

Study on extraction process of alcohol-soluble ingredients from Hellgrammites (Megaloptera: Corydalidae)

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Keywords: Hellgrammites, Alcohol extract, Fatty acid, Trace elements

Abstract: Study the alcohol soluble ingredient extraction process of Hellgrammites and the significance of whether it can be exploited the medicinal liquor. Using the crude product of Hellgrammites, the impact factors are the ethanol concentration, the extracting time and the ratio of the material liquid. These three factors are in the three level to do the orthogonal experiments. Using the alcohol extract of Hellgrammites as the sample, the meteorological mass spectrometry and the inductively coupled mass spectrometry, qualitatively detect the composition of fatty acid and quantitatively detect the content of trace elements of Zn and Mn. The best extraction technology conditions are: the ethanol concentration is 60 percent and the extraction time is 2.5 hours. The ratio of material liquid is one contrasting fifty. The compositions of fatty acid are: palmitic acid, stearic acid, oleic acid, linoleic acid, gamma linolenic acid, arachidonic acid, and eicosapentaenoic acid. The trace elements of Zn and Mn: the trace elements in the crude products are 140.15ug per gram and 67.69ug per gram, the trace elements in the alcohol extract are 48.87ug per gram and 11.58ug per gram. Experiments show that the alcohol process is reasonable, stable and feasible. Alcohol extraction of fatty acid and the trace elements of Zn and Mn have high contents. It can be further developed for efficacy to medicinal liquor products.

1. Introduction

Hellgrammites, the larval form of the dobsonfly, are a completely abnormal insect, scientific name: *Corydalus cornutus* (Linnaeus), belonging to the family Megaloptera. The insects are distributed in the Americas, Asia, and South Africa. It is a widely distributed insect in China^[1]. The hellgrammite insect is a common name for the larvae of the genus Polygonaceae, and it is a completely metamorphic insect^[2]. The hellgrammite insect is rich in protein and various mineral elements, including protein content of 75.5% and vitamin B2 content of 3.46mg/100g^[3]. Hellgrammite are not only a kind of food, but also have a good effect in treating the elderly with frequent urination and urinary bed in children^[4,5].

Chinese medicine believes that: hellgrammites have the effect of tonifying qi and kidney, suppressing deficiency and solidifying, nourishing and strengthening, and can be called medicine

and food, and people use sand reptiles to soak wine or match hellgrammites with three After the seventh class of soaking wine to treat rheumatism ^[6], it has the reputation of "animal ginseng". In recent years, with the in-depth study of hellgrammites, Yang DM et al ^[7,8] studied the fertility and growth of Drosophila extracts. The results showed that the extracts of hellgrammites can significantly increase the fruit fly progeny. The number increases the fecundity; it can significantly increase the body weight of Drosophila adults and promote their growth and development. China has abundant resources for hellgrammites and has formed artificial breeding industry. A number of related patents have been reported.

At present, in addition to the consumption of restaurants, there are no hellgrammite products on the market, and its development and utilization is still in a very primitive stage, and the market space is very large. Therefore, the subject is based on the direction of hellgrammites and alcohol extracts. The study of fatty acids, determination of the content of fatty acids, trace elements Zn and Mn in alcohol extracts, clear the extraction process, and develop its potential medicinal value, is of great significance for the effective and rational use of hellgrammite resources, The hellgrammites product "hellgrammites efficacy medicinal liquor" has a positive effect.

2. Materials and methods

2.1 Instruments

Pharmaceutical Ultrasound Processor (Model: FS-150N, Shanghai Shengsen Ultrasonic Instrument Co.), Freeze Dryer (Model: BT-85, Millrock Technology Company), Trace-1310 Gas Chromatograph (American Thermo Scientific), PSQ-8000 Mass Spectrometer (American Thermo Scientific), DB Digital Display Electrothermal Plate (Beijing Yongguangming Medical Instrument Co., Ltd.), 7700X Inductively Coupled Plasma Mass Spectrometry Instrument (Agilent, USA).

2.2 Materials

Fresh hellgrammites(HGM) were purchased from local farmers' markets in Sichuan Panzhihua, China. KOH, methanol, absolute ethanol, concentrated nitric acid, hydrogen peroxide(Sichuan Kelun Pharmaceutical Co).

