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Chemical Constituents of Chrysanthemum Morifolium in Food Processing

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Based on the study on the type of compounds and pharmacological activity of wild chrysanthemums, the chemical composition identification of wild chrysanthemums under terpenoids and flavonoids is discussed. X-4 type micro melting point analyzer, Bruker ACF-500 nuclear magnetic resonance instrument and Agilent 1100 MSD Trap electrospray mass spectrometer are used and the chemical composition of wild chrysanthemums is extracted and identified with analytical reagents. 12.3kg of dried wild chrysanthemums herb is crushed and different proportions of ethanol are heated and recycled for the extraction. It is finally divided into 4 types of chemical composition: chrysanthguaianolide A, chrysanthguaianolide B, chrysanthguaianolide C and apressin. This analysis of chemical composition of wild chrysanthemums has achieved good results and can provide an operable model for the extraction of chemical composition of plants.

1. Introduction

1.1 Literature review

Wild chrysanthemums is a kind of medicinal material used in folk medicine and its biological activity has diversified features. It is rich in resources and low in prices, which has been applied in food, medicine, cosmetics and other fields and has great development and application value. In addition to researches on the appearance, taste and medicinal value of wild chrysanthemums, the current academic community has conducted detailed studies on its chemical characteristics. Some scholars believe that the main chemical composition of wild chrysanthemums can be divided into terpenoids, flavonoids, volatile oil, etc. and wild chrysanthemums itself and its compound preparation s can be used treat infectious diseases such as skin and respiratory system (Shun et al., 2005). Based on the relevant theories, the author uses the modern chromatographic separation method to analyze the chemical composition of effective parts of wild chrysanthemums and obtains 10 monomer compounds (Wang et al., 2008). The final experimental results shows that the volatile oil of wild chrysanthemums contains 22 components and the optimal extraction process is ethanol for 3 times, which can be achieved by adding 70% ethanol for 12 times. Some experts in the field of medicine have analyzed the pharmacological effect and chemical composition extraction methods of wild chrysanthemums medicinal materials. Through the diagnosis of the application in the clinical disease treatment, it can be found that wild chrysanthemums has obvious pharmacological effect in clinical diseases and has certain application value (Hosni et al., 2013). Some scholars use silica gel and column gel to separate the chemical composition of wild chrysanthemums, thus determining the molecular structure of the compound. In addition, these scholars use the high performance liquid chromatography (HPLC) to separate the chemical substance of wild chrysanthemums and prepare a large number of related drugs, which has outstanding performance in natural plant medicine (Wu et al., 2010). Some scholars believe that the use of preparative HPLC for the separation and purification of wild chrysanthemums has the advantages of large amount of preparation and simple operation. It is then introduced to natural plant medicine chemistry and the preparation and purification have become an important method (Haouas et al., 2012).

By combing the above literature, it can be known that the current research by scholars on wild chrysanthemums is mainly focused on the preparation of wild chrysanthemums chemical reaction. Some scholars have adopted advanced methods to analyze the chemical composition of wild chrysanthemums and also achieved good results. In view of this, based on the existing literature, this paper will discuss the chemical

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composition of wild chrysanthemums in a relatively simple way in order to provide some operable models for other scholars and medical scholars.

1.2 Research purposes

The key of the research in this paper is how to analyze the chemical composition of feverfew such as wild chrysanthemums and extract the chemical factors in order to introduce it into more fields and bring better application effect to residents. According to the existing theories, wild chrysanthemums has good anti-inflammatory and anti-oxidation and can achieve good results in the prevention and treatment of respiratory disease. To this end, this paper first performs a preliminary analysis of the type of compounds and pharmacological activity of wild chrysanthemums. Secondly, chemical apparatus and related reagents are used and the wild chrysanthemums in the market of a certain city is selected to conduct the experiment. Different different proportions of ethanol and adjuvants are used conduct the experiment and finally the corresponding chemical composition type is obtained. This experiment can provide some reference for the extraction of other plant chemical components.

