REVIEW

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A review on the extraction and separation of andrographolide from *Andrographis paniculata*: extraction selectivity, current challenges and strategies

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Author contributions

Di Liang completed information retrieval, writing and drawing. Wei-Mei Zhang conducts information retrieval and writing. Xu Liang, Hong-Yue Tian and Xue-Min Zhang completed the information retrieval. Xia Li and Wen-Yuan Gao finished writing, reviewing and editing. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

AG, andrographolide; *A. paniculata*, *Andrographis paniculata* (Burm.f.) Nees; L/S, solid-liquid ratio; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction; SCFE, supercritical fluid extraction; SCF, supercritical fluid; SC-CO_2, supercritical CO_2; MSZW, the width of the metastable zone; PSD, prticle size distribution; MIPs, molecularly imprinted polymers; SPE, solid-phase extraction; CCC, countercurrent chromatography; HSCCC, high-speed countercurrent chromatography; AC, activated carbon.

Citation

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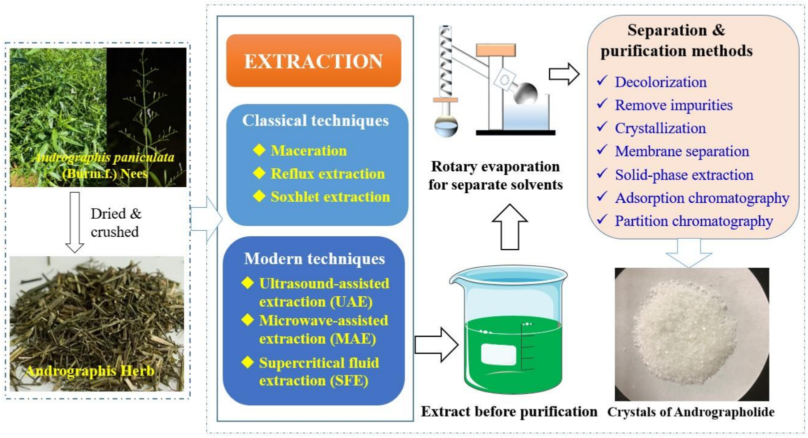
Abstract

Andrographolide is the main active ingredient of *Andrographis paniculata* (Burm.f.) Nees, known as “natural antibiotic”. Here, for the purpose of discovering a more efficient, low-cost extraction and separation method, the research status of andrographolide was reviewed. At present, researches only take extraction rate as the only index to optimize extraction parameters, but ignores the importance of extraction selectivity. It is usually meaningless to blindly pursue the extraction rate without considering the difficulty and cost of subsequent separation. So, factors affecting extraction selectivity such as solvent choice, temperature and physicochemical effects caused by extraction technique itself, are first discussed. Different extraction techniques for andrographolide were discussed by comparing the selectivity, efficiency and cost of extraction. The separation procedures of andrographolide such as decolorization, impurity removal, crystallization, membrane separation, solid-phase extraction and partition chromatography and their challenges and possible strategies are also discussed. It is hoped that this review can provide guidance for researchers who are committed to advancing the field of andrographolide extraction and purification.

Keywords: andrographolide; extraction techniques; extraction selectivity; separation



Highlights

A variety of andrographolide preparations have been marketed in China, andrographolide is in great demand.

The factors affecting extraction selectivity should be emphasized. A tailor-made activated carbon for andrographolide may be a strategy to solve the current high decolorization loss rate and poor decolorization effect.

It is still a challenge to control the crystallization quality by controlling the crystallization operation conditions.

Advanced separation and purification technologoies such as membrane separation, solid-phase extraction and high-speed countercurrent chromatography has broad prospects.

Medical history of objective

Andrographis paniculata (Burm.f.) Nees (*A. paniculata*) is native to India and Sri Lanka and now widely cultivated in China, Thailand, Indonesia, Laos, Malaysia for the treatment of upper respiratory infections, flu, snake-bite, sore throat and fevers (like malaria, dengue). In China, *A. paniculata* was first recorded in the *Materia Medica Collection in Lingnan* (1932 C.E.), written by Bu-Dan Xiao, and its functions were also recorded in local herbal books such as *Quanzhou Materia Medica* (1963 C.E.), *Chinese Herbal Medicine of Fujian* (1970 C.E.) and *Chinese Herbal Medicine of Guangxi* (1969 C.E.).

Andrographolide, the main active ingredient of *A. paniculata*, is used to treat sore throats caused by upper respiratory tract infections, chills, influenza and other ailments.

Background

Andrographis paniculata (Burm.f.) Nees (*A. paniculata*) is native to India and Sri Lanka and now widely cultivated in China, Thailand, Indonesia, Laos, Malaysia for the treatment of upper respiratory infections, flu, snake-bite, sore throat and fevers (like malaria, dengue) [1, 2]. The phytochemical constituents of *A. paniculata* can be roughly divided into diterpenoid lactones, flavonoids, terpenoids, phenolic acids, chalconoids and xanthones [3]. Andrographolide (Figure 1a), a diterpene lactone compound, is the major active ingredient of *A. paniculata* and is regarded as a “natural antibiotic” with unparalleled anti-inflammatory, anti-cancer, anti-viral and anti-fibrosis therapeutic potential [4, 5].

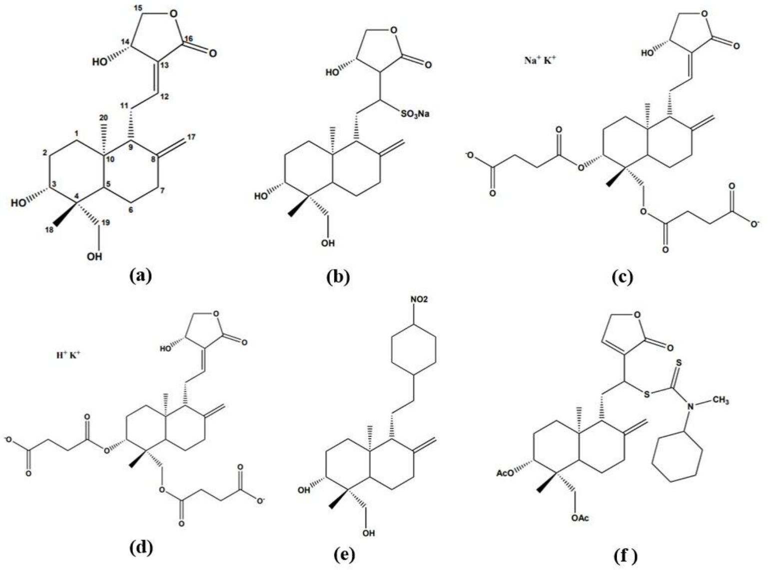
Now, Andrographolide (AG) tablets or dropping pills are already marketed as anti-inflammatory drugs in China. Moreover, extensive chemical modifications of AG have been used to develop derivatives with greater bioavailability and bioactivity. For example, water-soluble chemically modified injections such as Lianbizhi (Figure 1b), Yanhuning (Figure 1c) and Chuanhuning (Figure 1d) and are also available in market for the treatment of bacillary dysenter, viral pneumonia and upper respiratory tract infection [6]. Structural modification based on AG is also in full swing to develop richer and stronger pharmacological activities. Two series analogues of AG were synthesized using aromatic acids, phenolic and nitrogen-containing heterocyclic as bioisostere moiety of lactone ring. Figure 1e exhibited potent anti-inflammatory activity with half maximal inhibitory concentration of 3.38 ± 1.03 μM [7]. Arsakhans et al. synthesized a AG analogue (Fiure 1f) exhibited greater selective cytotoxic activity to human breast cancer cells (MCF-7) and human cholangiocarcinoma cells (KKU-055) than the parent AG and ellipticine (a well-known anticancer drug), with half maximal inhibitory concentration of 0.59 μM and 1.36 μM, respectively [8]. Additionally, AG can also act as a chemical sensitizer in combination with anticancer drugs (carboplatin) to tackle chemical drug resistance, which would represent a new drug combination strategy [9].

