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Fingerprint and multi-index content determination of ethyl acetate extract of *Sedum emarginatum* Migo.

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Abstract: *Sedum emarginatum* Migo (Aoyejingtian) is a perennial succulent herb of the sedum genus in the family Crassulaceae, which has the fountion of treating furuncle, swelling and haematemesis, hematochezia, menorrhagia and hepatitis. Preliminary studies of our research group had showed that the ethyl acetate extract of *Sedum emarginatum* Migo could inhibit the proliferation of liver cancer HepG2 cells. The establishment of a reasonable and feasible quality evaluation method for the effective parts of *Sedum emarginatum* Migo can provide a scientific basis for the further development and utilization of *Sedum emarginatum* Migo. In this study, a multi-wavelength conversion method was used to establish high-performance liquid chromatography (HPLC) fingerprints of the ethyl acetate extract of *Sedum emarginatum* Migo, and the method was also used to simultaneously determine the gallic acid, protocatechuic acid, caffeic acid, and ferulic acid, isoquercitrin and luteolin in the ethyl acetate extract of *Sedum emarginatum* Migo. The similarity of the fingerprints of the ethyl acetate extract of *Sedum emarginatum* Migo from different origins and the content of 6 components were compared. The established method was simple, accurate, table and reliable, which could provide a fast, accurate and reliable method for comprehensive evaluation of the quality of *Sedum emarginatum* Migo.

Keywords: *Sedum emarginatum* Migo. quality evaluation, fingerprint, HPLC.

INTRODUCTION

Sedum emarginatum Migo (Aoyejingtian) is a fleshy perennial herb of the genus Sedum in the family Sedum, it has a long history of medicinal use and has been written in several ancient texts .In China, it is mainly distributed in Guangxi, Jiangsu, Zhejiang, Jiangxi, Fujian, Hubei, Sichuan and other regions. It can be used as medicinal herbs, it is bitter, acrid taste, sour, cool, enters to heart, liver meridian (Sun *et al.,* 2016). It has the function of clearing away heat and detoxification, hemostasis and liver, and can be used to treat diseases such as snake bite, jaundice and iron injury. In the folk it was often used for freshing smashed external, curing boils, swelling pain, vomiting blood. It is also effective for the treatment of hepatitis and menorrhagia. Many studies have shown that the efficacy and quality of herbs vary depending on cultivated soil, climate and geographical origin, even from the same species (Zahiruddin *et al.,* 2020, Tamboli *et al.,* 2015).

The existing research basis had showed that *Sedum emarginatum* Migo was analysied in character identification, thin-layer identification (Xu *et al.,* 2008, Huang *et al.,* 2014), quercetin, kaempferin, isorhamnetin, glycyrrhizin and other flavonoids content determination (Qiu *et al., 2018,* Wei *et al.,* 2010*)* ultraviolet spectrum. In terms of quality analysis such as identification (Lv *et al., 2009,* Qiu *et al.,* 2017), chemical composition pre-experiment (Qiu *et al.,* 2017), certain researches have been carried out on anti-tumor (Hu 2012), antioxidant (Chen *et al.,* 2011), sedation and hypnosis (Chen *et al.,* 2013), hemostasis and analgesia (Chang *et al.,* 2017), had been researched. The traditional Chinese medicine *Sedum emarginatum* Migo is a complex whole. The above methods for evaluating the quality of *Sedum emarginatum* Migo lack fingerprints, which are not sufficient to control the quality of *Sedum emarginatum* Migo. It is not persuasive and cannot better interpret the characteristics of Chinese medicine. The content determination of *Sedum emarginatum* Migo does not involve other ingredients except for the above-mentioned ingredients, which is not comprehensive enough. With the continuous advancement of instrumental analysis methods, it is necessary to determine the content of a single medicinal material with multiple indicators. In view of the current situation, in order to better evaluate the quality of *Sedum emarginatum* Migo and promote the in-depth development and utilization of *Sedum emarginatum* Migo, it is necessary to conduct a more comprehensive study on the fingerprint and content determination of *Sedum emarginatum* Migo.

MATERIALS AND METHODS

Experimental Apparatus

KQ500DE Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), HWS-26 electric heating constant temperature water bath (Shanghai Qixin Scientific Instrument Co., Ltd.), SHB-III circulating water type multiple vacuum pump (Zhengzhou Great Wall Technology Industry and Trade Co., Ltd.), Simplicity

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ultrapure water system (Millipore China Co., Ltd.), Waters e2695 high performance liquid chromatograph, W2998PDA detector (American Waters company), Waters e2695 type high performance liquid chromatograph, 2489UV detector (Waters company, USA), DHG-9203A electric heating constant temperature blast drying oven (Shanghai Qixin Scientific Instrument Co., Ltd.), TGL-16G high-speed desktop centrifuge (Shanghai Anting Scientific Instrument Factory), High-speed universal crusher (Tianjin Test Instrument Co., Ltd.), SQP electronic balance (Sartorius Scientific Instruments (Beijing) Co., Ltd.).

Experimental Reagents

Petroleum ether (Chengdu Kelon Chemical Reagent, Chengdu, China), ethyl acetate (Chengdu Kelon Chemical Reagent, Chengdu, China, AR), n-Butanol (Tianjin Damao Chemical Reagent Factory, Tianjin, AR), 5% ethanol (Chengdu Kelon Chemical Reagent, Chengdu, China, AR), methanol (Chengdu Kelong Chemical Technology Co., Ltd., Chengdu, China, AR), chromatographic acetonitrile (American TEDIA company, USA), chromatographic methanol (American TEDIA company, USA), chosphoric acid is analytically pure, ultrapure water. Gallic acid (China Institute for Food and Drug Control, Beijing, China), protocatechuic acid (China Institute of Pharmaceutical and Biological Products, Beijing, China), caffeic acid (China Institute for Food and Drug Control, Beijing, China, purity of 99.7%), Ferulic acid (China National Institute of Pharmaceutical and Biological Products, Beijing, China), isoquercitrin (Chengdu Munster Biotechnology Co., Ltd., Chengdu, China), Luteolin (Chengdu Mansite Biotechnology Co., Ltd., Chengdu, China, HPLC≥98%).

