—

Note: '—'It indicates that the content of this element was not detected.

The changes in the main element contents of Ophicalcitum caused by different processing techniques were shown in the line Figure 1.

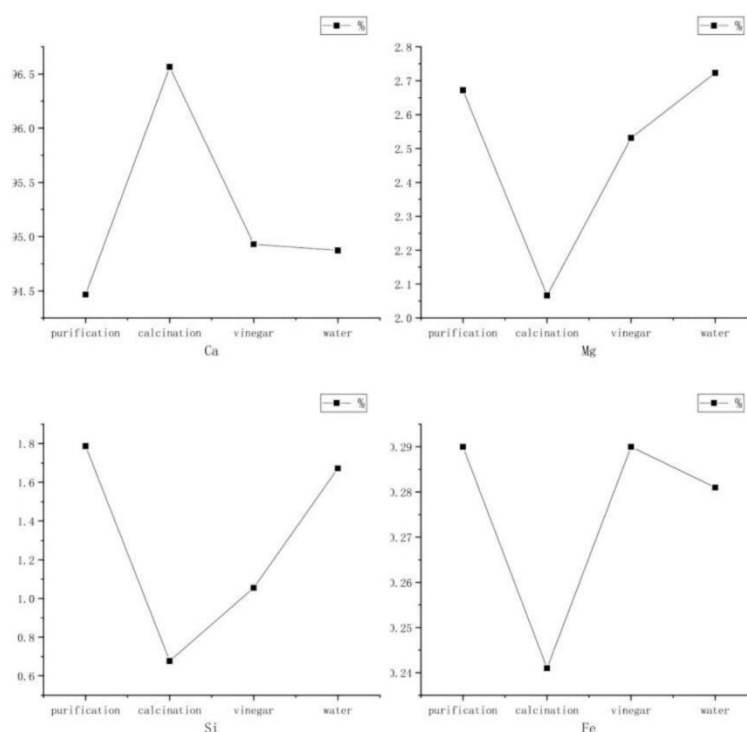


Figure 1. Line graph of the element (elemental) content of Ophicalcitung

The detection results of oxide content of Ophicalcitung processed by different techniques were shown in Table 3.

Table 3. Determination Results of XRF elements (oxides) in Ophicalcitung processed

Oxides	Purification (%)	Calcination (%)	Vinegar (%)	Water (%)
CaO	92.416	95.231	93.195	92.564
MgO	3.453	2.922	3.681	3.618
SiO ₂	3.093	1.242	1.816	2.963
Fe ₂ O ₃	0.263	0.210	0.255	0.249
K ₂ O	0.249	0.240	0.243	0.233
SrO	0.035	0.035	0.035	0.035
CuO	0.035	0.034	0.031	0.031
MnO	0.035	0.033	0.037	0.032
NiO	0.041	0.053	0.042	0.047
SO ₃	0.016	—	—	—
Al ₂ O ₃	0.272	—	0.257	0.255
Cl	0.092	—	0.471	—

Note: '—'It indicates that the content of this oxide was not detected The changes in the main oxide content of Ophicalcitung processed by different techniques were shown in line Figure 2.