2.3 Methods

2.3.1 Extraction of alcohol-soluble substances from hellgrammites

Fresh hellgrammites were purchased from local farmers' markets in Sichuan Panzhihua, China. Remove head, tail and viscera, freeze dry insect, get dry ingredients, crush and reserve. The dry powder of hellgrammite was accurately weighed 2g, extracted by condensation and reflux with a certain amount of ethanol, and recovered by vacuum rotary evaporation. The alcohol-soluble substances in the evaporator were dried repeatedly until the constant weight (the difference between the two weights was less than 2mg). The crude fat mass fraction of sample is calculated as follows 2-1:

$$X = (m_2 - m_1) / m \times 100\% \quad (2-1)$$

X———Crude fat mass fraction, %

m———Sample quality, g

m₁———evaporating dish quality, g

m₂———Crude fat and evaporating dish quality, g

2.3.2 Orthogonal design experiment of alcohol extract from hellgrammites

In the pre-experiment, considering the influence of the ratio of material to liquid, the extraction time and the concentration of ethanol on the yield of ethanol extract, the orthogonal experimental factors and levels were determined on the above three factors. The effects of various factors on fatty acid, Zn and Mn in ethanol extract were studied by L9(34) orthogonal experiment, and the optimum extraction conditions were selected.

Tab2-3 Design of the orthogonal of alcohol extracts of hellgrammites

Factors level	A Ethanol concentration / %	B Extraction time / h	C Ratio of material to liquid
1	50	1.5	1:50
2	60	2	1:60
3	70	2.5	1:70

2.3.3 Single factor experiment

Consider the ratio of material to liquid (1:40, 1:50, 1:60, 1:70), extraction time (1, 1.5, 2, 2.5), ethanol concentration (50%, 60%, 70%, 80%) The effect of three single factors on the yield of alcohol extracts. The filtrate was extracted by the orthogonal test of ethanol extraction. When other conditions were unchanged, the effect of a single factor on the yield of the medicinal ingredients of the alcohol extract of the hellgrammite was studied.

2.3.4 Hellgrammite extracts of GC-MS and Zn, Mn trace elements ICP-MS

2.3.4.1 Sample Pretreatment

The room temperature methyl esterification method^[9] qualitative analysis of fatty acids, take 0.1g of the larvae alcohol extract sample into a test tube, add 1:1 petroleum ether - benzene solution 2ml, the sample is fully dissolved, then add 0.4mol 2 ml of KOH-methanol solution was allowed to stand at room temperature for 10 min, and then purified water was added to stand for stratification, and the supernatant was taken for analysis. Pretreatment of samples by wet nitration method^[10] quantitative analysis of Zn, Mn trace elements. Separately weighed 0.1g of crude sand worm, 0.05g and 0.1g of sphagnum alcohol extract, 0.05g, placed in a small beaker that has been washed and dried, added 1~2ml of purified water, infiltrated, added 5ml thick Nitric acid, 2ml hydrogen peroxide, shake well, placed on a digital heating plate at 200°C for 10 ~ 20min, until the cup is nitrated to clarification, add 2% nitric acid to 100ml spare.

2.3.4.2 Gas chromatographic conditions

Column: TG-5MS capillary column (30m×25um×0.25um); inlet temperature: 260°C; split ratio: 30:1; carrier gas: high purity nitrogen, flow rate of 1.0ml / min; initial temperature 120°C, kept for 1 min, raised to 230°C at a heating rate of 4°C/ min, held for 25 min.

The fatty acid methyl ester is separated by a gas chromatography capillary column to obtain a total ion chromatogram of the mass spectrum. The fatty acid composition was determined according to the NIST mass spectrometry library and the retention time of the larvae alcohol extract sample,

and the relative content was calculated by the area normalization method.

2.3.4.3 Mass spectrometry conditions

EI source, electron energy 70eV; ion source temperature: 230°C; quadrupole temperature: 280°C; transmission line temperature: 230°C; mass range: 40 ~ 400 amu; solvent delay: 10 min.

2.4 Statistical analysis

The variance analysis was performed on the orthogonal test results using SPSS 17.0 software. P values <0.05 were considered significant.