Source of Experimental Medicinal Materials

The 10 different origins of *Sedum emarginatum* Migo were mainly from Guangxi Zhuang Autonomous Region, Zhejiang Province and Hubei Province, which was identified as the aerial part of *Sedum emarginatum* Migo by Ma Lifei, deputy director of Guangxi Yixin Medicine. The source of medicinal materials were as follows: S1(Ninghai, Zhejiang, 2017.12), S2(Lishui, Zhejiang, 2018.04), S3(Long'an County, Guangxi, 2018.05), S4(Nanning, Guangxi, 2018.05), S5(Wuming, Guangxi, 2018.05), S6(Guilin, Guangxi, 2018.05), S7 (Longsheng, Guangxi, 2018.05), S8(Jianshi County, Enshi, Hubei, 2018.04), S9(Xuan'en County, Enshi, Hubei, 2018.07), S10(Badong County, Enshi, Hubei, 2018.07).

Sample Preparation

Preparation of Reference Solution

Appropriate amount of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin reference substance were accurately weighed, methanol was added to dissolve and diluted to volume, a single reference substance stock solution was prepared. The above 6 reference substance stock solutions to make the concentrations of gallic acid 0.2892 mg mL^{-1}, protocatechuic acid 0.1721mg mL^{-1}, caffeic acid 0.2189 mg mL^{-1}, ferulic acid 0.0926mg mL^{-1}, and isoquercitrin 0.5016mg mL^{-1}, luteolin 0.0625mg ml^{-1}.

Preparation of Test Solution

150g crude powder of *Sedum emarginatum* Migo was taken, soaked with 10 times the amount of 70% ethanol for 30min, refluxeed and extracted 3 times for 2h, 1.5h, 1h respectively. It was filtered by gauze, the extracts were combined and concentrated under reduced pressure on a rotary evaporator to obtain a 70% ethanol extract concentrate. Then it was extracted 6-7 times with petroleum ether, ethyl acetate, n-butanol and concentrated under reduced pressure into petroleum ether, ethyl acetate, n-butanol extract and the remaining water layer and placed in a water bath (below 60^{o}C). Then it was dried under reduced pressure to prepare dry extract samples of petroleum ether, ethyl acetate, n-butanol, and water and stored them in a desiccator for later use 0.1g (equivalent to 7g of crude medicinal materials) sample extract of ethyl acetate extract of *Sedum emarginatum* Migo was accurately weighed. It was placed in a 2mL centrifuge tube, dissolved in methanol ultrasonically, and transferred to a 2mL volumetric flask and volumetrically adjusted to the mark, 13000r/min, centrifuged for 10min, filtered through a microporous membrane (0.45μm).

Determining Optimal Chromatographic Conditions

The chromatographic conditions for fingerprint and content determination were as follows: Column: Thermo ODS-2 HYPERSIL (4.6mm × 250mm, 5μm), mobile phase: acetonitrile (D)-0.1% aqueous phosphoric acid (B) with gradient elution, The gradient elution procedure: 0~20 min, 97% B→94%B;20~45 min, 94% B→88%B; 45~65min, 88%B→86%B;45~65 min, 86% B→65%B; 65~125in, 65% B→97%B; 125min~ 130min, 97% B. Column temperature 30^{o}C, flow rate was 0.8mL/min, the detection wavelength was 0-13min (260nm), 14-47min (321nm), 48-65min (255nm), 66-125min (355nm), and the injection volume was 5μL.

STATISTICAL ANALYSIS

Statistical analysis (Mean ± S.D) of the samples was performed on M.S. Excel® version 2010.

RESULTS

Method Validation

Reproducibility, stability and accuracy

0.1g of the ethyl acetate extract of *Sedum emarginatum* Migo from S1 origin was accurately weighed. The test solution according to the above method was prepared, 6 consecutive samples were injected according to the above chromatographic conditions, the HPLC profile was determined and the RSD was calculated with the 12th

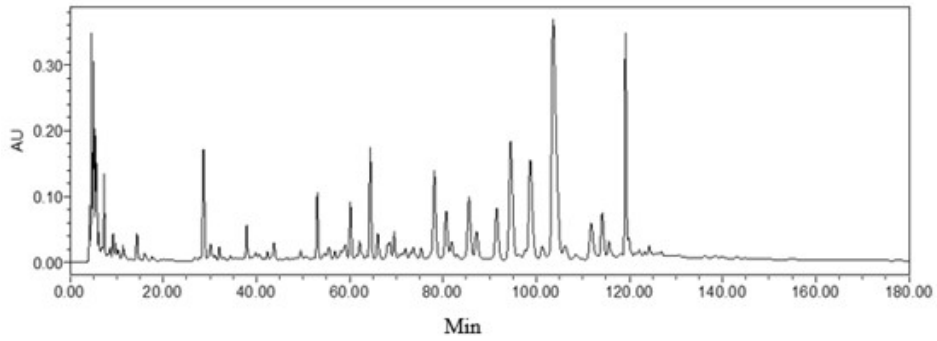

Fig. 1: The chromatogram of the ethyl acetate extract of in *Sedum emarginatum* Migo (extend the washing time by 180min)