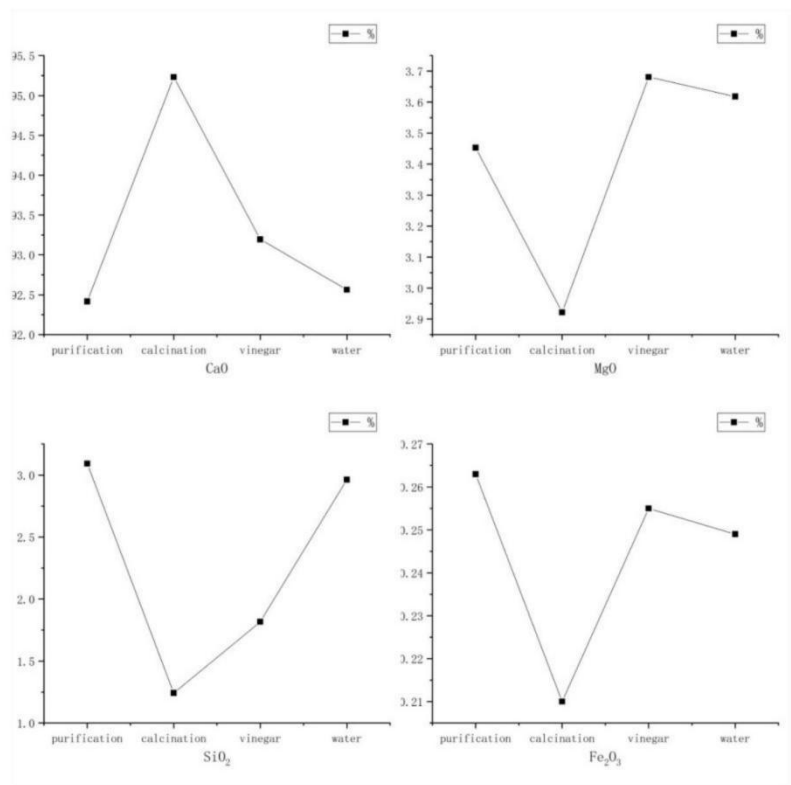


Figure 2. Line graph of main oxide content of Ophicalcitur

According to the data, significant differences were observed in major elemental and oxide contents among processing techniques. Calcined product showed the highest Ca and CaO, but the lowest Mg, Si, Fe, and their oxides. The contents of various elements in the vinegar-processed product were between those in the calcined product and the raw product, and the contents of various elements in the water-processed product were not

significantly different from those in the raw product. The differences in the contents of other elements and oxides were not significant.

3.2 Styplicity

The results of coagulation time and bleeding time in mouse hemostasis experiments were shown in Table 4.

Table 4. Measurement of coagulation time and bleeding time after administration in mice

	Blank (A)	Raw products (B)	Calcined products (C)	Vinegar products (D)	Water products (E)
Clotting time	52.35±7.14a	44.07±6.83	41.96±5.22	41.24±3.10	43.96±5.89
(capillary method)					
Clotting time	50.32±6.22	47.46±6.71	43.51±3.71	43.01±3.28	45.22±5.16
(slide method)	49.11±6.92	46.65±4.16	44.84±7.05	43.74±3.83	45.75±7.80
Bleeding time	83.32±9.41	71.02±7.16	68.87±9.83	67.39±7.37	70.91±9.49

Different processing techniques of Ophicalcitur exhibit hemostatic effects in the tests for clotting time (capillary method, slide method) and bleeding time (tail-cutting method). Compared with the blank control group (physiological saline), the aforementioned methods demonstrate the effectiveness of Ophicalcitur as a hemostatic mineral medicine. Hemostatic efficacy ranked: vinegar-quenched > calcined > water-quenched > raw. The

vinegar-quenched and calcined products showed better efficacy, though the difference was not significant.

3.3 Interactive analysis of element content and hemostatic efficacy

The contents of Ca and Mg and the changes in their oxides in this experiment were detailed in line Figure 3 and Figure 4.