However, production of AG by chemical synthesis is complicated and costly, so it is mainly obtained by isolation [10]. Nevertheless, traditional AG production methods frequently confront disadvantages such as high consumption of organic solvent, low extraction efficiency, poor extraction selectivity and cumbersome separation process. Skyrocketing demand for AG by the pharmaceutical industries and scientific researches make it extremely urgent to find a more efficient and more environmentally friendly AG production method. To our best knowledge, there is no systematically review on the extraction and separation of AG from *A. paniculata*. Reviews on AG, currently, mainly focus on the pharmacological effects, chemical modification, dosage form transformation and mechanisms of action [11–15]. Therefore, AG, extraction process, extraction selectivity, separation of AG were searched in the databases of Web of Science, PubMed and Google Academic. Different extraction and separation studies are focused on. Some articles focusing on analytical methods and pharmacological activities are not overconsidered. It is hoped that this paper can provide reference for the extraction and separation of AG.

Extraction

Mechanism of extraction

Naturally active ingredients are usually contained in the plant sample



(a) (b) (c)

(d) (e) (f)

Figure 1 Structural of andrographolide (a), Lianbizhi (b), Yanhuning (c), Chuanhuning (d) and its analogs (e) and (f)

[16]. Plant sample is made up of cells surrounded by a solid cellular wall. The more fragmented and porous the cell wall, the more easily solvents and solutes can be transferred [17]. The overall mechanism of solid-liquid extraction can be described as follows: first, the solvent is mixed with the plant material, diffused onto the solid surface, penetrated into the particles, and gradually dissolves the soluble ingredients. The subsequent processes can be viewed as a mass transfer from the interior of the cell to the surface (internal diffusion) and from the surface to the bulk solvent (external diffusion) [18, 19]. The driving force of mass transfer is the concentration difference between the local concentrate formed after the dissolution of the active component and the bulk solvent. Numerous emerging extraction techniques can accelerate cell wall rupture and mass transfer, and the principles for these extraction techniques will be discussed later.

Factors affecting extraction rate and extraction selectivity

The extraction rate of target compounds is an indispensable index for evaluating extraction techniques and parameters, however, it is meaningless to blindly pursue the extraction rate without considering the selectivity of extraction, which will inescapable augment the difficulty and cost of purification [20, 21]. As shown in Figure 2a, conventional factors affecting the extraction rate include solvent, temperature, solid-liquid ratio (L/S), etc. However, factors affecting the extraction selectivity can be mainly sort into two categories, one is the solvent which has the highest precedence, the other is the physicochemical effects produced by the extraction process itself, such as temperature and pressure (Figure 2b) [22].

Different solvents or solvent mixtures have different extraction capabilities. Whether the active compound can be dissolved hinges on the structure of the active compound and the properties of the solvent. In general, the rule of thumb for solubility “like dissolve like” is followed when selecting extraction solvents, that is, compounds of similar polarity are easily soluble [23]. As with most medium-sized bioactive molecules in plant matrices, AG molecules are highly polarized due to the presence of aromatic delocalized μ-electrons [24]. Kumoro et al. studied the effect of different solvent properties on the extraction of AG by Soxhlet, and the extraction performance of 16 kinds of solvents or solvent mixtures is as follows: 100% methanol > 75% methanol > 100% ethanol > 70% acetone > 50% methanol > 75% ethanol > ethyl acetate > 50% ethanol > chloroform > 100% acetone > 25% methanol > 25% ethanol > dichloromethane > water > petroleum ether > n-hexane [24].

Most studies prefer binary solvent systems for extraction of bioactive compounds to achieve better extraction performance compared to single-solvent systems. It’s because that solute solubility is at maximum when the dielectric constant (ε) of solute and solvent, a key index of polarity, are same and the corresponding extraction rate is usually the highest [25]. Greater ε, greater polarity [26]. Mixing two solvents whose ε is greater or smaller than the solute results in a mixed solution whose ε is close to the solute [24]. Furthermore, polar solvents help increase cell wall permeability, thereby improving the contact between solvent and solids, causing a higher extraction rate. In binary solvent systems, the combination of ethanol and water is the most popular due to their green solvent properties. Priyanka et al. found that the yield of AG in ultrasound-assisted extraction (UAE) was highest with 50% ethanol followed by 75% ethanol, 25% ethanol, and 100% ethanol. Rafi et al. also stated that the extraction effect of ethanol 50% was better than that of 100% ethanol for extraction without heating which is in contrast to that reported by Kumoro et al. [24, 27]. This may be due to the hydrolysis of AG due to the prolonged heating during the Soxhlet extraction. 50% ethanol seems to be the first-rank solvent for the extraction of AG, but the extraction solvent requires not only a high solubility for the desired component but also a low affinity for impurities. Plants inherently contain plentiful water-soluble substances, such as polyphenols, flavonoids, anthocyanins, tannins, aroma, gum, starch, pigments and other compounds in plants [17]. If the extraction selectivity of the solvent is ignored, it will unavoidable raise the difficulty and cost of subsequent separation, which is often ignored in the current research on AG extraction. Some patents control the ethanol concentration at 95% to balance the extraction rate and extraction selectivity [28, 29]. Therefore, in the future explore of AG extraction, solvent selection should not only consider solvent safety and extraction rate, but also pay attention to extraction selectivity.