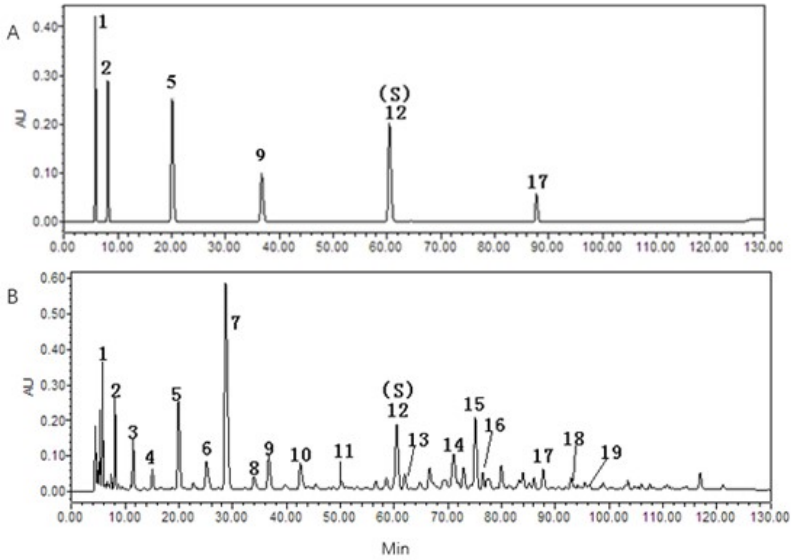

Fig. 2: HPLC chromatograms of mixed reference substances(A)and *Sedum emarginatum* Migo(B)

1-Gallic acid 2- Protocatechuic acid 5- Caffeic acid 9- Ferulic acid 12- Isoquercitrin 17- Luteolin

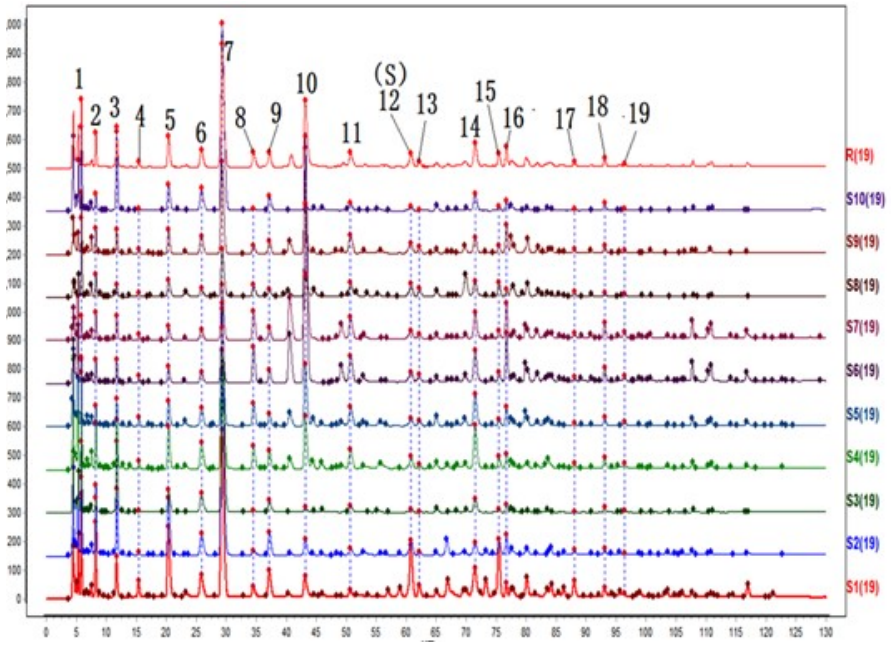

Fig. 3: HPLC fingerprint of 10 batches of *Sedum emarginatum* Migo

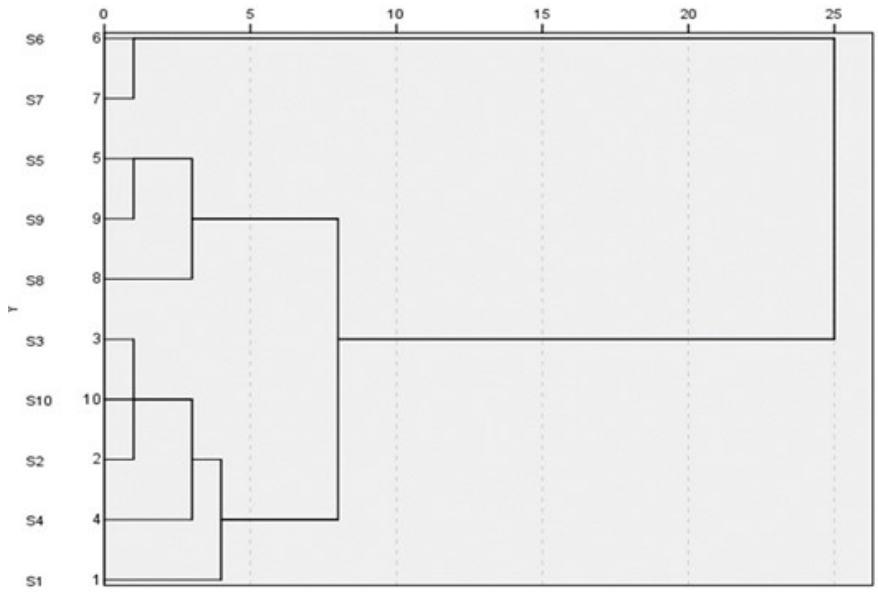

Fig. 4: Cluster analysis of fingerprints of 10 batches of *Sedum emarginatum* Migo

