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MINERAL ELEMENT LEVELS AND PROLIFERATIVE EFFECTS OF SEVERAL *DAVALLIOIDS* FROM DIFFERENT ZONES OF CHINA ON OSTEOBLASTIC-LIKE UMR-L06 CELL

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Abstract

We used ICP-MS to determine the contents of thirteen mineral elements (Na, Mg, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb) in 27 species of *Davallioid* from the Yunnan province in China and tested their ethanol extracts for proliferative effects on the osteoblastic-like UMR-l06 cell. The order of average concentrations of mineral elements was K>Ca>Mg>P>Na>Mn>Fe>Zn>Cu>Pb>Ni>Cr>Cd. The min and max element contents for different mineral elements were K(2439.37-36929.92 mg/Kg^{-1} dw), Ca(2108.02-10130.27 mg/Kg^{-1} dw), Mg(1166.75-6996.91 mg/Kg^{-1} dw), P(277.63-4091.87 mg/Kg^{-1} dw), Na(53.11-1874.58 mg/Kg^{-1} dw), Mn(37.64-2430.03 mg/Kg^{-1} dw), Fe(33.33-1747.84 mg/Kg^{-1} dw) , and Zn(22.12-178.36 mg/Kg^{-1} dw), etc. Sixteen ethanol extracts had promoting effects on cell proliferation, and three of them, *Humata platylepis* (**5,** P%=36.3), *Polypodisdesamoena* (**12,** P%=32.3), and *Drynaria rigidula* (**25,** P%=36.8), had the most proliferative effects. From the research, we found that the high content of Ca and low content of Mn could result ina positive effect on proliferation of the osteoblast-like UMR-106 cell, while the low content of Ca and high content of Mn could result in an inhibitive effect on the proliferation of the osteoblast-like UMR-106 cell. The mineral element contents and biological activity of *Davallioid* showed a wide variability among different species.Mineral elements and biological activity in different *Drynaria fortunei* varied in the range and we confirmed that this was the result of different geo-environmental conditions and soil characteristics.

Key words: *Davallioid*, *Drynaria fortunei*, Mineral element, Proliferation, Osteoblastic.

Introduction

Osteoblast-like UMR-106 cells are a widely-adopted model in research on bone growth and osteogenesis. During the proliferation, these cells experience the synthesis and secretion of organic substrates and can participate in bone mineralization. In the bone remodeling cycle, osteoclasts first dissolve the mineral and disrupt the substrates. Then, newly-obtained substrates are mineralized to shape new bone. Agents, such as growth, differentiation factors, and even mineral elements, can stimulate these cells to increase the formation of bone and prevent against osteoporosis. Recently, several anti-osteoporosis medical treatments were published and found to be potent, based on UMR-106 cell and herb extraction results, such as *Icariin* (Li *et al*., 2016), *Epimedii* (Xiao *et al*., 2014a), *Sambucus williamsii Hance* (Xiao *et al*., 2014b), *Ligustrum lucidum* (Huang *et al*., 2014), and *Naringin* (Pang *et al*., 2010). However, to date, there have been few reports on *Davallioids* on osteoblast cells, with the exception of *Drynaria baronii specie* (Wang *et al*., 2001).

The *Davallioid* comprises many species, such as *Lepisorus contortus*, *Leucostegia immerse,Colysis elliptica*, *Phymatopteris crenatopinnata*, *Humata platylepis*, *Drynaria delavayi*, *Phymatopteris ebenipes*, *Gymnogrammitis dareiformis*, *Drynaria propinqua*, *Polypodiastrum mengtzeense*, *Microsorum membranaceum*, *Polypodiodes amoena*, *Polypodiodes lachnopus*, *Arthromeris wallichiana*, *Phymatosorus cuspidatus*, *Phymatopteris trisecta*, *Pseudodrynaria coronans*, *Phymatopteris oxyloba*, *Arthromeris mairei*, *Humata tyermanni*, *Drynaria sinica*, *Drynaria quercifolia*, *Drynaria fortune*, *Drynaria bonii*, and *Drynaria rigidula*, etc, all ofwhich are famous herbal drugs in Traditional Chinese Medicines (TCMs). They have been used to cure many bone-related diseases in China, including bone fractures, arthritis, muscle injuries, tissue swelling pain, and rheumatism (Wang & Yang 2005). Among them, *Drynaria fortunei*is a traditional Chinese medicine that is widely used in the treatment of bone-related diseases, and modern pharmacological research has shown that the ethanol extractof the rhizomes of *Drynaria fortunei*exhibit various activities, such asconcentration-dependentactivity on bone marrow stromal cells, osteoporosis and hyperlipemia prevention, and an osteoarthritis cure.(Yang *et al*., 2013, Zhang *et al*., 2009, Wong *et al*., 2007, Jeong *et al*., 2005a, Jeong *et al*., 2005b). Meanwhile, phytochemical investigations found that the main components of *Drynaria fortunei*are flavonoids, phenylpropanoids, triterpenes, and many mineral elements (Wang *et al*., 2008, Zhang *et al*., 2009, Liu *et al*., 1999). According to the literature, many mineral elements are plant nutrients ,vitally important for various metabolic processes of the human body and play a significant role in the formation of the active components responsible for their curative properties. Human growth and general health areclosely related to these elements, and deficiencies or imbalances could cause physiological disorders (Arain *et al*., 2017, Kanias *et al*., 1993, Yamashita *et al*., 2005, Naidu *et al*., 1999, Balaji *et al*., 2000). The present investigation revealed that calcium contents will help the local people in hypertension, osteoporosis and in bones strength.(Dastagir *et al*., 2017).