What is worth mentioning is that “like dissolve like” only

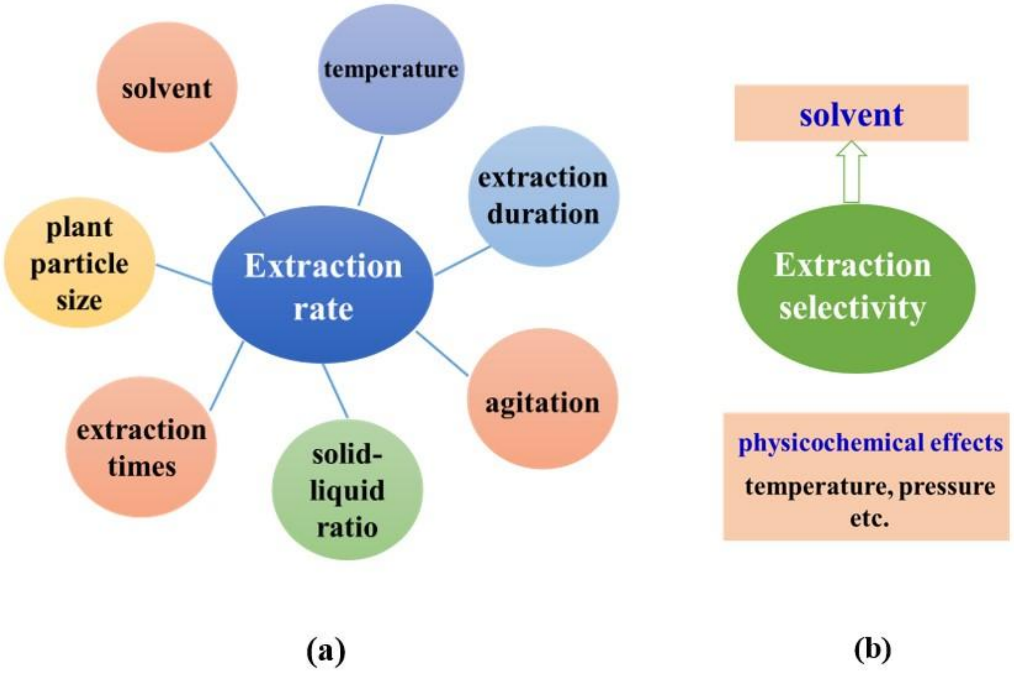


Figure 2 Factors affecting extraction rate (3a) and extraction selectivity (3b)

qualitatively describes the relationship between solute and solvents. Solubility parameters (δt) may more accurately explain the extraction ability of diverse solvents and is also beneficial to the extraction and purification process design of AG. δt proposed by Hildebrand, which concretized the abstract concept of polarity and can be seen as a physical constant to predict intermolecular forces [30]. Free miscibility is most easily achieved when the intermolecular forces are close. That is to say, the closer the solubility parameters of solvent and solute are, the better the mutual solubility effect will be [31]. However, δt reflects the sum of all forces between molecules, but in fact the forces between the molecules are not a single force, which means that the solvent remains dissolve the solute even while the solubility parameters differ widely between the two substances (Table 1) [27]. A typical example, the δt of water is 47.8, while that of ethanol is 26.5, but they are miscible with each other due to strong the hydrogen bond adhesion force, however, AG is soluble in ethanol but almost insoluble in water. In 1966, Charles M. Hansen decomposed the δt into three components, δd, δp and δh, which stand for the dispersive force, the polar force and the hydrogen bond adhesion force, respectively. It allow to distinguish and measure the individual components separately, and knowing their relationship and ratio to the total force allows us to judge solubility more accurately [32]. So far, δd, δp and δh for most substances have been obtained either computationally or experimentally [31]. If the basic physical data of a molecular is foregone, the group contribution method can be put to reckon parameters for specific substances which not listed in the table [33]. Unfortunately, there are no reports on the solubility parameters of AG at present and further research is needed.

Temperature is another dominating factors that affecting extraction rate and extraction selectivity. Generally, if the critical temperature leading to thermal degradation of the compound is not considered, an increase in temperature is associated with an increase in the extraction rate until equilibrium is reached [34]. This phenomenon is mainly on account of an increase in compound solubility and solvent diffusion rate [35]. The problem, however, is that almost all reactions speed up with increasing temperature, including all unwanted side effects [34]. Selecting an appropriate temperature can greatly reduce the presence of impurities and ensure a relatively high extraction rate.

The greater L/S, the higher the concentration gradient between the solvent and the plant material, and the stronger the driving force for mass transfer [16, 36]. However, due to the limited amount of active components in the plants, the effect of increasing L/S on improving extraction rate tends to weaken. Further increase in L/S will cause the waste of solvent, the extraction of impurities, the reduction of the feed amount, the reduction of the utilization rate of extraction equipment and the difficulties of downstream processing [16]. Agitation is an assisted diffusion method that disperses the concentrated solution that has gathered around the particle surface and brings the fresh solvents to the particle surface and thus improve the concentration gradient and ameliorate the extraction efficiency [37]. However, when agitation is applied on an industrial scale with large sample containers, it is often operationally difficult. The development of circulating extraction and multi-stage extraction equipment gives full play to the extraction capacity of solvents and controls the production cost at a lower level and it also makes up for the disadvantage of difficult stirring to a certain extent [37]. The increase in surface area caused by particle size reduction and destruction of the cell structure integrity both facilitate the diffusion of the solvent [38]. In general, however, plant material in fine powder form is not used, as it causes the excessive absorption of solute in solid and makes clarification of subsequent extractions difficult [37, 39]. Small batch extraction can precipitate powder by centrifugation, but centrifugation is more difficult in mass production. Furthermore, a batch of medicinal materials usually needs to be extracted two or three times to obtain a satisfactory extraction rate and time is also required for solvent to penetrate plant substrate, dissolve the active compound and subsequent mass transfer. Increasing the extraction duration will increase the extraction rate until the concentration in the plant material equilibrates with the concentration in the bulk solvent.

Extraction techniques

Parameter optimization is the key to improve extraction yield and obtain high extraction selectivity. However, an increase in extraction efficiency requires a change in extraction technique. The optimal extraction parameters of AG under different extraction processes was shown as Table 2.

Maceration. Maceration is considered a simple steady-state extraction technique with minimal experimental setup in which the whole or powdered medicinal materials by placing whole or powdered medicinal materials in an airtight container with solvent and allowing to remain at room temperature for at least three days with frequent stirring until the soluble compound dissolves [37,49]. The extraction selectivity of maceration is determined only by the extraction solvent, and its disadvantages are long extraction duration and high solvent consumption. Only 1.8% w/w of AG was obtained from 10 g of *A. paniculata* with 100 mL methanol at room temperature with occasional stirring for 7 days [40].