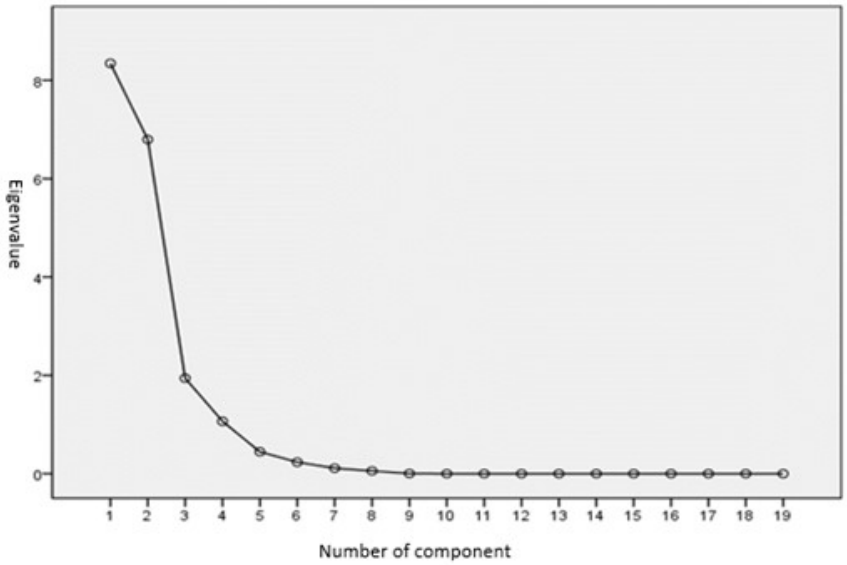

Fig. 5: Crushed stone diagram of principal component analysis of 10 batches of *Sedum emarginatum* Migo

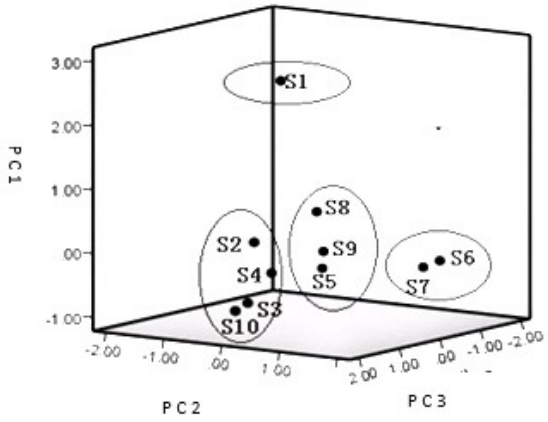

Fig. 6: The main component 3D scores of 10 batches of *Sedum emarginatum* Migo

Table 1: The relative retention time of the common peaks of 10 batches of *Sedum emarginatum* Migo

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	RSD (%)
1	0.0958	0.0963	0.0964	0.0968	0.0969	0.0967	0.0969	0.0964	0.0969	0.0963	0.37
2	0.1340	0.1361	0.1348	0.1369	0.1369	0.1366	0.1367	0.1349	0.1367	0.1347	0.82
3	0.1896	0.1940	0.1904	0.1950	0.1950	0.1948	0.1948	0.1907	0.1949	0.1902	1.23
4	0.2486	0.2544	0.2512	0.2556	0.2557	0.2553	0.2555	0.2514	0.2556	0.2562	1.02
5	0.3295	0.3364	0.3319	0.3381	0.3384	0.3373	0.3373	0.3325	0.3384	0.3316	1.01
6	0.4154	0.4286	0.4167	0.4301	0.4303	0.4305	0.4302	0.4175	0.4303	0.4161	1.66
7	0.4743	0.4830	0.4754	0.4853	0.4858	0.4865	0.4865	0.4775	0.4864	0.4748	1.11
8	0.5619	0.5701	0.5625	0.5721	0.5716	0.5707	0.5708	0.5622	0.5722	0.5623	0.83
9	0.6065	0.6124	0.6207	0.6152	0.6146	0.6143	0.6145	0.6192	0.6150	0.6174	0.63
10	0.7243	0.7121	0.7218	0.7145	0.7137	0.7099	0.7090	0.7198	0.7141	0.7188	0.72
11	0.8479	0.8333	0.8442	0.8371	0.8369	0.8364	0.8365	0.8431	0.8369	0.8423	0.55
12S	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
13	1.0234	1.0164	1.0240	1.0216	1.0215	1.0266	1.0219	1.0238	1.0222	1.0241	0.26
14	1.1873	1.1693	1.1833	1.1746	1.1749	1.1753	1.1755	1.1830	1.1761	1.1812	0.46
15	1.2422	1.2327	1.2420	1.2397	1.2397	1.2397	1.2402	1.2479	1.2403	1.2464	0.33
16	1.2662	1.2519	1.2680	1.2592	1.2589	1.2477	1.2591	1.2683	1.2592	1.2674	0.56
17	1.4513	1.4371	1.4493	1.4452	1.4450	1.4443	1.4455	1.4500	1.4455	1.4497	0.29
18	1.5365	1.5191	1.5333	1.5274	1.5265	1.5266	1.5279	1.5349	1.5278	1.5338	0.34
19	1.5934	1.5745	1.5856	1.5829	1.5823	1.5806	1.5831	1.5867	1.5830	1.5858	0.30

Table 2: The relative peak area of the common peaks of 10 batches of *Sedum emarginatum* Migo