The main purpose of this study is to explore the proliferative effects of osteoblastic-like UMRl06 cells on twenty-seven species of *Davallioids*, which we collected from different zones in Yunnan Province, China, using the bone remodeling cycle and the idea that mineral elements play an important role in bone formation, and then build the relationship between mineral element levels of plants (the whole plant) and the biological activities of their ethanol extracts.

Materials and Methods

Sample preparation for ICP-MS analysis: The acquisition sites of 27 samples of *Davallioid*are listed in Table 1. Dr. Lu Shugang, a botany professor of the Institute of Ecology and Geobotany, Yunnan University, authenticated the samples and deposited the voucher specimens for future reference. We dried the samples at 60°C to constant weight, and accurately weighed (0.5000±0.0005 g) them in the Erlenmeyer flasks (25 mL), added 5 mL of conc. HNO_3 and 1 mL H_2O_2 (30%), left them standing overnight, and then heated and volatilized them to dry. We heated the residual solid at 550°C again for 4h and dissolved it in deionized water to 100.00 mL. We repeated this process five times with every sample. We purchased the reference substances and analytical reagents from Beijing Beihua Fine Chemical Co., Ltd.

Plant material extraction: We extracted the dried whole plant samples (each 500g) with 95% ethanol three times at room temperature, and then concentrated the filtrates under vacuum conditions to obtain extracts (about 100g for each). We weighted 20mg of extract and NaF (as a positive control) (Wang *et al*., 2001), and obtained 0.01 mg/ml concentration of solution from the dilution.

Cell culture and proliferation assay: The methods can refer to literature (Wang *et al*., 2001, Meng *et al*., 2004). We wrote proliferation data as the mean±standard deviation from the Student's t-test for statistical significance. The value of $p<0.05$ implied that a value was significant. We obtained proliferation ratios using the equation: $\% = (A_{sample} - A_{blank})/A_{blank} \times 100\%$, where A was the absorbance of three wells.

Table 1. Sample origins in Yunnan province.

No.	Plant name	Site	Longitude (ºE)	Latitude (ºN)	Source
1.	*Lepisorus contortus*	Xinping, Yuxi	101.98	24.06	Wild picking
2.	*Leucostegia immersa*	Xinping, Yuxi	101.98	24.06	Wild picking
3.	*Colysis elliptica*	Xinping, Yuxi	101.98	24.06	Wild picking
4.	*Phymatopteris crenatopinnata*	Xinping, Yuxi	101.98	24.06	Wild picking
5.	*Humata platylepis*	Xinping, Yuxi	101.98	24.06	Wild picking
6.	*Drynaria delavayi*	Shiguzhen, Lijiang	100.14	26.52	Wild picking
7.	*Phymatopteris ebenipes*	Xinping, Yuxi	101.98	24.06	Wild picking
8.	*Gymnogrammitis dareiformis*	Xinping, Yuxi	101.98	24.06	Wild picking
9.	*Drynaria propinqua*	Xinping, Yuxi	101.98	24.06	Wild picking
10.	*Polypodiastrum mengtzeense*	Xinping, Yuxi	101.98	24.06	Wild picking
11.	*Microsorum membranaceum*	Xinping, Yuxi	101.98	24.06	Wild picking
12.	*Polypodiodes amoena*	Xinping, Yuxi	101.98	24.06	Wild picking
13.	*Polypodiodes lachnopus*	Xinping, Yuxi	101.98	24.06	Wild picking
14.	*Arthromeris wallichiana*	Xinping, Yuxi	101.98	24.06	Wild picking
15.	*Phymatosorus cuspidatus*	Xichouxian, Wenshan	104.68	23.42	Wild picking
16.	*Phymatopteris trisecta*	Xinping, Yuxi	101.98	24.06	Wild picking
17.	*Pseudodrynaria coronans*	Xinping, Yuxi	101.98	24.06	Wild picking
18.	*Phymatopteris oxyloba*	Xinping, Yuxi	101.98	24.06	Wild picking
19.	*Arthromeris mairei*	Xinping, Yuxi	101.98	24.06	Wild picking
20.	*Humata tyermanni*	Weishanxian, Dali	100.33	25.23	Wild picking
21.	*Drynaria sinica*	Habaxueshan, Diqing	99.72	27.78	Wild picking
22.	*Drynaria quercifolia*	Menglaxian, Xishuangbanna	101.56	21.48	Wild picking
23.	*Drynaria fortunei*	Menglaxian, Xishuangbanna	101.56	21.48	Wild picking
24.	*Drynaria bonii*	Xinping, Yuxi	101.98	24.06	Wild picking
25.	*Drynaria rigidula*	Jinghong, Xishuangbanna	100.25	21.27	Wild picking
26.	*Drynaria fortunei*	Institute of Botany, The Chinese Academy of Science	102.73	25.04	Artificial cultivation
27.	*Drynaria fortunei*	Institute of Botany, The Chinese Academy of Science	102.73	25.04	Wild transplant