2. Type of compounds and pharmacological activity of wild chrysanthemums

2.1 Type of compounds of wild chrysanthemums

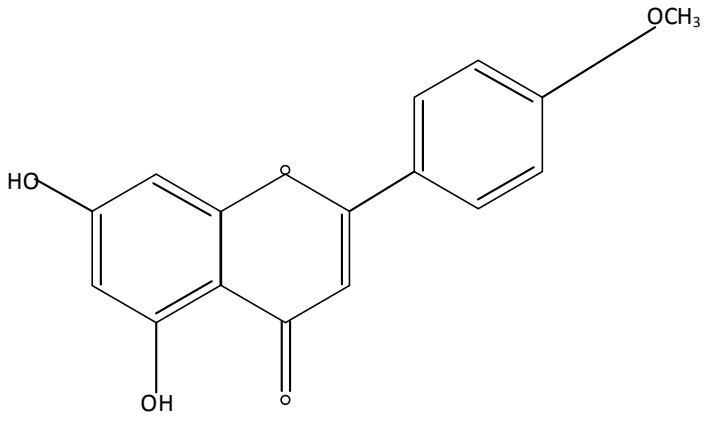
At present, according to the existing literature on the classification of the chemical composition of wild chrysanthemums, the main types are terpenoids and flavonoids. Based on this, the specific content of these two types of compounds separated from wild chrysanthemums plants is summarized.

2.1.1 Terpenoids

The wild chrysanthemums contains relatively large quantity of terpenoids and the major proportion is the monomer compounds dominated by sesquiterpenoids. At the same time, some researchers have conducted comprehensive synthesis of the sesquiterpenoids separated from wild chrysanthemums (Wang et al., 2014). The specific classification name of the so-called sesquiterpenoids is shown in Table 1. Here, some typical names of sesquiterpenoids of wild chrysanthemums are listed, which are mainly distributed in the root, stem and leaf of wild chrysanthemums.

Table 1: Names of sesquiterpenoids in wild chrysanthemums (part)

Serial number	Name	Ref.	Serial number	name	Ref.
1.1	Lactone of wild chrysanthemums	[3]	1.10	Kikkanol B	[9]
1.3	Angeloylcumambrin B	[4]	1.11	Kikkanol C	[9]
1.6	chrysanthemol chrysanthemol	[6]	1.12	Clovanediol	[9]
1.9	Kikkanol A	[9]	1.14	INdicumenone	[10]



2.10

Figure 1: Molecular structure of flavonoids 2.10

2.1.2 Flavonoids

During the further classification of wild chrysanthemums, it is found that the flavonoids are far more than the terpenoids. At this stage, the flavonoids obtained from the chemical separation of wild chrysanthemums mainly include hedgehog and luteolin. In the research on the quality control of wild chrysanthemumss, the content of these compounds is also used as the judgement standard (Lograda et al., 2013). In addition, other types of flavonoids have also been discovered. Here, the 2.10 Acacetin compound is taken as an example. The Ref. position is [16] and its chemical structure is shown in Figure 1.

2.2 Pharmacological activity of wild chrysanthemums

According to the analysis of the pharmacology of wild chrysanthemumss by the Institute of Materia Medica, Chinese Academy of Medical Sciences, after the decoction of wild chrysanthemum, the obtained liquid can have different inhibitory effects on pneumococcus, Escherichia coli and gold grapes; it has a reduced effect on typhoid fever; it has significant inhibitory effect on the platelet aggregation induced by ADP and collagen inside and outside the human body (Chang et al., 2010). Up to now, the pharmacological effect of wild chrysanthemums mainly include the following aspects.

Firstly, the effect of anti-pathogenic microorganisms. Ethanol is used to extract the wild chrysanthemum liquid with the concentration of 8mg/ml, which has different degrees of inhibition effect on 19 kinds of pathogenic fungi. The inhibitory effect on the evaluation rot bacteria and coniothyrium diplodiella is relatively good, reaching more than 80% and the inhibitory effect on pythium aphanidermatum and EC50 of wheat root rot pathogen is 0.476 and 1.524 respectively. It can be seen that wild chrysanthemums, as a new source of medicinal plants, can inhibit the production of pathogens (Li et al., 2015).