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	RSD (%)
1	0.6095	1.6678	5.5325	2.8631	2.0595	0.7650	0.8823	1.6778	1.6881	6.2210	81.68
2	0.5392	1.8668	1.5896	1.4783	0.8966	0.7601	1.0788	0.6101	0.8276	1.7621	43.12
3	0.3921	2.1650	8.8532	1.9734	1.5377	0.8902	1.2866	0.4288	1.0294	9.8869	122.92
4	0.1822	0.1152	0.2386	0.3752	0.5673	0.3400	0.3028	0.1415	0.2104	0.2797	48.13
5	1.0059	2.7179	4.1404	2.4948	2.0768	0.9630	1.0503	0.8135	1.3682	4.6263	64.39
6	0.4630	1.4841	4.8089	2.1546	2.0571	0.8752	1.0388	0.5503	1.4651	5.3704	84.63
7	3.0534	11.7189	39.0604	12.4374	11.9702	3.9654	4.9815	3.0922	6.8462	43.5598	105.58
8	0.1778	0.2578	0.4245	1.5775	2.1928	2.5294	2.4843	0.7156	0.5717	0.4288	84.34
9	0.4458	1.3031	2.6300	1.1947	1.1890	0.8656	1.0179	0.4452	0.8504	2.9380	65.50
10	0.3994	0.9757	1.1991	3.9658	6.5676	17.0860	20.1408	1.4523	3.9714	0.4085	127.50
11	0.1112	0.4455	1.5689	1.6624	2.4134	2.4341	2.3981	0.9758	1.6792	1.8175	52.51
12S	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.00
13	0.2065	0.2103	0.2293	0.3047	0.5539	0.5824	0.5212	0.4827	0.4376	0.2308	41.06
14	0.6363	0.9192	3.4460	3.5977	3.8005	2.6746	2.7846	0.9441	1.3874	3.9878	54.59
15	0.9338	0.7942	0.6336	0.7451	0.5262	0.6222	0.6106	0.8163	0.4804	0.6777	20.32
16	0.1626	0.8218	1.2145	0.7367	1.3692	2.4945	2.3185	0.3197	1.4392	1.4392	62.13
17	0.2479	0.2266	0.2232	0.2602	0.2342	0.2785	0.3906	0.2363	0.2284	0.2394	19.51
18	0.1451	0.3005	0.8758	0.5811	0.6500	0.7825	1.0205	0.1662	0.4185	1.0125	55.34
19	0.0529	0.0822	0.3395	0.2482	0.2939	0.3751	0.4642	0.1424	0.1539	0.3720	55.11

peak isoquercitrin as the reference peak. The measured relative retention time RSD value of each chromatographic peak was 0.12%~0.77%, and the relative peak area RSD value was 0.03%~1.92%, indicating that the precision of the instrument was good. 0.1g of the ethyl acetate sample of *Sedum emarginatum* Migo from S1 origin was accurately weighed. The test solution was prepared according to the above method, according to the above chromatographic conditions, the samples were injected 7 times at 0h, 2h, 4h, 15h, 20h, 22h, 24h and the HPLC profile was determined. The RSD was calculated with the 12th peak isoquercitrin as the reference peak. The results showed that the relative retention time RSD value of each chromatographic peak was 0.01%~0.68%, and the RSD value of the relative peak area was 0.03%~2.95%, indicating that the stability of the test solution was good.

Table 3: The results of the matching data of the common peaks of 10 batches of *Sedum emarginatum* Migo

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
1	4255283	3339794	2902023	4592038	2339470	1198843	1125417	3203221	2644221	3419279
2	3764300	3738345	833829	2370974	1018499	1191128	1375980	1164735	1296421	968502
3	2737404	4335563	4643859	3165025	1746713	1394952	1641039	818642	1612537	5434130
4	1271850	230652	125167	601771	644407	532822	386204	270151	329533	153753
5	7023086	5442721	2171809	4001372	2359037	1509085	1339641	1552997	2143238	2542752
6	3232329	2971981	2522470	3455632	2336745	1371404	1324951	1050571	2294915	2951717
7	21317660	23467740	20488760	19947941	13597154	6213991	6353897	5903457	10724191	23941883
8	1241192	516188	222672	2530173	2490832	3963633	3168702	1366125	895456	235678
9	3112050	2609479	1379547	1916079	1350564	1356411	1298318	849875	1332162	1614797
10	2788581	1953885	628956	6360665	7460204	26774551	25689760	2772693	6220945	224501
11	776639	892198	822959	2666307	2741427	3814367	3058793	1862879	2630429	998969
12S	6981559	2002559	524541	1603864	1135917	1567044	1275506	1909133	1566433	549632
13	1441465	421225	120260	488771	629219	912659	664855	921593	685527	126838
14	4442579	1840842	1807553	5770293	4317095	4191178	3551771	1802432	2173197	2191820
15	6519126	1590495	332335	1195030	597711	975085	778871	1558406	752446	372512
16	1135016	1645624	637059	1181632	1555338	3909035	2957310	610384	2254392	791053
17	1731020	453817	117070	417364	266050	436356	498183	451106	357716	131555
18	1013223	601734	459400	932015	738400	1226174	1301625	317247	655485	556475
19	369250	164603	178082	398010	333890	587845	592144	271828	240997	204443

Table 4: The eigenvalues and contribution rate results of the principal component analysis of 10 batches of S*edum emarginatum* Migo

Principal	Eigenvalues	Contribution (%)	Cumulative contribution rate (%)
1	8.346	43.925	43.925
2	6.797	35.773	79.698
3	1.942	10.221	89.919
4	1.066	5.608	95.527
5	0.445	2.345	97.871
6	0.234	1.23	99.102
7	0.112	0.589	99.691
8	0.056	0.293	99.984
9	0.003	0.016	100

6 samples of the ethyl acetate extract of *Sedum emarginatum* Migo in S1 origin in parallel, each 0.1g, were accurately weighed. The test solution was prepared according to the above method, the sample was injected according to the above chromatographic conditions, the

HPLC profile was determined, the RSD was calculated, taking the 12th peak isoquercitrin as the reference peak. The measured relative retention time RSD value of each chromatographic peak was 0.01% to 0.16%, and the relative peak area RSD value was 0.24% to 3.09%, indicating that the test solution had good repeatability.

Establishment of Fingerprint

Extended flush test

In order to verify whether there was a signal peak after 130 min, the gradient elution time of this experiment was extended from 130 min to 180 min. The experimental results showed that no interference peaks appeared after 130 minutes, and the chromatographic peaks were mainly concentrated within 130 minutes, so the set acquisition time was reasonable. The results were shown in fig. 1.