Table 2. The detection limits, operating conditions, and standard curves of the mineral elements.

Element	DL ($\mu g \cdot mL^{-1}$)	Operating condition		Standard curve	R^2
		Detection wavelength (nm)	Passband (nm)		
Na	0.06333	589.0	0.2	*A* =6.6153X + 3.6892	1.0000
Mg	0.001343	285.2	0.5	*A* =2.9020X + 0.0094	1.0000
P	0.02202	213.6	0.5	*A* =0.0943X + 0.0022	1.0000
K	0.01127	766.5	0.5	*A* =3.9397X + 0.5069	0.9999
Ca	0.1123	422.7	0.5	*A* =0.0115X + 0.0030	0.9999
Cr	$9.652*10^{-5}$	357.9	0.5	*A* =46.5311X + 0.0484	1.0000
Mn	$8.107*10^{-5}$	279.5	0.5	*A* =31.5192X + 0.0170	0.9999
Fe	0.0003842	248.3	0.2	*A* =44.1711X + 0.1566	0.9999
Ni	0.0001415	232.0	0.2	*A* =23.7972X + 0.0594	0.9999
Cu	$8.076*10^{-5}$	324.8	0.5	*A* =64.8529X + 0.0334	0.9999
Zn	0.0007235	213.9	0.5	*A* =11.3639X + 0.0262	0.9999
Cd	$4.4*10^{-5}$	228.8	0.5	*A* =6.6153X + $5.8339*10^{-5}$	0.9999
Pb	$1.718*10^{-5}$	283.3	0.2	*A* =9.7174X + 0.0023	1.0000

Table3. Determined and certified values of elements, n = 4.

Element	Certified value (mg kg^{-1})	Determined value (mg kg^{-1})	Recovery (%)
K	1677.50 ± 121.35	1670.00 ± 135.47	94.0
Ca	1741.25 ± 61.38	1742.5 ± 79.31	100.3
Mg	557.00 ± 15.34	667.00 ± 29.46	98.0
P	248.06 ± 9.64	243.37 ± 8.76	96.7
Na	256.96 ± 5.82	251.00 ± 12.87	96.0
Mn	17.11 ± 1.32	17.41 ± 3.42	104.0
Fe	184.08 ± 21.68	181.63 ± 18.37	97.2
Zn	45.75 ± 0.68	44.61 ± 0.45	93.9
Cu	1.91 ± 0.15	1.86 ± 0.56	92.0
Pb	5.13 ± 0.26	4.98 ± 0.58	94.0
Ni	0.81 ± 0.08	0.79 ± 0.13	95.0
Cr	3.93 ± 0.43	3.94 ± 0.28	92.5
Cd	0.58 ± 012	0.55 ± 0.34	92.0

Element levels analysis: We used ICP-MS (Agilent-770X, Japan) to perform simultaneous multi-element detection of Na, Mg, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb.Table 2 showsthe optimal instrumental conditions for ICP-MS. Table 3 showsthe emission wavelengths and detection limits for each element. We measured every sample with three replicates. We prepared blank experiments in the same way. We used the certified reference material to validate the methods, to ensure the precision of the experiment (Table 3). The recovery values we obtained were in the range of 92-104%, while the relative standard deviation (RSD) was found to be below 8%. We also confirmed the method had a good recovery.