Reflux extraction. Reflux extraction is a process in which solvent evaporates and condenses repeatedly in a certain time at a constant temperature without loss of solvent. Under 50% ethanol (v/v), plant leaf-liquid ratio of 1:9, 73 °C and 1.9 hours, Chua et al. achieved a crude extraction rate of 17.2% w/w with the concentration of AG of 140.5 ppm by reflux extraction [41]. Reflux extraction system is extensively applied in herbal medicine industry owing to its high extraction rate and simple operation. However, the long-time heating often leads to a large number of impurities.

Soxhlet extraction. To use soxhlet extraction, the sample is repeatedly contacted with a fresh portion of solvent, thus providing greater a mass transfer. Soxhlet extraction is capable of extracting more target compounds than other extraction methods, and the plant matrix is independent, requiring no filtration after extraction is complete [50]. However, the temperature of the system is kept at a high level, which may have an effect on the selectivity of extraction [51]. Furthermore, ideal samples for Soxhlet are limited to dry and finely segmented solids and it uses hazardous and flammable organic solvents. Sharma et al. loaded 1 g of *A. paniculata* material into

Table 1 Solvent properties, Hildebrand parameter and Hansen parameters

Solvent	Dielectric constant ε	Hildebrand parameter δt ($MPa^{1/2}$)	Hansen parameter δd ($MPa^{1/2}$)	Hansen parameter δp ($MPa^{1/2}$)	Hansen parameter δh ($MPa^{1/2}$)
Water	80.0	47.8	15.6	16.0	42.3
Methanol	32.7	29.6	15.1	12.3	22.3
Ethanol	24.5	26.5	15.8	8.8	19.4
Acetone	21.0	20.0	15.5	10.4	7.0
Ethyl acetate	6.4	18.1	15.8	5.3	7.2
Chloroform	4.8	19.0	17.8	3.1	5.7
Methylene chloride	9.1	20.3	18.2	6.3	6.1
n-Hexane	1.9	14.9	14.9	0.0	0.0
Benzene	2.3	18.6	18.4	0.0	2.0

Table 2 The optimal extraction parameters of andrographolide under different extraction techniques

Extraction Techniques	Parameters	Yield	Reference
Maceration	ES: methanol; L/S: 10:1; ED (d):7	1.8%	[40]
Reflux extraction	ES: 50% ethanol; L/S: 9:1; T (°C): 73; ED (h): 1.9	Crude yield: 17.2%; AG concentration: 140.5 ppm	[41]
Soxhlet extraction	ES: methanol; L/S: 100:1; ED (h): 1.5	Crude extract yield: 15.684%; AG concentration: 1.790%	[42]
Ultrasound-assisted extraction	ES: 50% ethanol; L/S: 40:1; ED (min): 10; P (W): 134; f (kHz): 22	27.97 mg/g	[43]
Microwave-assisted extraction	ES: 85% ethanol; L/S: 20:1; ED (min): 4; P (W): 140; PS: 125–250 μm	10.926 ± 0.053 mg/g	[44]
	ES: 150 mL chloroform plus 30 mL water; L/S: 18:2 (20 g sample); P (W): 210; ED (min): 40	0.589%	[45]
	ES: (bmim) Cl (2.84 mol/L); L/S: 10:1; T (°C): 70; P (W): 600; ED (min): 6	4.71%	[46]
Supercritical fluid extraction	ES: SF-CO$_2$; T (°C): 40; CO$_2$ flow rate: 2 mL/min; P’ (MPa): 10	0.0174 g/g per hour	[47]
	ES: SC-CO$_2$-ethanol mixture containing 12.5 mol % ethanol; T (°C): 50; flow rate: 2 mL/min; P’ (MPa): 15; particle size: 0.375 mm	0.715 g/g after 4 h of extraction.	[48]

ES, extraction solvent; L/S, liquid to solid ratio; T, temperature; ED, extraction duration; P, power; F, frequency; P’, pressure; PS, particle size; AG, andrographolide; SF-CO$_2$, supercritical fluid CO$_2$; SC-CO$_2$, supercritical CO$_2$.

thimble and used 100 mL methanol for Soxhlet extraction for 1.5 h obtaining the highest crude extraction rate of 15.684% and AG content of 1.790% [42].

Ultrasound-assisted extraction. UAE is a fresh and mature method applied in the extraction active compounds in recent years [52]. The main mechanism of UAE is acoustic cavitation [53]. When the ultrasound waves travel through the medium, the negative pressure during rarefaction exceeds the attractive force holding molecules together, pulling them apart and creating cavitation bubbles [54]. As the sheer number of expansion and compression cycles in the medium increases, these bubbles suddenly implode creating instantaneous hot spots and extreme local environments with temperature up to 5,000 K and pressure up to 1,000 atm which leading to cell disruption and particle rupture facilitating the release of active compounds and improving mass transfer from matrix to solvent [54].

AE can be divided into two forms: ultrasonic water bath and ultrasonic probe [53]. Ultrasonic bath is economical and easy to operate, requiring only the plant matrix to be placed in the extraction solvent in a stainless steel container. However, if the volume of the extraction tank is over-sized, there will be an ultrasonic blank area affecting the reproducibility and limiting its application in industrial extraction process [55]. Rao et al. confirmed that the actual power in the ultrasonic bath varies in different regions through the weight loss test of aluminum foil and precise placement of the extract in the tank helps to obtain the highest extraction rate [43]. The best yield of AG was 27.97 mg/g obtained at position 11 (this article does not give its specific location), depth of 2.54 cm, ultrasonic extraction for 10 min, 50% ethanol as solvent, L/S ratio 40:1, power of 134 W and frequency of 22 kHz. An ultrasonic probe may be able to compensate for this deficiency, which consisting of one or more probes or horns and can be immersed in the extraction vessel to deliver ultrasound through medium with minimal energy loss and is the first choice in the industry.

In UAE, the strong acoustic cavitation effect, thermal effect and agitation effect caused by ultrasonic wave could improve extraction efficiency [56, 57]. The low temperature generated by ultrasonic waves in a short time ensures the extraction selectivity which will undoubtedly bring convenience to the subsequent separation. At present, there are industrial-scale ultrasonic extraction equipment with a processing capacity of 3,000 L on the market, and the application of UAE in AG will definitely improve efficiency and comprehensive economic benefits.