The fingerprint obtained from each production area were automatically integrated and then imported into the "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System 2012.1 Software" for analysis. S1 was supposed the reference spectrum, after multi-point calibration, automatic matching, the Mark peak adopted to match the chromatographic peak and generated the control fingerprint spectrum R by the median method. Fig. 2 to fig. 3 showed the superimposed fingerprint of 10 samples from the different origins, the chromatogram of the mixed reference substance and sample chromatogram of the ethyl acetate extract of *Sedum emarginatum* Migo.

According to the measurement results of 10 batches of

Table 5: Rotation matrix of principal component analysis of 10 batches of *Sedum emarginatum* Migo

Peak number	Compounds			
	1	2	3	4
Peak 1	0.862	0.008	0.024	-0.421
Peak 2	0.671	0.55	0.104	0.263
Peak 3	0.636	-0.467	0.505	0.218
Peak 4	0.269	0.901	-0.037	-0.241
Peak 5	0.847	0.478	0.122	0.105
Peak 6	0.814	0.032	0.511	-0.173
Peak 7	0.868	-0.193	0.438	0.036
Peak 8	-0.738	0.572	0.238	-0.174
Peak 9	0.771	0.516	0.248	0.261
Peak 10	-0.83	0.441	0.22	0.241
Peak 11	-0.877	0.293	0.17	-0.218
Peak 12	0.525	0.788	-0.299	0.062
Peak 13	-0.009	0.861	-0.495	-0.039

Table 6: Investigation of the linear relationship of the 6 components of ethyl acetate in *Sedum emarginatum* Migo.

Compositions	Linear equations	r	Linear range (μg mL^{-1})
Gallic acid	Y=1.58e+004X+4.46e+004	0.9997	15.20~304.0
Protocatechuic acid	Y=2.33e+004X+3.76e+004	0.9997	8.700~173.0
Caffeic acid	Y=3.40e+004X+4.59e+004	0.9997	11.40~220.8
Ferulic acid	Y=3.56e+004X+1.86e+004	0.9996	4.900~98.10
Isoquercitrin	Y=2.73e+004X+9.55e+003	0.9998	24.20~484.1
Luteolin	Y=2.73e+004X+9.55e+003	0.9997	3.200~63.10

Sedum emarginatum Migo ethyl acetate samples, the retention time of peak 12 as the reference peak (S) were calculated as the sum of the relative retention times and peak area of each common peak, as shown in table 1. The results showed that the RSD of the relative retention time of each common peak was 0.26% to 1.66%, the RSD value of the relative peak area was 19.51%~127.50%, indicating that the peak time of the common peak of the samples from each producing area was relatively table.

Similarity Evaluation

The 2012.1 version software of the Chinese medicine chromatographic fingerprint similarity evaluation system was used, the matching data and similarity of the ethyl acetate extract of *Sedum emarginatum* Migo were calculated. The results are shown in table 3. The similarity results showed that the similarity of the samples from each producing area was between 0.677 and 0.991, indicating that the quality of the ethyl acetate extract of *Sedum emarginatum* Migo in each producing area was relatively table, their chemical components were basically the same, and the content of each component was quite different. Among them, the similarity of Guilin, Guangxi and Longsheng, Guangxi was relatively low, while the similarity of samples from other areas was relatively high, all above 0.88.

Cluster Analysis

SPSS 21.0 was used to process the original data of samples from 10 different producing areas of *Sedum emarginatum* Migo ethyl acetate. SPSS cluster analysis was used to deal with 19 common peaks from 10 producing areas between groups was adopted and the cosine distance was used as the distance formula between samples by clustering method (Mazina *et al.,* 2015) .The clustering results are shown in fig.4. It could be seen from the fig.4 that when the discrimination distance was 5, the ethyl acetate extract of *Sedum emarginatum* Migo could be divided into 3 categories. Ninghai Zhejiang, Lishui Zhejiang, Long'an Nanning, Guangxi were grouped into one category, En Shiba East County Hubei, Longsheng Guilin were grouped into one category, Wuming, Jianshi County, Enshi, Enshi Xuan'en Hubei were were grouped into one category. Through cluster analysis, it was found that there were some differences in the samples of ethyl acetate extract from 10 different producing areas of *Sedum emarginatum* Migo, which might be related to the different harvest seasons, climate, soil and other external factors.

Principal Component Analysis

In order to further explore the differences between the ethyl acetate extract of *Sedum emarginatum* Migo from different producing areas, the principal component analysis of the fingerprint data was carried out on the basis of cluster analysis (Rafi *et al.,* 2020). The peak areas of 19 common peaks from 10 different producing areas of *Sedum emarginatum* Migo ethyl acetate extract were

Table 7: The recovery test results (n=6)