Results and Discussion

Table 6 showsthe mineral element contents in the 27 samples of *Davallioid* that we analyzed. The orders of the average conc. for thirteen elements were as follows: K>Ca>Mg>P>Na>Mn>Fe>Zn>Cu>Pb>Ni>Cr>Cd.We determined all of the element contents on a dry weight basis. Table 4 showsthe ranges and mean elemental contents in 27 samples of *Davallioid*.The min and max levels of Na were 53.11 and 1874.58 mg/kg, respectively. And the ranges of the elements were 1166.75-6996.91mg/kg for Mg, 277.63-4091.87mg/kg for P, 2439.37-36929.92mg/kg for K, 2108.02-10130.27mg/kg for Ca, 1.16-4.13mg/kg for Cr, 37.64-2430.03mg/kg for Mn, 33.33-1747.84mg/kg for Fe, 1.95-13.96mg/kg for Ni, 4.50-61.27 mg/kg for Cu, 22.12-178.36 mg/kg for Zn, 0.12-9.07 mg/kg for Cd, and 0.59-46.44mg/kg for Zn. The 27 samples of *Davallioid* contained larger contents of K, Ca and Mg, whereas they contained lower contents of P, Fe, Na, Cr, Cd, Ni, Cu, Pb,Mn, andZn. Our mineral element values were in agreement with those found in previous studies (Lifan & Zhao 2014). Table 5 shows that there was a significant difference in the concentrations of Ca, Mn, Fe, and K in three zones at the 0.05 level. The highest concentrations of Ca and Mn in the middle zone of Yunnan were 7459.78±8281.99 and 644.32±543.22 mg/kg. The highest concentrations of Fe and K were 624.23±794.51 (in the northwest zone) and 19828.54±5448.00 (in the southeast zone) mg/kg, in Yunnan. Moreover, the contents of Mg, Na, P, Cr, Cu, Ni, Cd, and Pb were not significantly different in three zones ($p>0.05$), as shown in Table 5. We found the highest levels of Ni (6.31±3.31 mg/kg), Cu (19.16±13.10 mg/kg), Zn (51.88±32.64 mg/kg), and Cd (1.56±1.98 mg/kg) in the middle zone, while we found the lowest levels of Ni (3.34±1.07 mg/kg), Cu (9.22±1.42 mg/kg), Zn (36.23±12.27 mg/kg), and Cd (0.26±0.16 mg/kg) in the southeast zone. In the northwest of Yunnan, the highest concentrations of Na and Pb were 595.23±137.65 and 14.89±19.86 mg/kg. In the southeast, the highest concentration of Mg and P were (3720.44±1895) and (1635.92±540.52).

Table 4. Contents (mg kg^{-1} dw) ofthemineralelementsin davallioidssamples (n = 27)

Element	Min.	Max.	Mean	SD
Na	53.11	1874.58	533.22	473.71
Mg	1166.75	6996.91	3038.67	1354.78
P	277.63	4091.87	1332.21	822.06
K	2439.37	36929.92	11405.52	7803.33
Ca	2108.02	10130.27	6839.33	7264.42
Cr	1.16	4.13	2.40	0.71
Mn	37.64	2430.03	520.55	516.84
Fe	33.33	1747.84	412.09	470.12
Ni	1.95	13.96	5.69	3.11
Cu	4.50	61.27	17.50	11.91
Zn	22.12	178.36	47.93	29.62
Cd	0.12	9.07	1.23	1.81
Pb	0.59	46.44	8.38	12.16

Table 6 shows that the mineral element contents among the different species of *Davallioid*from Yunnan widely varied . We found the highest contents of Zn (178.36 mg/kgdw), Cd (9.07 mg/kgdw), Pb (46.44 mg/kgdw), and Cr (4.13 mg/kgdw) in *Lepisorus contortus* **(1)**, whereas we found thelowest contents in *Arthromeris wallichiana* (**14,** Zn: 22.12 mg/kgdw; Cr: 1.16 mg/kgdw), *Arthromeris mairei* (**19**, Cd: 0.20 mg/kgdw), and *Drynaria sinica* (**21,** Pb: 1.10 mg/kgdw). We found the highest contents of Cu (61.27 mg/kgdw) and Ca (40,293.71 mg/kgdw) was found in *Microsorum membranaceum* **(11)**, and we found the highest contents of Mg (6,966.91 mg/kgdw) and K (26,851.83 mg/kgdw) in *Drynaria bonii* **(24)**. For the trace elements, the contentsalso differed among the species of medicinal fern used as *Drynaria fortunei*. We found the highest contents of Fe in *Humata tyermanni* (**5**, 1747.84 mg/kgdw), whereas we found the lowest contents in *Polypodiodes lachnopus* (**13**, 33.33 mg/kgdw). We found the highest contents of Mnin *Pseudodrynaria coronans* (**17**, 2430.03 mg/kgdw), whereas we found thelowest contents in *Drynaria fortunei* (**23**, Wild picking) (37.64mg/kgdw).We found the highest contents of Ni in *Arthromeris mairei* (**19**, 13.96 mg/kgdw), whereas we found thelowest contents in *Drynaria fortunei* (**26**, artificial cultivation) (1.95mg/kgdw).