3. Results

3.1 Single factor experiment results of alcohol extraction from hellgrammites

3.1.1 Effect of material to liquid ratio on crude fat content of alcohol extract

Under the condition of 50% alcohol concentration and 1.5 h extraction time, the crude fat content of ethanol extract increased obviously from 1:40 to 1:60, and the crude fat content of ethanol extract decreased obviously at 1:70. It shows that the crude fat content of alcohol extract has been basically dissolved in 1:60, and the solvent is added to cause waste. Material 1:60 is the best. The results are shown in Figure 1.

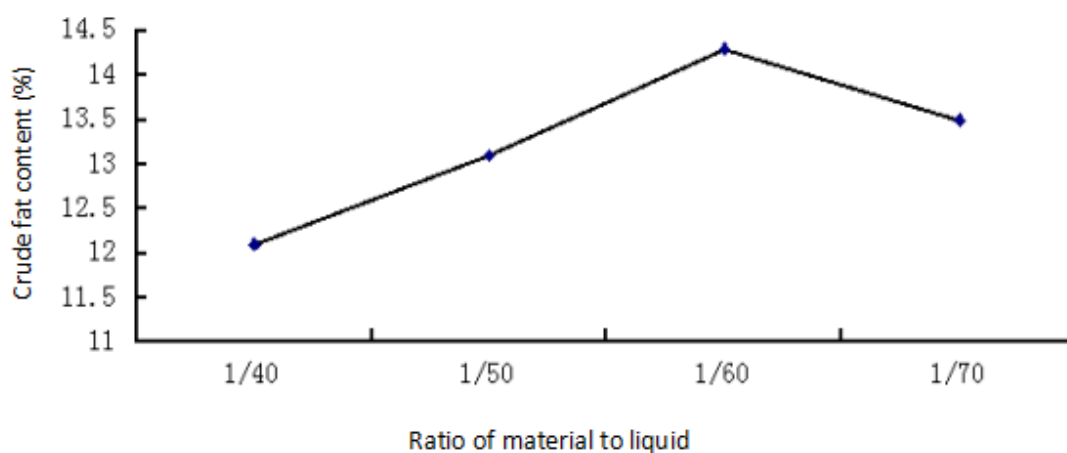


Fig.1 Effect of solid-liquid ratio on the content of crude fat of alcohol extracts

3.1.2 Effect of extraction time on crude fat content of alcohol extract

Under the condition of 1:60 ratio of material to liquid and 50% alcohol concentration, different extraction time was selected. It can be seen from Figure 2 that the effect of extraction time on the crude fat content of alcohol extracts is increasing, and the extraction time is 2h and 2.5h. The content is relatively high, but the content growth is obviously slow after 2h. Considering the improvement of efficiency and cost saving, the extraction time is 2h as the best extraction time.

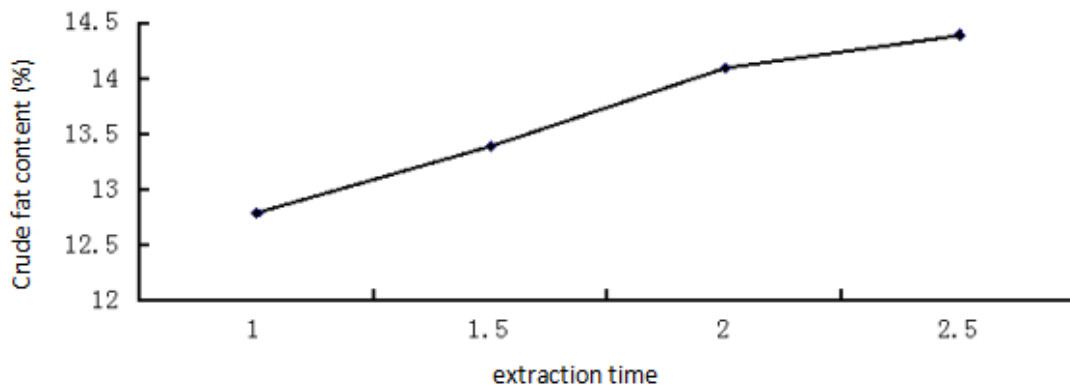


Fig. 2 Effect of extraction time on the content of crude fat of alcohol extracts

3.1.3 Effect of ethanol concentration on the content of crude fat of alcohol extracts

The results are shown in Figure3. When the ratio of material to liquid is 1:60 and the extraction time is 2 hours, different alcohol concentration is selected. From Figure 2-3, it can be seen that the alcohol concentration has a great influence on the crude fat content. When the concentration of ethanol reaches 60%, the crude fat content reaches 21.8%, which indicates that the dissolution content of crude fat is the highest when the concentration of ethanol is 60%. Ethanol concentration of 60% is the best.