Components	Known content (g)	Added amount (g)	Measured amount (g)	Sample recovery (%)	Average recovery (%)	RSD (%)
Gallic acid	0.2858	0.2892	0.5691	97.96	98.43	1.18
	0.2880	0.2892	0.5775	100.10		
	0.2869	0.2892	0.5692	97.61		
	0.2847	0.2892	0.5705	98.82		
	0.2923	0.2892	0.5791	99.17		
	0.2907	0.2892	0.5709	96.89		
Protocatechuic acid	0.2163	0.2189	0.4360	100.37	97.67	1.69
	0.2179	0.2189	0.4364	99.82		
	0.2171	0.2189	0.4360	100.00		
	0.2155	0.2189	0.4374	101.37		
	0.2212	0.2189	0.4415	100.64		
	0.2200	0.2189	0.4463	103.38		
Caffeic acid	0.2140	0.1970	0.4170	103.08	100.93	1.31
	0.2210	0.1970	0.4270	104.54		
	0.2240	0.1970	0.4300	104.80		
	0.2190	0.1970	0.4240	103.85		
	0.2180	0.1970	0.4200	102.53		
	0.2180	0.1970	0.4190	101.96		
Ferulic acid	0.0915	0.0926	0.1844	100.27	102.26	2.39
	0.0922	0.0926	0.1895	105.08		
	0.0919	0.0926	0.1839	99.34		
	0.0912	0.0926	0.1849	101.17		
	0.0936	0.0926	0.1910	105.13		
	0.0931	0.0926	0.1880	102.54		
Isoquercitrin	0.4958	0.5016	0.9879	98.11	96.95	2.76
	0.4995	0.5016	0.9684	93.48		
	0.4976	0.5016	0.9882	97.81		
	0.4939	0.5016	1.0000	100.9		
	0.507	0.5016	0.9930	96.89		
	0.5042	0.5016	0.9782	94.50		
Luteolin	0.0618	0.0625	0.1231	98.08	98.51	1.71
	0.0623	0.0625	0.1250	100.32		
	0.062	0.0625	0.1237	98.72		
	0.0616	0.0625	0.1223	97.12		
	0.0632	0.0625	0.1260	100.48		
	0.0628	0.0625	0.1230	96.32		

imported into SPSS 21.0 to standardize the peak areas of common peaks and perform principal component analysis. Value greater than 1 was a characteristic extraction principle, the results could be showed in table 4, the feature value was greater than 1 has four main components, which was 8.346%, 6.797%, 1.942%, 1.066%, 43.925% and 35.773%, 10.221%, 5.608%. The total variance contribution rate was 95.527%, which could fully reflected the basic characteristics of the chemical composition of the ethyl acetate extract of *Sedum emarginatum* Migo. At the same time, observing the gravel diagram in fig. 5, it could be found that the first four principal components were steeper, and the other components were relatively smooth. Because the eigenvalues of the first three principal components were relatively large, the scores of the first three principal components were different. The three-dimensional distribution diagram of the principal components of the acetic acid B of *Sedum emarginatum* Migo in the producing area was shown in fig. 6. The results showed that, Ninghai and Zhejiang were grouped into one category, Lishui, Zhejiang, Long'an Nanning, Guangxi, En Shiba East County Hubei were grouped into one category, Guilin, Longsheng Guangxi were grouped into one category. Wuming, Jianshi County, Enshi, En Shi Xuanen Coun Hubei were grouped into one category. There were some differences between the results and cluster analysis. It might be because the principal components extracted by principal component analysis had not yet fully reflected all the information about the medicinal materials.

The contribution value of the principal component mainly depends on the absolute value of the load. It could be seen

Table 8: Determination results of 6 components in ethyl acetate samples from 10 batches of *Sedum emarginatum* Migo

Number	Gallic acid (mg g^{-1})	Protocatechuic acid (mg g^{-1})	Caffeic acid (mg g^{-1})	Ferulic acid (mg g^{-1})	Isoquercitrin (mg g^{-1})	Luteolin (mg g^{-1})
S1	5.333	3.200	4.100	1.737	9.375	1.258
S2	4.107	3.117	3.117	1.429	2.642	0.3235
S3	3.555	0.6927	1.239	0.7527	0.6893	0.0831
S4	5.306	2.033	2.356	1.079	2.176	0.3060
S5	2.918	0.8615	1.371	0.7504	1.520	0.1924
S6	1.504	1.013	0.8818	0.7580	2.109	0.3174
S7	1.423	1.186	0.7938	0.7348	1.739	0.3670
S8	3.992	0.9843	0.9014	0.4717	2.552	0.3259
S9	3.311	1.109	1.250	0.7430	2.104	0.2597
S10	4.224	0.8114	1.463	0.8860	0.7285	0.0942

from table 5 that peak 1 and peak 7 had the highest load value in principal component 1, peak 4, peak 12, peak 13, and peak 17 had the highest load value in principal component 2 and peak 6 had the highest load value in principal component 3. Peak 16 had the highest loading value in principal component 4. It showed that the components of peak 1, peak 4, peak 6, peak 7, peak 12, peak 13, peak 16 and peak 17 had a greater contribution to the quality of *Sedum emarginatum* Migo. Peak 1, peak 12 and peak 7 are gallic acid, isoquercitrin and luteolin, respectively and their content may reflected the quality of *Sedum emarginatum* Migo.

Methodological Validation

Mapping of Standard Curves

The above six reference substance stock solutions were precisely drawed to make the concentrations of gallic acid 0.2892 mg mL^{-1}, protocatechuic acid 0.1721 mg mL^{-1}, caffeic acid 0.2189 mg mL^{-1}, ferulic acid 0.0926 mg mL^{-1}, and isoquercitrin 0.5016 mg mL^{-1},luteolin 0.0625 mg mL^{-1},they were gradually diluted with methanol into a series of mixed reference solutions of different concentrations. According to the above-mentioned optimal chromatographic conditions, 5μL of sample was injected for determination. In sample concentration (X) as the abscissa, the peak area (Y) for the vertical axis for linear regression, the regression equation to obtain the results shown in table 6. The experimental results showed that the 6 components had a good linear relationship within the corresponding range.

Reproducibility, stability, and accuracy

5μL each of the same reference substance mixture was precisely drawn, according to the above mentioned optimal chromatographic conditions with 6 consecutive injections, respectively.The peak area of each chromatographic peak was measured separately. The results calculated that the RSD values of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin, and luteolin were all less than 2%, indicating that the instrument had good precision.

0.1 g of the ethyl acetate sample of *Sedum emarginatum* Migo at S1 origin was accurately weighed, and the test solution was prepared according to the above method. Samples at 0h, 2h, 4h, 15h, 20h, 22h, 24h according to the above chromatographic conditions were injected and the peak area of each chromatographic peak was determined. Results showed the RSD values of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin were all less than 1.80%.