Microwave-assisted extraction. The mechanism of microwave-assisted extraction (MAE) is the microwave radiation causes dipole rotation of molecules in the extraction vessel [58]. Friction between polar (solvent) dipoles and polarizable material molecules (plant samples) creates the localized internal superheating leading to an increase in pressure within plant cells, causing the cells to rupture, promoting the release of active compounds, thus leading to a faster mass transfer [44, 45]. The effects of microwave power and ethanol concentration on the extraction yield of AG were investigated under the conditions of constant particle size (125–250 μm), L/S ratio (20:1) and extraction time (4 min), and the highest AG yield (10.926 ± 0.053 mg/g dry weight) was obtained with microwave power of 140 W and ethanol concentration of 85% [44].

Dielectric constant (ε) of the solvent plays an important role in MAE [59]. The higher the ε of the solvent, the more microwave energy absorbed, and correspondingly, the higher the extraction rate [60]. Since chloroform is a microwave transparent solvent, a mixed solvent of chloroform and water was applied to compare the difference between Soxhlet extraction and MAE for AG [45]. The heat generated by water absorbing the microwaves to rupture the cells, releasing the plant components and chloroform present then dissolves AG. The results indicating that the yield of AG provided by MAE was 0.589% at the extraction 40 min, microwave power 210 W and L/S ratio 18:2 (150 mL chloroform plus 30 mL water, 20 g sample), while conventional Soxhlet extraction was known to provide a yield of 0.4452%, and its L/S ratio was 15:2 (150 mL pure chloroform, 20 g sample) and the extraction time was 6 h.

Ionic liquids are a novel type of extraction solvent and are characterized by virtually no vapor pressure, good electrical conductivity, extensive electrochemical stability and high fluidity [61]. The yield of AG was improved from 1.5% w/w at 7 days (Maceration) and 1.8% w/w at 3 hours (Soxhlet) to 4.71% w/w by using ionic liquid-MAE at only 6 minutes, temperature of 70 °C, L/S ratio of 10:1, (bmim) Cl concentration of 2.84 mol/L and microwave power of 600 W [46]. Although MAE has many advantages, the effect of the heat generated by the absorption of microwaves by the solvent on the extraction selectivity and stability of active compounds needs to be further explored.

Supercritical fluid extraction (SCFE). The use of SCFE as solvents benefits from the ability to alter density and induce a shift in solubilization by changing either the pressure or temperature [62]. The viscosity of supercritical fluid is much lower and its diffusivity is one or two orders of magnitude higher than any other liquid, allowing for a faster mass transfer rates. Supercritical CO_2 (SC-CO_2) is the most attractive, mainly because of its low price, a near-ambient critical temperature, non-toxicity, non-flammability and a relatively easy CO_2 separation by a simple depressurization [63]. Kumoro and Hasan reported that the highest extraction yield 0.0174 g/g per hour of operation of AG was achieved at a temperature of 313 K, a pressure of 10 MPa and a SC-CO_2 rate of 2 mL/min [47].

However, SC-CO_2 also has its own shortcomings. Most of the less volatile high molecular weight organic compounds are almost insoluble in SC-CO_2, and the same is true for AG, which would result in the lack of selectivity of SC-CO_2 for polar organic compounds [64]. It has been known that adding a small fraction of polar co-solvents to SC-CO_2 will inherently increase the polarity and density of the supercritical fluid (SCF) mixture, thus, the ability to dissolve polar compounds is enhanced. Chen et al. found that non-toxic co-solvents ethanol, water and acetic acid could improve the yield of AG, and the order is: acetic acid > ethanol > water [63]. Kumoro et al. reported the highest yield of AG was 0.715 g/g after extraction for 4 h with SC-CO_2-ethanol mixture containing 12.5% mol ethanol, flow rate of 2 mL/min, 15 MPa, 323 K and particle size of 0.375 mm, respectively [48].

In SCFE, a cross-property is the result of an equilibrium between solvent density and solute vapor pressure as a function of pressure and temperature [64]. In isothermal extraction process, the density of SCF augment with the increase of pressure, while the vapor pressure of the solute reduced. As the solvation ability of SCF is enhanced, the extraction rate of AG increases with the increase of pressure. In addition, the extraction selectivity is also a function of pressure, that is, with increasing pressure, different types of solutes are also extracted. The mechanism of temperature effect on extraction rate is different from other extraction techniques. The solubility of solutes initially increases with temperature. However, as the temperature exceeds a certain value, the density of SF will decrease conspicuously, resulting in a decrease in the solubility of AG, which in turn leads to a decrease in the extraction rate [63].

The extraction capacity of SCF on temperature and pressure and the selectivity of extraction can be guaranteed by selecting the best critical conditions. But, SCFE requires expensive financial input and the presence of water in the sample can easily lead to clogging [48]. Moreover, when changing products, it is difficult to clean the equipment.

Separation

Enhancing extraction selectivity is the most effective methods to reduce impurities, but considering the types and characteristics of phytochemical components in herbs, impurities are unavoidable, so proper method of removing impurities is necessary. The principle applied in AG is mainly based on the differences in adsorption, solubility, partition coefficient and molecular size of coexisting components. It should be emphasized multiple principles are constantly used in combination to obtain high-purity products, and the purification strategies of extracts obtained by different extraction techniques and extraction parameters are also different.

Decolorization

The extracts of *A. paniculata* usually contains a large amount of pigments, and if the extract is directly concentrated, dark-green sticky lumps will be produced, which affecting the appearance and purity of the product. Hence, the first operation executed on the extract is normally decolorization.

Kilambi et al. initially degreased and decolorized *A. paniculata* powder with toluene at room temperature, followed by extraction [65]. However, due to the toxicity of residual toluene, this method is not fit for pharmaceutical production. “Alcohol extraction and water precipitation” used to be used to remove pigments in the traditional process, however, this operation usually requires the addition of a mass of water, resulting in extremely high energy consumption for solvent recovery. Additionally, the reclaimed liquid generally contains plenty of water, if it serves as an extraction solvent once again, rectification is required, requiring a large quantity of energy again. Therefore, “alcohol extraction and water precipitation” is yet not a suitable method for decolorization of *A. paniculata* extract. Macroporous anion exchange resins have been applied to decolorization of polysaccharides, however, since complicated operation and high cost, it is not optimal choice either [66].

Activated carbon (AC) is a widely used adsorbent in separation of natural products, which has the advantages of low cost , enormous internal surface area, exceptionally high porosity, adjustable pore size, and high adsorption capacity [67]. Decolorization of the extracts of *A. paniculata* using AC has been adopted as the standard extraction protocol in many schoolbooks in China [68]. However, it was found that the decolorization effect of AC produced by different manufacturers varies greatly and AC also adsorbs a large amount of AG while adsorbing impurities. The iodine value has always been the main indicator of AC selection, but, it often occurs that the iodine value of AC varies greatly, but the pollutant removal efficiency is not much different [69]. Consequently, it is crucial to confirm the key properties of AC for decolorization of *A. paniculata* extracts. So, what properties should high-quality AC have?