Secondly, anti-inflammatory and immune effect. After extracting wild chrysanthemums with petroleum ether and n-butanol, it can have anti-inflammatory and immune effect on many inflamed wounds and can effectively enhance the immunocompromised humoral immunity and cellular immunity function.

Thirdly, the antihypertensive effect. Anesthetized cats are injected intraperitoneally with 16g of wild chrysanthemum solution, which reduces the arterial pressure and slows down the heart rate for 2 hours. According to the experimental results, ethanol extract refined products can have antihypertensive effect on hypertensive animals such as anesthetized cats (Luyen et al., 2015).

Fourthly, antioxidant effect. Wild chrysanthemums have good inhibitory effect on the erythrocytes hemolysis induced by H_2O_2 and the inhibitory rate of the two is the same.

3. Analysis of chemical composition of wild chrysanthemums

By combing the existing theories, it can be seen that wild chrysanthemums are rich in volatile oils, terpenoids, flavonoids and other ingredients, which have a variety of pharmacological effects, such as antiviral, antihypertensive effect, anti-bacterial and anti-inflammatory (Wang et al., 2010).

At present, wild chrysanthemums have been widely applied in the clinic treatment of cardiovascular diseases and various kinds of inflammation. In order to make full use of the medicinal properties and plant resources of wild chrysanthemums, the chemical composition of wild chrysanthemums is analyzed (Basta et al., 2010). Three compounds are separated and identified with view to providing a certain reference for medical application.

3.1 Selection of instrument, medicament and reagent

Before the analysis of chemical composition of wild chrysanthemums, it is necessary to conduct chemical reaction on the plant, which requires the screening of the production equipment, wild chrysanthemums medicament and related reagent so as to obtain accurate chemical composition results. Firstly, the melting point of wild chrysanthemums is measured using an X-4 type micro melting point tester. At this time, the stability has not been corrected. Secondly, the NMR performance is tested using a Bruker ACF-500 nuclear magnetic resonance instrument; after that, the Agilent 1100 MSD Trap electrospray mass spectrometer is used to measure mass spectrum.

Finally, during the chemical reaction process, the thin-layer and column chromatography silica gel used are produced by Qingdao Ocean Chemical Plant. Wild chrysanthemums are purchased from the medicinal material market in a city (Chang et al., 2010). It is identified as wild chrysanthemum plant by a professor at the School of Pharmacy of Nanjing University. The standard of the certificate is stored in the teaching and research office of the Nanjing Medical University Hospital. In addition, the reagents used in this experiment are all analytically pure.

3.2 Extraction and separation of chemical composition of wild chrysanthemums

12.3kg of dried wild chrysanthemum medicinal material is crushed, followed by the extraction with heating reflux of 88% and 64% of ethanol respectively. The reduced pressure recovery is performed using extractum such as petroleum ether, ethyl acetate and extraction solvent. Finally, 280g of ethyl acetate extractum and 360g n-butanol extractum are obtained. At this time, the chloroform-methanol obtained is extracted to obtain four fractions (A-D). Fraction A is subjected to silica gel column chromatography and the alternative chemical petroleum ether-acetone is eluted to obtain compound 1 (18 mg).

The proportion of the compound in the fraction B is chloroform/acetone=100/1. After silica gel analysis, the petroleum ether-acetone gradient is eluted to obtain the pure compound 2 (25 mg), 3 (45 mg). The proportion of the compound in the fraction C is chloroform/acetone=50/1. After silica gel chromatography, the petroleum ether-acetone gradient is also eluted to obtain the LH-20 gel column. The obtained compound 4 (30 mg), 5 (22mg). The proportion of the compound in the fraction D is chloroform/acetone = 30/1. At this time, After silica gel column chromatography, the chloroform-acetone gradient is eluted to obtain the compound 12 (55 mg) and 13 (15 mg). At this time, the flow and results of the chemical composition of wild chrysanthemums are shown in Figure 1. Due to the large number of types, it is only necessary to conduct detailed analysis and chemical composition analysis on the above four types.