6 samples of the ethyl acetate part of *Sedum emarginatum* Migo at S1 origin, each 0.1g, were accurately weighed. The test solution according to the above method was prepared, the sample according to the above chromatographic conditions was injected and the peak area of each chromatographic peak was determined.The RSD values of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin were all less than 2.08%, which indicated that the method had good repeatability.

Recovery

A total of 6 samples of the known content of the ethyl acetate extract of *Sedum emarginatum* Migo from the above S1 origin, each 0.05g, were accurately weighed. The reference solution and the sample test solution were prepared according to the above preparation method, appropriate amount of mixed reference solution to each part was added, the sample was analyzed according to the above chromatographic conditions, the sample recovery rate was determined and calculated. The RSD of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin were all less than 2.76%, which indicated that the sample recovery rate were good. The sample recovery test results were shown in table 7.

Determination of Content

0.1g samples of ethyl acetate extrcat from 10 different origins of *Sedum emarginatum* Migo were accurately weighed, the reference solution and the test solution were prepared according to the above method,the samples was

analyzed according to the above optimal chromatographic conditions , and the peak areas of each sample were determined. The contents of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin in 10 batches of ethyl acetate extract samples of *Sedum emarginatum* Migo were calculated according to the external standard method. The results showed that the content of gallic acid in the ethyl acetate extract of *Sedum emarginatum* Migo from 10 different producing areas was 1.423~5.333mg g^{-1}, the content of protocatechuic acid was 0.6927~3.200mg g^{-1}, and the content of caffeic acid was 0.7938~4.100mg g^{-1} .The content of ferulic acid was 0.4717~0.7374mg g^{-1}, the content of isoquercitrin was 0.6893~9.375mg g^{-1}, and the content of luteolin was 0.0942~1.258mg g^{-1}. The results are shown in table 8.

DISCUSSION

Six chromatographic peaks were identified from the 19 common peaks in the ethyl acetate extract of *Sedum emarginatum* migo, which were gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin. The experiment also identified hyperoside, quercetin, kaempferin and isorhamnetin in fingerprint of the ethyl acetate extract. Since these 4 components were low in some samples from the origin, the resolution was poor, and some batches cannot be detected, so they were not identified in the common peak.The method of fingerprinting of ethyl acetate extract established in this experiment could achieve a good separation of the main components in *Sedum emarginatum* Migo within a certain period of time. And the operation method was simple to operate, high sensitivity, good repeatability, accurate results, and could be used to comprehensively evaluate the quality of *Sedum emarginatum* Migo. The similarity analysis results showed that the similarity of the ethyl acetate extract of *Sedum emarginatum* Migo. from 10 different producing areas was 0.677~0.991. There were certain differences in the quality of *Sedum emarginatum* Migo. medicinal materials from different origins. The reason for the differences may be due to differences in climate, environment, and geographic location that affect their quality. The results of cluster analysis and principal component analysis showed that there were some differences in the cluster analysis and principal component analysis results of the ethyl acetate part of *Sedum emarginatum* Migo. but the difference was not very large. This may be because the main components extracted by the principal component analysis had not fully reflected all the information of the medicinal materials (Chang *et al.,* 2016; Zhang *et al.,* 2021).

According to the literature, gallic acid has anti-tumor effects (Lima *et al.,* 2016), protocatechuic acid has antioxidant (El-Sonbaty *et al.,* 2019, Yuksel *et al.,* 2017, Menezes *et al.,* 2017) and antibacterial effects (Abou Aitah *et al.,* 2021), protection against acute lung injury (Zhang *et al.,* 2015) and protection against myocardial ischemia/reperfusion injury (Tang *et al.,* 2014), caffeic acid can anti-cancer (Aguilar *et al.,* 2020, Fırat *et al.,* 2019) ,lower blood sugar (Xu *et al.,* 2020), ferulic acid and isoquercitrin have antioxidant effects (Sim *et al.,* 2020, Yokohira *et al.,* 2008), luteolin has anti-tumor effects (Chakrabarti *et al.,* 2016, Liu *et al.,* 2017). These show that these components of the ethyl acetate extract of *Sedum emarginatum* Migo have certain pharmacological activity, which provides a research foundation for further research on the correlation between the chemical components and efficacy of *Sedum emarginatum* Migo, and the development, application and quality control of resources of *Sedum emarginatum* Migo. There is a complex content in *Sedum emarginatum* Migo. and the six index components to be tested have different maximum absorption wavelengths, the same detection wavelength cannot guarantee that each component is the best absorption (Zhang *et al.,* 2013), which might affect the measurement results. Therefore, this study was conducted to establish the fingerprints of the ethyl acetate part of *Sedum emarginatum* Migo and to determine the contents of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin in the ethyl acetate extract of *Sedum emarginatum* Migo simultaneously by multi-wavelength conversion method.Six components in the ethyl acetate extract of *Sedum emarginatum* Migo from different origins were determined, it was expected to provide a simple method for multiple indicators to control the quality of the herbs of *Sedum emarginatum* Migo.

CONCLUSION

The method of fingerprinting of ethyl acetate extract established in this experiment could achieve a good separation of the main components in *Sedum emarginatum* Migo in a certain period of time, and the operation method was simple, high sensitivity, good repeatability, accurate results, and was more comprehensively evaluated the quality of the *Sedum emarginatum* Migo. An HPLC multi-wavelength method was established to simultaneously determine the contents of six components of gallic acid, protocatechuic acid, caffeic acid, ferulic acid, isoquercitrin and luteolin in the ethyl acetate part of *Sedum emarginatum* Migo. The method was simple, reliable, accurate, repeatable and stable,which provides a research foundation for further research on the correlation between the chemical components and efficacy, application and quality control of *Sedum emarginatum* Migo.

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