More and more studies have shown that the adsorption capacity of AC is related to its specific surface area, pore size, surface charge, pore size distribution and surface functionality [70, 71]. Pore diameter must be sufficiently large to accommodate the size of the adsorbed molecule. Different pore structures have different functions. Micropores (≤ 2 nm) mainly adsorb low-molecular weight compoun, mesopores (2–50 nm)mainly adsorb large-molecular weight organics and macropores (≥ 50 nm)are suitable for adsorbing microorganisms. For example, the size (1.5–3.0 nm) of dyes and humic acids favors their adsorption in mesopores. For adsorbates with molecular diameters of about 0.5 nm, a predominantly microporous AC is usually sought [67]. Appropriate surface functional groups will create a positively, neutral or negatively charged surface capable of attracting the adsorbing species (or a large surface area may become secondary want). Furthermore, the sizable porous network also plays an important role, as it allows higher diffusion rates, which are essential for adsorption kinetics and process design [67].

It is exciting that the adsorption capacity and characteristics of AC are fantastically related to its activation method, activation conditions and raw material sources [72]. Physical activation typically involves heating the feedstock in the presence of an inert gas, carbon dioxide or steam at temperatures ranging from 500 to 1,000 °C. The chemical activation method is to add chemical activators such as zinc chloride, phosphoric acid and other chemical activator to the precursor prior to the thermal treatment [67]. Changes in chemical activator type, impregnation time, carbonization temperature or chemical composition of raw materials will obviously affect the structure of AC, and at this time, their adsorption properties and adsorbed target impurities will also changer (Figure 3).

On the premise that the intended use of AC is clear, the adsorption mechanisms need to be systematic and comprehensive studied to determine which excellent properties should be possessed by AC with optimal adsorption capacity. AC has a hexagonal structure formed by covalent bonds with a large π bond structure properties of aromatic rings. Most pigments have a conjugated structure, so the adsorption of pigments using AC is expected to be due to π-π interactions between the π-electrons of the adsorbent and the π-electrons of the aromatic rings of the adsorbates [73]. In addition, many chemical modifications have been applied to increase the density of functional groups on the AC surface, stressing the significance of the interaction of functional groups with polar substances from solution [74]. However, the current research on AC decolorization mainly focuses on the effect of AC dosage, decolorization temperature and time on the decolorization rate and retention rate, the effect of source of raw materials, activation methods and activation conditions on the structure and adsorption performance of AC should be the focus of following research [20, 75]. A tailor-made AC for AG may be a strategy to solve the current high decolorization loss rate and poor decolorization effect.

Impurity removal of AG by solvent method

Separation of AG by solvent method is easy to operate and suitable for industrial production and is currently the most commonly used. The solubility of AG in various solvents is critical to select the appropriate solvent and design the best production process. Chen et al. systematically investigated the solubility of AG in water and commonly organic solvents in the temperature range of 288.2–323.2 K (Figure 4) [76]. In all selected solvents, AG had the highest solubility in methanol and the lowest in water, with a difference of several hundred times. Furthermore, the solubility of AG increased with increasing temperature in all selected solvents and temperature ranges. Therefore, using the difference in solubility between AG and other impurities for purification is the most common operation, and its operation is mainly divided into modifying the temperature and (or) polarity of the mixtures. For example, in the patent of Shi et al., the decolorized solution is concentrated to a relative density of 1.3–1.4, firstly [29]. Then an appropriate amount of 90–95% ethanol was added to the concentrate, heated, stirred and filtered, and the filtrate is maintained at 70 °C and purified water was added to make the final ethanol concentration to be 30%–40%. Afterwards, chilled and filtered to attain the crude AG. In this process, impurities were removed by changing the temperature and the polarity of the concentrate. Rajani et al. reported a rapid method for the isolation of AG from *A. paniculata* leaves by using cold maceration (dichloromethane and methanol 1:1) and Soxhlet extraction (methanol and 95% ethanol, respectively) [77]. Vacuum removal of solvents and the dark green crystals obtained were washed several times with toluene until most of the pigment was removed from the crude crystals. The remaining crystalline material was re-crystallized in hot methanol several times a colorless crystal with a constant melting point of 230–231 °C were obtained.

Crystallization

Crystallization refers to the separation of solid material in the form of crystals from solution mixtures and is often used as the last step to obtain pure monomer compound [78]. The separation by crystallization is based on the different solubility of target compounds and impurities and crystallization operation can separate the crystals with ordered molecular or ion arrangement directly [79]. Therefore, even if the active compound and the impurity solubility are not much different, they are also separated by the difference of the crystal lattice [80].

The crystallization process includes three steps: the formation of supersaturation, nucleation and crystal growth. Supersaturation is that the concentration of the solute has exceeded the solubility, but the solute still does not precipitate. This phenomenon results from that although crystallization is a spontaneous process accompanied by a drop in Gibbs free energy, the process needs to overcome a nucleation energy barrier [81]. The solute will crystallize only when the concentration of the solution over critical value, and the curve formed by critical values in different states is called the supersolubility curve (Figure 5) [82]. For a specific system, the solubility curve is unique, but the supersolubility curve is uncertain and it is affected by many external factors, such as the cooling rate, stirring intensity, whether to add seeds or anti-solvent, etc. Therefore, the width of the metastable zone (MSZW) also changes [83, 84].

Supersaturation is the only driving force for crystallization, which controls nucleation and growth [85]. Therefore, the point of crystallization is to increase supersaturation in a certain way. For example, cooling crystallization by manipulating the temperature of the crystallizer to change the saturation concentration of the crystalline material. Anti-solvent crystallization is supersaturated by adding anti-solvents with low affinity for the solute but high affinity for the solvent, thereby making the crystal easy to precipitate. As mentioned before, AG is a substance whose solubility increases significantly with increasing temperature [76]. Accordingly, the cooling crystallization is widely applied to separate AG from other substances. During the cooling crystallization of AG, the process conditions of such as the initial temperature of the crystallization solution, the type of solvent, and the solvent ratio are usually roughly determined by the yield and purity, which results in often multiple crystallizations to obtain a satisfactory product. Moreover, crystals with a particle size distribution (PSD) are desired that facilitates efficient downstream processing such as filtration, washing and drying [86]. Research shows that AG is a fast nucleation-fast crystal growth molecule [87]. Therefore, crystallization processes generally operate in the metastable zone to avoid spontaneous explosion nucleation occurs [79]. If the state of the solution is controlled in Metastable zone, only minimal nuclei can be generated, and crystal growth will dominate. At this time, a crystal product with a large and uniform PSD can be obtained. On the contrary, if the solution state is located far from the solubility curve or even in the unstable region, explosive nucleation occurs, so the crystal grains in the obtained product must