Some research has shown that there are differences among the contents of these elements in the different producing areas of *Drynaria fortunei* (Li *et al*., 2014). We conducted in-depth research on artificial cultivation and wild transplants of *Drynaria fortunei*, to study the effects of the geo environmental conditions and local soil characteristics on the zones. As illustrated in Table 6, we found higher contents of Ni (3.08 mg/kgdw), Zn (45.01mg/kgdw), Cd (1.57 mg/kgdw), Pb (4.42 mg/kgdw), and Ca (5977.35 mg/kgdw) in the samples of wild transplants of *Drynaria fortune* than in the wild or artificial cultivation. However, we found higher contents of Na (3.08 mg/kgdw), P (4091.87 mg/kgdw), Mn (136.29 mg/kgdw), and Cu (19.38 mg/kgdw) in the artificial cultivation of *Drynaria fortunei*. We found the highest contents of Fe(96.28 mg/kgdw), Mg(6,996.91 mg/kgdw), K(26,851.83 mg/kgdw), and Cr(3.24 mg/kgdw) in the wild *Drynaria fortunei*, whereas we found the lowest contents of Fe(75.44 mg/kgdw), Mg(3775.74 mg/kgdw), and Cr (1.97 mg/kgdw) in the artificial cultivation of *Drynaria fortunei*, and the lowest contents of K(9415.63 mg/kgdw) in the wild transplant of *Drynaria fortunei*.The content results of the mineral elementsin different *Drynaria fortunei* varied, confirming that different local geo-environmental conditions and soil characteristics played an important role.

We tested all twenty-seven extracted samples for their proliferation effects on the osteoblast-like UMR-106 cell. As shown in Table 7, the positive control, NaF, expressed significant promoting effects on cell proliferation (P%=38.30). Almost half of the other samples showed effects on positive cell proliferation, ranging from medium to strong biological activity in concentration 1.0×10^{-2}mg/mL, such as *Lepisorus contortus* (**1,** P%=16.4), *Leucostegia immerse* (**2,** P%=9.9), *Colysis elliptica* (**3,** P%=13.4), *Phymatopteris crenatopinnata* (**4,** P%=11.4), *Humata platylepis* (**5,** P%=36.3), *Drynaria delavayi* (**6,** P%=3.4), *Drynaria propinqua* (**9,** P%=0.5), *Polypodiastrum mengtzeense* (**10,** P%=3.9), *Polypodisdesamoena* (**12,** P%=32.3), *Polypodiodes lachnopus* (**13,** P%=26.3), *Phymatosorus cuspidatus* (**15,** P%=6.9), *Phymatopteris oxyloba* (**4,** P%=16.4), *Humata tyermanni* (**4,** P%=12.4), *Drynaria rigidula* (**25,** P%=36.8), *Drynaria fortune* (AC) (**26,** P%=5.4), and *Drynaria fortune* (WT) (**27,** P%=9.0) respectively. It is noteworthy that three samples, *Humata platylepis* (**5**), *Polypodisdesamoena* (**12**), and *Drynaria rigidula* (**25**), had the best effects on cell proliferation and were almost equivalent to NaF in concentration 1.0×10^{-2}mmol/L. Meanwhile, three samples, *Microsorum membranaceum* (**11,** P%=-11.4), *Pseudodrynaria coronans* (**17,** P%=-17.9), and *Drynaria bonii* (2**4,** P%=-8.4), produced obvious inhibition effects on UMR-106 cell proliferation. It is interesting that the same species of *drynaria fortunei* (**23, 26, 27**) had different effects on cell proliferation under different cultivating circumstances. Wild picking *drynaria fortunei* (**23,** P%=-2.9) had inhibiting effects, while Artificial cultivation *drynaria fortunei* (**26** P%=5.49) and wild transplant *drynaria fortunei* (**27** P%=9.0) had promoting effects. When considered in conjunction with the mineral element levels, we found it was very amazing that promoting effects on cell proliferation had a positive correlation with Ca element concentration, while they had a negative correlation with the Mn element concentration. The best three samples, *Humata platylepis* (**5,** P%=36.3, Ca=10130.27, Mn=255.44), *Polypodisdes amoena* (**12,** P%=32.3, Ca=10068.51, Mn=504.72), and *Drynaria rigidula* (**25,** P%=36.8, Ca=9275.90, Mn=97.35), had the highest Ca concentration, but had relatively low Mn concentration. Conversely, *Microsorum membranaceum* (**11,** P%=-11.4, Ca=4293.71, Mn=1098.71), *Pseudodrynaria coronans* (**17,** P%=-17.9, Ca=2108.02, Mn=2430.03), and *Drynaria bonii* (2**4,** P%=-8.4, Ca=2836.01, Mn=980.84), which had inhibition effects on cell proliferation, had the highest Mn concentration but had relatively low Ca concentration. The other samples were also mostly consistent with this rule. We found that Calcium is advantageousto the mineralization of osteoblasts growth, while Manganese ions have an inhibiting effect. These results could be further explored in future research on the biological mechanism.

Table 5. Concentration (in mg/kg dw) of mineral elements in the different of zones in Yunnan (N=5).