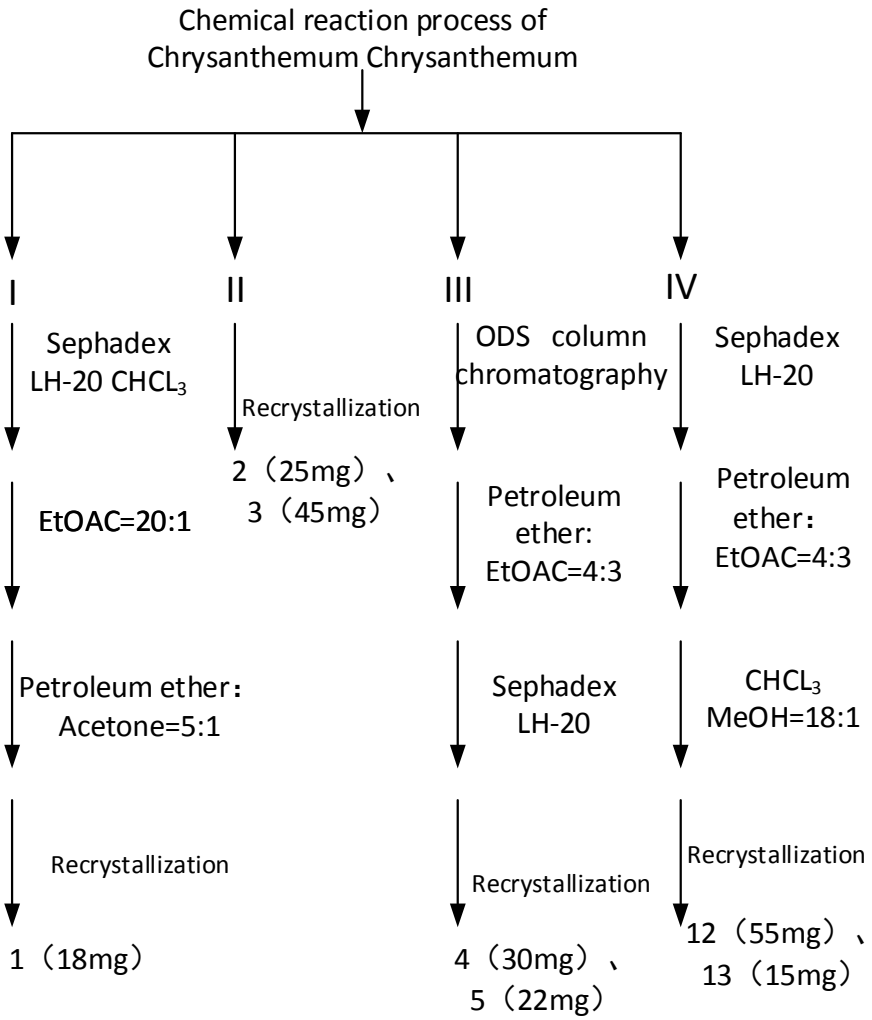


Figure 2: Separation process of the chemical composition of petroleum ether phase wild chrysanthemums

3.3 Analysis and identification of compound structure

Through chemical experiments on wild chrysanthemums, dozens of chemical components can be obtained. In this section, four typical chemical composition structures are mainly discussed. The specific contents are as follows.

3.3.1 Structure discussion compound 1 (FCIL-14)

This compound is in the form of blue in anisaldehyde-sulfuric acid at mp. 50-52°C. At the same time, there is a strong absorption at both nodes 1785 and 1807 of *IR*(KBr) cm^{-1} and there is a hydroxyl absorption at 3486, suggesting that the existence of ester hydroxyl in the compound. Through the spectrum display of ^{1}*H-NMR* and ^{13}*C-NM*R, d_H2.02(3*H, d, J*=7.2*Hz*), 6.16-6.22*(m*) and d_C166.9,145.3,16.2,20.4 are typical angelica acyl signals.