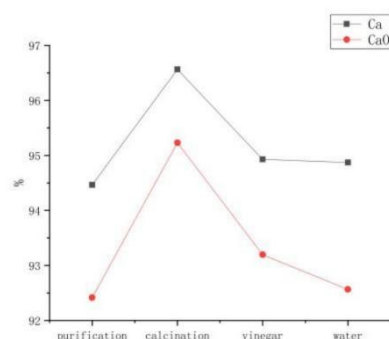


Figure 3. Shows the changes in the contents of Ca and CaO elements

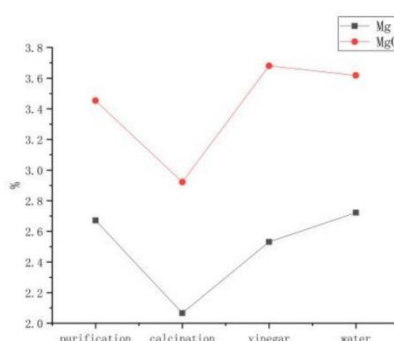


Figure 4. Shows the changes in the contents of Mg and MgO elements

Based on the results in 3.1 and 3.2, given the high calcium content, the hemostatic effect is likely mediated by Ca^{2+} (coagulation factor IV), which increases blood calcium upon absorption. After the drug enters the mouse body, was absorbed and distributed into the blood circulation, it can effectively increase the blood calcium concentration, promote blood coagulation, and exhibit a hemostatic effect. After undergoing processing techniques (calcination, vinegar quenching, and water quenching), the calcium, magnesium, and their oxides in the Ophicalcium undergo significant changes. Magnesium ions (Mg^{2+}) can reduce vascular permeability, prevent plasma extravasation, and thereby assist in hemostasis. Therefore, magnesium ions (Mg^{2+}) and calcium ions (Ca^{+}) may exhibit an interactive coagulation effect in hemostasis.

According to the results of hemostatic efficacy in mice in section 3.2, the hemostatic effects of different processing techniques of Ophicalcium were as follows: vinegar quenching > calcination > water quenching > purification. Among them, calcination and vinegar quenching had the best hemostatic effects, but the difference between the two were not significant. According to the graph of calcium and its oxide content changes in Figure 3, the content of Ca and CaO was the highest in the

calcined product, followed by vinegar-processed product. CaO was soluble in water, which can increase the calcium concentration in mice after oral administration, thereby enhancing the blood coagulation process. According to the graph of magnesium and its oxide content changes in Figure 4, the vinegar-processed sample had the highest MgO content, with Mg content slightly lower than that of the water-processed and raw sample, and higher than that of the calcined sample. MgO was slightly soluble in water. Following oral administration and entry into the murine systemic circulation, the magnesium concentration increases, which can induce blood coagulation and thereby exert a hemostatic effect in mice. Consequently, the hemostatic effects of Ophicalcium processed by different techniques are may mainly attributed to calcium (Ca), magnesium (Mg), and their oxides.

4 Summary and discussion

This article conducts chemical element analysis of Ophicalcium under different processing techniques and studies its hemostatic effect on mice, and analyzes the interaction between element content and hemostatic effect.

Ca, Mg, and their oxides were the predominant components across all processed products of Ophicalcium.

The content of Ca and its oxide CaO was the highest in the calcined product, while the content of Mg and its oxide MgO was lowest in the calcined product. The hemostatic experiment on mice demonstrated that all the processed products of Ophicalcitum showed hemostatic effectiveness compared to the control group. Among them, the vinegar-processed and calcination processes exhibited better hemostatic effects, but the difference in hemostatic effects between the two processes was not significant. Through a comprehensive interactive analysis of element content and hemostatic efficacy, it was found that the calcium content in Ophicalcitum was abundant. The hemostatic efficacy of Ophicalcitum processed by different techniques may mainly relies on the role played by Ca, Mg and their oxides in the circulation of mice. Combined with the interactive effects of other elements such as iron, nickel, chlorine, and copper, they promote blood coagulation and prevent plasma extravasation, exhibiting a hemostatic effect.

5 Conclusions

In conclusion, the effectiveness of the hemostatic effect of the mineral medicine Ophicalcitum can be analyzed from the perspective of chemical element content and pharmacological experiments, providing valuable experimental data and results for the clinical application of the mineral drug Ophicalcitum in hemostasis. However, as a mineral-based medicine, the clinical application of Ophicalcitum requires comprehensive consideration of the actual symptoms of patients and the complete mechanism of its action in the body. Further research can be conducted on the interactive effects between the remaining pharmacological activities and chemical element content of Ophicalcitum, and currently, mineral medicines such as Ophicalcitum can be deeply studied in the context of the extensive development of nanomedicine as nanocarrier delivery systems.

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Author contributions

Zhangxian Xu: Writing – original draft, Methodology, Formal analysis. **Zhenkuo Liu:** Writing – review & editing, Supervision, Project administration, **Yan zhang:** Writing – review, Funding acquisition. **Junshan Mo, Hongmei Liao:** Visualization, Formal analysis. **Ru Li:** Formal analysis.

Conflicts of interest

The authors declare no conflicts of interest.

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