Element	Northwest zone		Middle zone		Southeast zone	
	Dili, Diqing, Lijing		Yuxi, Kunming		Xishuangbanna, Wenshan	
	Mean	SD	Mean	SD	Mean	SD
Na	595.23	137.65	503.01	537.79	418.61	89.98
Mg	2817.07	618.70	2935.55	1261.23	3720.44	1895.72
P	1504.38	316.16	1245.63	899.16	1635.92	540.52
K	11546.25	1387.71	9501.73	7632.95	19828.54	5448.00
Ca	3714.69	1628.40	7459.78	8281.99	5949.19	2299.66
Cr	2.57	0.23	2.37	0.77	2.78	0.52
Mn	274.27	137.18	644.32	543.22	86.41	54.43
Fe	624.23	794.51	436.56	424.13	130.65	54.65
Ni	4.65	1.31	6.31	3.31	3.34	1.07
Cu	17.50	4.63	19.16	13.10	9.22	1.42
Zn	37.19	13.81	51.88	32.64	36.23	12.27
Cd	0.42	0.03	1.56	1.98	0.26	0.16
Pb	14.89	19.86	8.97	11.06	1.28	0.42

Table 7. Proliferation of ethanol extracts on osteoblast-like UMR-106 cell.

No.	Sample name	Concentration mg/mL	$A_{595/655nm}$ ($\bar{x}$ ± SD)	Proliferation (%)
	Blank control	0	0.201 ± 0.015	
	NaF	1.0×10^{-2} mmol/L	0.278 ± 0.014	38.3**
1.	*Lepisorus contortus*	1.0×10^{-2} mg/mL	0.234 ± 0.012	16.4
2.	*Leucostegia immersa*	1.0×10^{-2} mg/mL	0.221 ± 0.16	9.9
3.	*Colysis elliptica*	1.0×10^{-2} mg/mL	0.228 ± 0.015	13.4*
4.	*Phymatopteris crenatopinnata*	1.0×10^{-2} mg/mL	0224 ± 0.023	11.4
5.	*Humata platylepis*	1.0×10^{-2} mg/mL	0.274 ± 0.012	36.3**
6.	*Drynaria delavayi*	1.0×10^{-2} mg/mL	0.208 ± 0.015	3.4
7.	*Phymatopteris ebenipes*	1.0×10^{-2} mg/mL	0.187 ± 0.17	-6.9
8.	*Gymnogrammitis dareiformis*	1.0×10^{-2} mg/mL	0.190 ± 0.013	-5.4
9.	*Drynaria propinqua*	1.0×10^{-2} mg/mL	0.202 ± 0.023	0.5
10.	*Polypodiastrum mengtzeense*	1.0×10^{-2} mg/mL	0209 ± 0.015	3.9
11.	*Microsorum membranaceum*	1.0×10^{-2} mg/mL	0.176 ± 0.018	-11.4*
12.	*Polypodiodes amoena*	1.0×10^{-2} mg/mL	0.266 ± 0.018	32.3**
13.	*Polypodiodes lachnopus*	1.0×10^{-2} mg/mL	0.254 ± 0.015	26.3**
14.	*Arthromeris wallichiana*	1.0×10^{-2} mg/mL	0.200 ± 0.016	-0.5
15.	*Phymatosorus cuspidatus*	1.0×10^{-2} mg/mL	0.215 ± 0.013	6.9
16.	*Phymatopteris trisecta*	1.0×10^{-2} mg/mL	0.184 ± 0.015	-8.4
17.	*Pseudodrynaria coronans*	1.0×10^{-2} mg/mL	0.165 ± 0.014	-17.9**
18.	*Phymatopteris oxyloba*	1.0×10^{-2} mg/mL	0.234 ± 0.016	16.4
19.	*Arthromeris mairei*	1.0×10^{-2} mg/mL	0.190 ± 0.015	-5.4
20.	*Humata tyermanni*	1.0×10^{-2} mg/mL	0.226 ± 0.017	12.4
21.	*Drynaria sinica*	1.0×10^{-2} mg/mL	0.191 ± 0.016	-4.9
22.	*Drynaria quercifolia*	1.0×10^{-2} mg/mL	0.187 ± 0.018	-6.9
23.	*Drynaria fortunei (WP)*	1.0×10^{-2} mg/mL	0.195 ± 0.023	-2.9
24.	*Drynaria bonii*	1.0×10^{-2} mg/mL	0.184 ± 0.015	-8.4*
25.	*Drynaria rigidula*	1.0×10^{-2} mg/mL	0.275 ± 0.017	36.8**
26.	*Drynaria fortunei (AC)*	1.0×10^{-2} mg/mL	0212 ± 0.017	5.4
27.	*Drynaria fortunei (WT)*	1.0×10^{-2} mg/mL	0.219 ± 0.013	9.0

* p<0.05; ** p<0.01 significant as compared to blank control using student's t-test

Table 6. Mean ± RSD of mineral element content (in milligrams per kilogram, dry weight basis) in wild and artificial cultivation davallioidssamples.