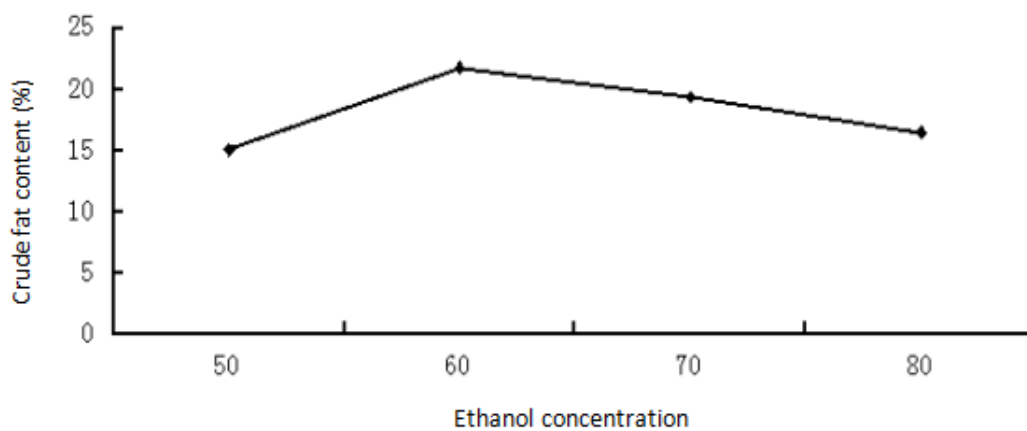


Fig.3 Effect of alcohol concentration on the content of crude fat of alcohol extracts

3.2 Orthogonal experimental data processing of crude fat of hellgrammites

According to the principle of statistics, Table1 shows that the greater the R value, the greater the effect of this factor on the experiment, $R_A > R_C > R_B$, so the primary and secondary factors affecting the crude fat yield of hellgrammite are $A > C > B$.

Table 1 The result of the orthogonal test

Factors Test number	A	B	C	Crude fat content (%)
1	1:50	1.5	50	12.01
2	1:50	2	60	13.32
3	1:50	2.5	70	10.46
4	1:60	1.5	60	16.12
5	1:60	2	70	16.02
6	1:60	2.5	50	22.18
7	1:70	1.5	70	17.58
8	1:70	2	50	19.05
9	1:70	2.5	60	16.84
K ₁	35.79	45.71	53.24	-
K ₂	54.32	48.39	46.28	-
K ₃	53.47	49.48	44.06	-
R	18.53	3.77	9.18	-

Table 1 shows that K2 is the most important factor in factor A, K3 is the most important factor in factor B, K1 is the most important factor in factor C, and A2B3C1 is the best matching level for extracting crude fat from hellgrammites. The yield of crude fat reached 22.18% at the optimum combination.

Table 2 ANOVA of the content of crude fat of alcohol extracts

Factors	Sum of squares of deviations	Freedom	mean square	F	F _a	Saliency
A	73.54	2	36.77	23.87	19.00	P<0.05
B	3.08	2	1.54	1	19.00	-
C	33.62	2	16.81	10.81	19.00	-
Error	3.08	2	1.54	-	-	-

$F_{0.05(2, 2)}=19.00$

According to Table 2, on the basis of orthogonal test, the ratio of ethanol extract to liquid is significant, which shows that the ratio of extract to liquid has the greatest influence on the extraction rate of crude fat of hellgrammites. Through orthogonal experiment, the optimum conditions for extracting crude fat from hellgrammites are as follows: alcohol concentration 60%, extraction time 2.5 h, material-liquid ratio 1:50. That is, A₂B₃C₁. This is similar to the results of Li SM^[42] in the extraction of insect oil, fatty acid composition analysis and purification of alpha-linolenic acid.