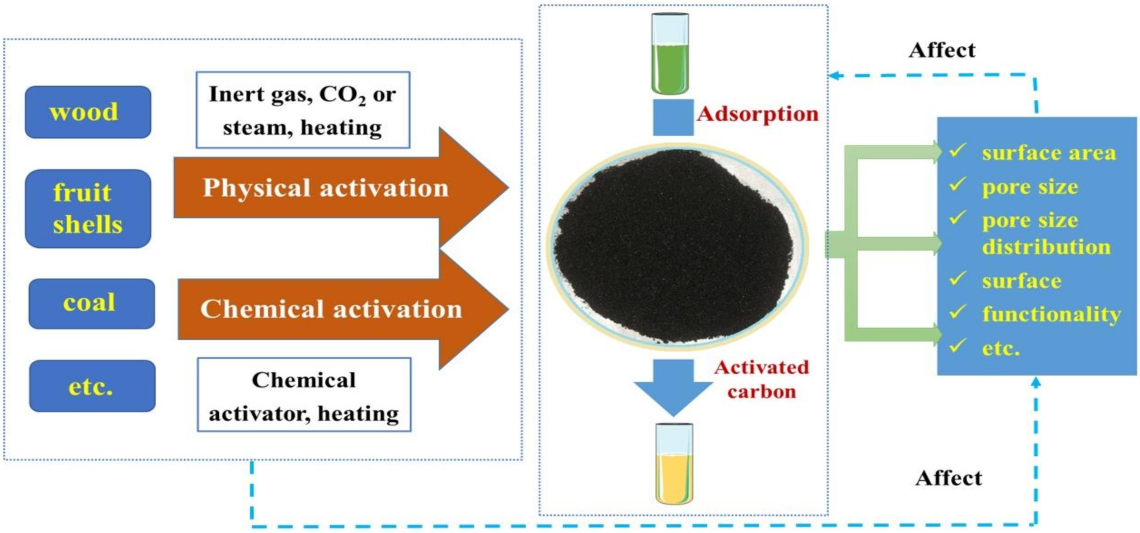


Figure 3 The source of raw materials, activation methods and activation conditions affect the structure of activated carbon, and the structure of activated carbon affects the decolorization.

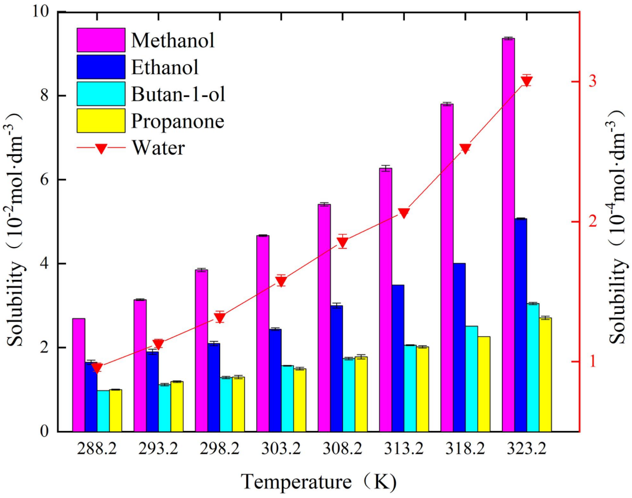


Figure 4 Solubility of andrographolide in water and commonly organic solvents in the range of 288.2–323.2 K.

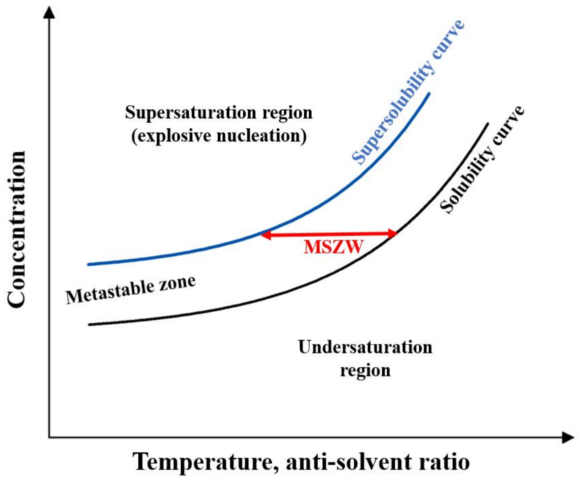


Figure 5 Solution concentration profile during cooling crystallization and anti-solvent crystallization.

be very small [88]. In general, it is elusive to regulate the crystallization quality by controlling crystallization process due to the lack of research on crystallization kinetics, crystallization thermodynamics, the optimal cooling curve and the MSZW under different conditions.

With the pursuit of nontoxic property in industrial, some green solvents and gases are progressively applied in the pharmaceutical industry. Trisilanun and co-workers used CO_2 as the anti-solvent to precipitate AG from four organic solvent (methanol, ethanol, acetone, and N, N-dimethylformamide) extracts of *A. paniculata*, and the highest recovery rate of 96.88% was obtained in the acetone extract at 35 ℃ and the flow rate of CO_2 of 5 mL/min [89]. SC-CO_2 was also used as anti-solvent to precipitate AG from ethanol extracts and at pressure 10 Mpa, temperature 318 K, SC-CO_2 flow rate 1.5g/ min, the highest precipitation rate is 99.94% [90]. In the anti-solvent crystallization, different operating conditions also affect the purity, yield and PSD of the product. At high pressure, CO_2 is denser and its ability to dissolve is increased which results in the formation of a high supersaturation and the acquisition of crystal grains of small size. Furthermore, with the increase of pressure, the morphology of AG particles changed from flakes to columns [91, 92]. As the temperature raised, the density of SC-CO_2 declined, which leads to an increase in the diffusivity of SC-CO_2, resulting in an increase in mass transfer rate and a decrease in particle size. As the CO_2 flow rate increases, the kinetic energy of the dense gas atomization and the mass transfer between CO_2 and solution droplets also increase, resulting in smaller droplets, higher supersaturation and smaller particles [90]. Furthermore, the addition of anti-solvent will lead to local extreme supersaturation and possibly lead to the precipitation of some impurities. It was found that the of gas-precipitation was not a dry powder, but a viscous substance. This is because other substances present in *A. paniculata* were precipitated at the same time as AG [89]. Consequently, it is necessary to carefully consider the addition rate and the amount of anti-solvent and its impacts on PSD, purity and yield.