In addition, the compound contains 26 H and 20 C and the dissimilarity is 8, indicating that this compound contains 2 double bonds and 4 rings. It can be judged that this compound contains a sesquiterpene lactone of the intracyclic double bond. Therefore, the compound 1 here is identified as $la-hydroxy-3a,4a-epoxy-6\beta,7a-guaianon-9,10-en-6,12-olide$, which is named chrysanthguaianolide A.

3.3.2 Structure discussion compound 2 (FCIL-09-01)

This compound is in red form in anisaldehyde-sulfuric acid at mp. 169-160°C (expressed as a mixture). There is ester hydroxyl substance at 1706 and 1820 of *IR*(KBr) cm^{-1}. It is then identified that FCIL-09 is a mixture and only the structure identification is only conducted on the FCIL-09-01. The compound contains angelica acyl d_H2.02(*d,J*=7.2),1.80*s*,6.11-6.14(*m*); d_C166.7,142.3,18.2,20.4.

At this time, the double bond in the first ring, C-3, and C-4 respectively form a three-membered ring with oxygen. Here, there lacks one carbon atom with oxygen and the number of oxygenated carbon becomes four of the current value, indicating that there is one less hydroxyl in the compound. The compound 2 is identified as $8a-angeloyl-3a,4a-epoxy-6\beta,7a-guaianon-1,0-en6,12-olide$, which is named chrysanthguaianolide B.

3.3.3 Structure discussion of compound (FCIL-09-02, FCIL-16)

The compound here is a white long needle crystal (acetone) at mp. 64-66°C, which is in red form in anisaldehyde-sulfuric acid. ^{1}*H-NMR* and^{13}*C-NM*R. At this time, d_H1.84(3*H,d,J*=7.2*Hz*),1.85(3*H*,*br*,*s*),6.84-6.99(*m*); d_C166.9,138.3,13.2,13.4. By the comparison with the literature, it is eventually shown as tigloyl. The compound here is identified as $8a-tigloyl-3a,4a-epoxy-6\beta,7a-guaianon-1,10-en-6,12-olide$, which is named chrysanthguaianolide C.

3.3.4 Structure discussion of compound 4 (FCIL-03)

The compound here is white powder (acetone) at mp. 170-171°C, which is in brown form in anisaldehyde-sulfuric acid. Compared with compound 1, they are both in a similar state. In the spectrum ^{1}*H-NMR*, there are two carbon protons nearby *d*6.11 and *d*5.33 and this compound is a sesquiterpene lactone containing a double bond. There is a double bond outside the ring.

Thus, it can be identified that this compound is apressin.

By chemical analysis and judgement of the compound of chrysanthemum chrysanthemum, the name, structure type and some chemical properties of the chemical constituents of chrysanthemum chrysanthemum were obtained, as shown in Table 2.

Therefore, combined with the current academic research on the chemical composition of chrysanthemum chrysanthemum, the results obtained in this experiment are more accurate, which can explain the specific chemical composition of chrysanthemum chrysanthemum, and on this basis can be more accurate to distinguish other chemical components.

Table 2: Chemical composition types of Chrysanthemum Chrysanthemum

Compound name	Temperature setting	Reagent	Colour	Called
FCIL-14	mp.50-52°C	Anisaldehyde sulphuric acid	Blue	chrysanthguaianolide A
FCIL-09-01	mp.169-160°C		Red	chrysanthguaianolide B
FCIL-09-02, FCIL-16	mp.64-66°C		Red	chrysanthguaianolide C
FCIL-03	mp.170-171°C		Brown	apressin