No.		Element (mg·Kg^{-1}dw)												
		Na	Mg	P	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
1.	Mean	200.31	2651.68	1561.25	11163.72	5358.60	4.13	891.24	721.92	8.83	15.19	178.36	9.07	46.44
	RSD	11.37	7.06	7.71	7.32	5.70	8.54	7.47	7.31	0.75	1.43	1.06	7.42	6.17
2.	Mean	556.45	2108.60	1156.65	7476.11	6196.32	3.29	484.07	1303.06	10.85	28.10	56.68	0.97	24.37
	RSD	6.26	5.18	6.56	5.04	5.83	5.50	5.81	6.19	1.76	1.42	1.67	6.44	3.48
3.	Mean	533.79	4184.61	1322.64	12414.31	6277.14	2.73	231.04	332.74	5.89	18.30	65.25	1.01	4.83
	RSD	5.89	5.30	4.96	5.32	6.68	5.32	5.53	5.76	1.93	1.11	0.29	5.20	3.09
4.	Mean	441.56	1256.76	681.39	3934.87	2636.26	3.04	293.07	826.10	8.93	22.44	43.48	0.67	5.52
	RSD	8.28	6.91	6.19	6.69	6.32	7.39	6.73	6.80	2.91	1.84	2.19	7.55	5.11
5.	Mean	676.49	2442.61	700.78	10976.65	10130.27	2.33	255.44	1132.95	8.31	25.64	40.53	1.00	8.57
	RSD	6.37	5.35	6.34	5.62	5.15	6.49	6.27	5.95	3.32	3.13	3.20	5.50	3.39
6.	Mean	100.40	3110.42	1880.95	10777.40	2335.18	2.30	220.34	61.27	3.12	10.96	24.69	0.39	0.59
	RSD	15.91	7.80	9.43	7.16	8.65	10.41	7.56	9.87	4.32	3.23	3.04	15.05	8.51
7.	Mean	53.11	1703.70	780.53	4095.75	2738.83	2.11	1460.54	117.27	4.36	9.83	47.10	1.96	8.90
	RSD	57.18	18.53	22.02	17.41	18.05	20.90	17.87	18.48	8.81	9.26	8.95	18.87	15.24
8.	Mean	83.56	1166.75	627.89	2439.37	2866.75	2.35	550.43	397.41	5.56	7.56	87.12	4.68	22.90
	RSD	17.29	7.72	8.79	7.27	9.33	8.30	7.60	7.31	4.16	4.06	3.73	9.32	5.69
9.	Mean	440.13	2709.13	578.88	10474.27	2750.56	1.91	493.81	60.37	4.34	6.98	24.97	0.62	3.04
	RSD	6.50	5.83	7.08	5.55	3.89	6.60	5.48	6.03	2.18	1.61	1.93	5.41	6.48
10.	Mean	593.23	3206.77	1549.26	7151.01	7675.37	2.45	2430.03	48.57	5.75	18.01	63.17	1.05	2.19
	RSD	24.11	19.89	20.91	19.26	21.08	21.86	20.26	21.09	14.22	12.20	11.80	20.82	20.77
11.	Mean	513.92	2890.45	1729.57	4531.30	40293.71	1.78	372.82	318.40	11.05	61.27	42.71	0.19	2.90
	RSD	7.00	5.81	7.71	5.82	6.04	6.92	6.63	6.18	4.18	4.29	5.16	6.11	3.68
12.	Mean	523.26	4246.53	3077.85	7058.32	14168.51	1.49	504.72	77.90	2.86	20.15	54.35	0.34	2.63
	RSD	7.08	5.38	4.98	5.11	5.24	7.15	4.97	5.78	1.39	1.62	2.34	6.15	4.51
13.	Mean	69.12	3160.13	1840.70	9589.27	8286.38	1.35	482.71	33.33	3.14	12.72	46.43	0.92	1.95
	RSD	16.91	6.74	6.37	6.60	8.01	7.84	6.96	7.78	2.76	2.98	2.69	8.29	7.99
14.	Mean	435.76	4293.96	812.44	15751.46	5816.34	1.16	495.06	247.80	5.51	8.29	22.12	0.58	5.69
	RSD	11.46	10.26	9.87	9.05	10.35	12.93	9.17	9.83	8.50	8.12	8.98	11.96	6.34
15.	Mean	459.17	2820.60	846.86	14498.24	6303.88	1.39	171.03	108.07	2.69	10.22	31.64	0.20	1.81
	RSD	8.70	6.83	8.07	6.94	6.54	7.24	7.08	7.38	3.72	3.44	3.01	10.83	3.94
16.	Mean	79.93	1239.08	619.74	3516.34	7985.14	3.10	1211.48	1125.48	9.89	27.82	36.98	1.37	5.44
	RSD	14.94	6.05	5.99	5.98	6.19	5.57	5.78	5.78	1.78	2.99	3.34	7.48	4.33
17.	Mean	373.81	3101.00	277.63	13454.96	2108.02	1.72	1098.71	41.56	2.73	4.50	39.55	2.00	1.70
	RSD	30.44	22.47	26.59	22.59	23.32	28.52	21.60	25.74	15.42	14.72	13.54	26.88	22.36
18.	Mean	715.59	1395.46	968.31	4244.92	7180.55	2.48	687.46	591.51	6.55	13.71	51.70	1.72	15.75
	RSD	9.17	7.82	7.09	7.62	8.52	9.73	7.94	8.61	3.09	3.00	3.59	8.27	6.83
19.	Mean	428.13	2567.44	495.80	5828.63	4035.49	3.83	557.77	1056.37	13.96	40.34	27.11	0.20	6.09
	RSD	6.48	5.20	5.69	4.42	6.07	5.65	4.47	4.89	2.96	3.04	2.13	8.94	3.65
20.	Mean	732.87	1956.51	1107.34	10366.94	6001.40	2.87	139.85	1747.84	4.53	20.45	56.44	0.45	42.97
	RSD	0.96	1.71	1.39	1.10	2.04	0.41	1.14	1.20	1.68	1.32	0.98	5.87	1.25
21.	Mean	457.58	3384.29	1524.86	13494.41	2807.50	2.54	462.63	63.58	6.31	21.08	30.44	0.37	1.10
	RSD	5.86	4.22	4.32	4.54	6.40	6.26	4.55	4.94	2.97	1.20	2.13	8.55	10.25
22.	Mean	404.97	2586.07	2215.92	23429.01	5370.38	2.06	39.60	93.41	3.03	11.01	30.77	0.12	1.28
	RSD	6.14	6.13	5.79	5.11	5.72	6.34	5.63	6.33	3.26	2.78	3.48	7.21	4.34
23.	Mean	528.01	6996.91	1434.04	26851.83	2846.61	3.24	37.64	96.28	2.48	7.86	25.43	0.17	0.63
	RSD	5.38	4.55	4.95	3.93	4.37	5.88	5.35	5.23	3.04	0.66	2.03	9.26	1.91
24.	Mean	224.56	4717.79	1053.10	36929.92	2836.01	2.17	180.84	136.11	2.66	4.74	31.37	0.81	1.90
	RSD	8.92	5.91	9.11	5.97	7.01	7.52	5.45	6.81	2.07	1.86	2.20	4.50	4.13
35.	Mean	282.27	2478.17	2046.86	14535.09	9275.90	3.05	97.35	224.82	5.17	7.79	57.07	0.53	1.38
	RSD	9.70	6.63	6.09	5.87	5.44	6.85	6.44	6.58	3.81	3.57	3.42	11.80	4.71
26.	Mean	2614.41	3775.74	4091.87	13549.19	4403.34	1.97	136.29	75.44	1.95	19.38	33.54	0.37	1.30
	RSD	4.59	4.03	4.37	3.99	2.22	5.31	4.49	4.95	0.48	1.09	1.15	8.02	4.58
27.	Mean	1874.58	5892.89	986.41	9415.63	5977.35	2.10	68.82	86.99	3.08	18.20	45.01	1.57	4.42
	RSD	7.41	7.12	7.69	7.19	6.66	7.76	7.33	7.58	4.50	3.28	3.40	6.41	4.14