3.3 Verification of orthogonal experiment results

Under the optimum conditions, the crude oil content of hellgrammites was 21.6%, 21.2%, 21.1% and 21.3%, respectively. The results showed that the above methods were feasible and the crude fat content was stable.

3.4 Analysis of fatty acid composition of oil extracts from hellgrammites by GC-MS

Table3 The fatty acid content of hellgrammites (% of total fatty acids)

component	Content(%)
Palmitic oleic acid	14.1
Palmitic acid	17.2
stearic acid	6.9
oleic acid	24.6
linoleic acid	5.7
Gamma linolenic acid	0.81
Arachidonic acid four	2.8
Twenty carbon five enoic acid	21.5
Monounsaturated fatty acids	48
Polyunsaturated fatty acids	33

According to Table 3, the fatty acids of hellgrammites are mainly palmitic acid, oleic acid (C18:1n-9), eicosapentaenoic acid (EPA), and the content of stearic acid is low (C18:0). Among them, reptiles are also rich in arachidonic acid, eicosapentaenoic acid (EPA), which plays an important role in human nutrition, development, health and the content of EPA is as high as 21.5%.

3.5 Determination of hellgrammites Zn and Mn in alcohol extract by ICP-MS

The results of Zn and Mn content are shown in table 4.

Table 4 Hellgrammites Zn, Mn trace element detection results

Element	Name	Coarse hellgrammites ug/g	Alcohol extract of hellgrammites ug/g
Zn		140	48.8
Mn		67.6	11.6

4. Discussion

As a kind of medicine and food insect, hellgrammite has the effect of invigorating lung, strengthening kidney yang, treating phlegm, asthma and cough, and treating deficiency of deficiency and strain^[2]. Myrica rubra et al. have proved that the extract of hellgrammites can improve the fecundity of *Drosophila melanogaster* and promote its growth and development.

Fat is one of the important nutrients to maintain human health. The performance and function of fat mainly depend on fatty acid^[12]. Most unsaturated fatty acids have many physiological functions, such as anti-tumor, improving immunity and treating schizophrenia^[13-15]. They are also beneficial to reducing blood sugar, regulating blood lipids, reducing cholesterol content and protecting the heart. They also have the effect of enhancing memory^[7,16-18]. They are essential

nutrients for human body. Hellgrammite oil can not only maintain relatively low stearic acid, but also relatively high palmitic acid and oleic acid, and hellgrammites also rich in EPA, its content is as high as 21.5%. Zhu LY^[19] and others found that EPA is a very important resource, can prevent cardiovascular diseases, and has anti-cancer, anti-inflammatory effects, promote the development of the nervous system and visual system.

In addition, the content of Zn, Mn hellgrammites can be used as a quality standard of kidney tonic drugs, lack of zinc, manganese will lead to endocrine dysfunction or gland atrophy, the occurrence of kidney reproductive development pathological changes. According to Xu NC^[20], it is pointed out that hellgrammites, such as zinc and manganese, which are closely related to the pharmacodynamics. It can be used as reliable reference for screening kidney tonics for the treatment of kidney deficiency. The results showed that the contents of Zn and Mn were 140.15ug/g and 67.69ug/g, respectively, and the contents of Zn and Mn were 48.87ug/g and 11.58ug/g, respectively. It can be seen that hellgrammites medicinal liquor is a product worthy of development.

5. Conclusions

In a word, through orthogonal design experiment, the optimum process of ethanol extract was determined. The ethanol concentration was 60%, the ratio of material to liquid was 1:60, the extraction time was 2.5 h, the crude fat content was 22.18%, the ethanol extraction process was reasonable and the yield was stable. The content of hellgrammites Zn and Mn in crude and ethanol extracts were 140.15 ug/g, 67.69 ug/g and 48.87 ug/g, 11.58 ug/g, respectively. The prospect of utilization and development of hellgrammites was very wide. The development of hellgrammites medicinal liquor products was very promising. It will be very meaningful. Because there is no in-depth study on the hellgrammites in the literature at present, so there is no efficacy test. It is only a preliminary study on the preparation process of the ethanol extract for the detection of the ethanol extract of hellgrammites.

Acknowledgements

This work was supported by Sichuan Provincial Department of Education (17ZB0303), Graduate student innovation fund(y2017039) and this work was supported by Zigong City Science and Technology Innovation Seedling Project (2017cxm03), China.

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