4. Conclusion

In summary, the separation analysis of the chemical composition of wild chrysanthemums is dconducted by means of polyamide chromatograph and ethanol with different proportions. finally, four compounds are obtained through Sephadex LH-20 purification. After spectral analysis, four compounds are identified as chrysanthguaianolide A, chrysanthguaianolide B, chrysanthguaianolide C and apressin. In this study, the flavonoids and terpenoids in wild chrysanthemums are used as the research object and the chemical composition of wild chrysanthemum s are analyzed and identified on the basis of ethanol concentration, frequency of use and appropriate time. The content of the final four compounds of wild chrysanthemums are further determined from the extracting solution. Finally, the optimal best extraction process is selected based on the identified chemical composition of wild chrysanthemums. This experiment obtains the total flavonoids of wild chrysanthemums with high degree of purity, which lays a good foundation for its comprehensive utilization.

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References

Basta A., Milica P., Couladis M., 2010, Essential oil composition of the flowerheads of Chrysanthemum coronarium, L. from Greece, Flavour & Fragrance Journal, 22(3), 197-200.

Chang K.M., Choi E.M., Kim G.H., 2010, Chemical constituents of Chrysanthemum indicum, L. flower oil and effect on osteoblastic MC3T3-E1 cells, Food Science & Biotechnology, 19(3), 815-819, DOI: 10.1016/j.phymed.2013.10.019

Chang K.M., Choi E.M., Kim G.H., 2010, Chemical Constituents of Chrysanthemum Indicum, L. Flower Oil and Effect on Osteoblastic MC3T3-E1 Cells, Food Science & Biotechnology, 19(3), 815-819.

Haouas D., Halima-Kamel M.B., Flamini G., 2012, Chemical Composition and Bioactivities of Three Chrysanthemum Essential Oils Against Tribolium Confusum (Du Val) (Coleoptera: Tenebrionidae), Journal of Pest Science, 85(3), 367-379, DOI: 10.1007/s10340-012-0420-7

Hosni K.H., 2013, Secondary Metabolites from Chrysanthemum Coronarium (Garland); Flowerheads: Chemical Composition and Biological Activities, Industrial Crops & Products, 44(2), 263-271.

Li Y.L., Wang S., Zhu J.J., 2015, Study on Effects of Sulfur Fumigation on Chemical Constituents of Chrysanthemum Morifolium CV. Boju, China Journal of Chinese Materia Medica, 40(13), 2624-2628.

Lograda T., Ramdani M., Chalard P., 2013, Chemical Composition, Antibacterial Activity and Chromosome Number of Algerian Populations of Two Chrysanthemum Species, Journal of Applied Pharmaceutical Science, 3(8), S6-S11.

Luyen B.T.T., Tai B.H., Thao N.P., 2015, The Anti-Osteoporosis and Antioxidant Activities of Chemical Constituents from Chrysanthemum indicum Flowers, Phytotherapy Research Ptr, 29(4), 540-548, DOI: 10.1002/ptr.5281

Shun Y.Z., Yang Y., Huaidong Y., 2005, Chemical Composition and Antimicrobial Activity of the Essential Oils of Chrysanthemum Indicum, Journal of Ethnopharmacology, 96(1), 151-158.

Wang J., Chen D., Liang L., 2010, Chemical constituents from flowers of Chrysanthemum indicum, China Journal of Chinese Materia Medica, 35(6), 718.

Wang S., Hao L.J., Zhu J.J., 2014, Study on the Effects of Sulfur Fumigation on Chemical Constituents and Antioxidant Activity of Chrysanthemum Morifolium CV, Hang-ju. Phytomedicine, 21(5), 773-779, DOI: 10.1016/j.phymed.2013.10.019

Wang Y.J., Yang X.W., Guo Q.S., 2008, Studies on Chemical Constituents in Huangjuhua (Flowers of Chrysanthemum Morifolium), China Journal of Chinese Materia Medica, 33(5), 526.

Wu L.Y., Gao H.Z., Wang X.L., 2010, Analysis of Chemical Composition of Chrysanthemum Indicum Flowers by GC/MS and HPLC, Journal of Medicinal Plant Research, 4(5), 421-426.