Values followed by the same letter within each column are not significantly different ($p>0.05$, Scheffee's test)

Conclusions

Firstly, we determined the contents of thirteen elements of traditional Chinese medicine *Davallioid* that were collected from China. Samples from the middle of Yunnan were plentiful in Ca(7459.78 ± 8281.99 mg/kg), P (1245.63±899.16 mg/kg), Mn(644.32±543.22 mg/kg), Ni(6.31±3.31 mg/kg), Cu(19.16±13.10 mg/kg), Cd(1.56±1.98 mg/kg), and Zn(51.88±32.64 mg/kg). Samples from the southeast were especially enriched in K(19828.54±5448.00 mg/kg), but did not have enough Pb. Samples from the northwest were enriched in Na(595.23± 8137.65 mg/kg), Fe(624.23±794.51 mg/kg), and Pb(14.89±19.86 mg/kg). We found that the concentrations of the mineral elements in the samples differed from site to site. By comparing the contents of mineral elements in wild, artificial planting or transplanting *Drynaria fortunei*, we found that different environments and soil compositions had a significant impact on the contents of mineral elements in plants. Herein, we reported that the average concentrations of thirteen elements were in the following order: K>Ca>Mg>P>Na>Mn>Fe>Zn>Cu>Pb>Ni>Cr>Cd. On the other hand, we tested twenty-seven extracts of samples with the concentration of 1×10^{-2} mg/mL for proliferative effects on the osteoblast-like UMR-106 cell, and found that sixteen of them could promote proliferative effects, while eleven samples had inhibitive effects. Three samples, *Humata platylepis* (**5,** P%=36.3), *Polypodisdesamoena* (**12,** P%=32.3), and *Drynaria rigidula* (**25,** P%=36.8), had the most proliferative effects. Meanwhile, we found that high contents of Ca and low contents of Mn could result inpositive effectson the proliferation of the osteoblast-like UMR-106 cell. In contrast, low contents of Ca and high contents of Mn could result in inhibitive effects on the proliferation of the osteoblast-like UMR-106 cell.The study of mineral element contents in *Davallioid* provided a scientific basis forits usefulness in traditional Chinesemedicine development.

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