Membrane separation

Membrane separation has always been a potent tool for separation in laboratory and pharmaceutical industries. Chen et al. decolorized the extract of *A. paniculata* by AC, and the supernatant obtained by centrifugation was filtered with an inorganic ceramic microfiltration membrane at a temperature range of 20–70 ℃ and a pressure range of 0.2–0.7 Mpa, next filtered again using an ultrafiltration membrane, and finally concentrated with a reverse osmosis membrane until the AG content in the solution reaches more than 30%, a product with a purity of 98.98% can be obtained by vacuum drying the above-mentioned concentrated solution [93]. Preparation of AG by membrane separation has high purity and no organic solvent used, which has great industrial application prospect. But the high price of instruments and filter media will undoubtedly enhance the production cost, and the occurrence of clogging is a challenge in industrialization.

Solid-phase extraction

Molecularly imprinted polymers (MIPs) are polymeric materials which are often loaded into solid-phase extraction (SPE) columns as adsorbent for separation and enrichment of active ingredients from herbal medicines [94]. Yin et al. prepared a special MIPs with AG, acrylamide and ethylene glycol dimethacrylate as templates, functional monomers and cross-linkers, respectively [95]. The MIPs displays high selectivity and good affinity for AG with a column capacity of 11.9 ± 0.6 μmol/g, 2–3 times that of C18-SPE and non-imprinted polymer SPE.

Different functional monomers have great influence on the adsorption performance of MIPs. The stronger the interaction between template molecules and functional monomer molecules, the higher the adsorption performance and the selectivity of the recognition site of the prepared MIPs [96]. Among α-methacrylic acid, acrylamide and 1-ethylene imidazole, the adsorption performance of MIPs is the weakest when α-methacrylic acid is the functional monomer because of the weakest interaction betweenα-methacrylic acid and AG, while the adsorption properties of MIPs with 1-vinylimidazole and acrylamide as functional monomers were similar because of their similar interactions with AG [96]. Krishnan et al. further optimized the ratio of template and functional monomer using computational quantum chemical approach by software, and the best results is 149.59 μg/g when the ratio of AG, α-methacrylic acid and ethylene glycol dimethacrylate was 1:3:20 [97].

Adsorption chromatography

Column chromatography is a common technique for the separation of highly complex plant samples [98]. The crude extract of AG was obtained by reflux extraction technique and further purified by silica gel filled column [42]. When using 20–100% (v/v) ethanol aqueous solution as the eluent, the difference of other concentrations of ethanol is the consumption of elution solvent except that 20% ethanol has poor elution effect. The minimum volume of 40% ethanol elution system is 950 mL. It may be the polarity of AG is also close to the 40% ethanol elution system. The scavenging process increased the AG content from 0.1% in the crude extract to 1.9% w/w in the fractionated extract [42]. *A. paniculata* of 8.5 kg were exhaustively extracted with 95% ethanol at room temperature by Wen et al [99]. 980 g residue was obtained by evaporating the filtrate under vacuum, a portion of 900 g was suspended in water (2.0 L) and partitioned with ethyl acetate and n-butanol to afford 280 g and 140 g, respectively. Afterwards, ethyl acetate extract was subjected to column chromatography eluted successively with dichloromethane/methanol from 70:1 to 0:1 to give fractions E1–E9, and 36 g of AG crystal was obtained from E2 fraction [99].

At present, there are few studies on purification of AG by various resins, which may be related to the high solvent consumption, complex elution procedure and irreversible adsorption of large polar compounds of column separation.

Partition chromatography

Partition chromatography follows the principle of liquid-liquid extraction and separates target compounds based on the relative solubility in immiscible liquids [100]. However, the original countercurrent chromatography (CCC) was rarely used because of its disadvantages such as poor fixation, long separation time and high labor intensity [101]. Extrusion-eluting CCC and High-speed countercurrent chromatography (HSCCC) significantly ameliorate these shortcomings and performed well in the separation of AG.

Hexane-ethyl acetate-ethanol-water (5:5:4:6, v/v) was used as solvent in an on-demand solvent preparation mode by Wu et al. who combined regular chromatographic elution with stationary-phase extrusion to extend the hydrophobicity window of CCC, and after preparation, sweep elution and extrusion, 9.5 mg of AG with a purity of 97.8% was obtained from a 234 mg injection sample [68]. Zhang et al. obtained 1.02 mg of AG from 2 g crude powder of *A. paniculata* in one step [102]. First, the crude powder was ultrasonic extracted with 20 mL methanol/water (2.5:5) for 1 h and the extracts was pumped into Sephadex LH-20 column directly to cut the non-target fractions followed by the second-dimensional HSCCC with n-hexane/ethyl acetate/methanol/water (2.5:5:2.5:5, v/v) as flow phase at a speed of 800 r/min and a flow rate of 2 mL/min, hyphenated by a six-port valve equipped at the post-end of Sephadex LH-20 column for the final purification. The advantage of HSCCC is that both stationary phases and mobile phases are liquids, so it eliminates the irreversible adsorption loss of target compounds on the solid support matrix used in conventional chromatography [103]. However, the separation of AG by HSCCC has only been found in experimental studies, and its practical application needs further study.

Conclusion

Changing extraction techniques can improve extraction efficiency, but the optimization of extraction parameters is the key to obtaining good extraction quality. At present, most of the extraction researches on AG unilaterally pursue the highest extraction rate by changing extraction techniques and parameters. It is usually meaningless to blindly pursue the extraction rate without considering the difficulty and cost of subsequent separation. Therefore, the influence of extraction conditions on extraction selectivity should be fully considered. The factors affecting the extraction selectivity, such as solvent composition, temperature and pressure should be the focus of future research. In terms of extraction techniques, maceration, reflux extraction and Soxhlet extraction are simple to operate and no strict requirements for equipment, but they often face challenges such as low extraction efficiency and poor extraction selectivity. UAE, MAE and SCFE have high extraction efficiency and great application potential.

Decolorization of *A. paniculata* extract using AC has been accepted as a standard extraction protocol. The use of AC decolorization is often faced with poor decolorization effect and high decolorization loss rate. This is because the raw material source of AC, activation methods and conditions affect the structure of AC, AC structure and then affect the decolorization effect. A tailor-made AC for AG may be a strategy to solve the current high decolorization loss rate and poor decolorization effect. Cooling crystallization and anti-solvent crystallization are often used as the final step to obtain AG with high-purity. At present, it has been possible to achieve higher crystallization yield and purity by optimizing the pre-crystallization operations and crystallization conditions. But, it is still difficult to control the crystallization quality, which requires in-depth research on the crystallization kinetics, crystallization thermodynamics, optimal cooling curve and MSZW of AG under different conditions. Some advanced separation technologies, such as Membrane separation, SPE and HSCCC have great application potential with the characteristics of fast processing, high yield and high purity, but also face the high cost of production equipment and industrialization difficulties. With the continuous development of separation technology and related materials, the product quality of AG and production efficiency must be significantly improved.

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