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A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation

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Abstract

Medicinal plants are gaining much interest recently because their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. The study on medicinal plants started with extraction procedures that play a critical role to the extraction outcomes (e.g. yield and phytochemicals content) and also to the consequent assays performed. A wide range of technologies with different methods of extraction is available nowadays. Hence, this review aim to describe and compare the most commonly used methods based on their principle, strength and limitation to help evaluating the suitability and economic feasibility of the methods.

Keywords: Methods; Maceration; Soxhlet extraction; Microwave-assisted extraction, Ultrasound-assisted extraction, Accelerated solvent extraction, Supercritical-fluid extraction, Medicinal plants

Introduction

Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention [1]. Modern Mediterranean and DASH (Dietary Approaches to Stop Hypertension) incorporate a phytochemicals rich diet from fruit and vegetable sources as the plant based diet has shown to extend life span in Okinawan people, that has the highest number of centenarians [2,3]. Interest in utilizing natural sources in the development and formulation of skin products, as an alternative to conventional drugs and synthetic products, contribute to increase interest in research and industrial application of medicinal plants [4]. High content of phenolic and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of age-related disease, particularly cause by oxidative stress. With regards to the beneficial phytochemicals in medicinal plants and the shift towards natural products in pharmaceuticals and cosmeceuticals industry, the research on medicinal plants particularly are as important as the research on conventional drugs.

The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Traditional methods such as maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Significance advances have been made in the processing of medicinal plants such as the modern extraction methods; microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), in which these advances are aimed to increase yield at lower cost. Moreover, modifications on the methods are continuously developed. With such variety of methods present, selection of proper extraction method needs meticulous evaluation. This review describes the principle, strength and limitation of the commonly used methods with examples in recent years to help in the selection of proper methods.

Pre-extraction preparation of plant samples

The initial stage in studying medicinal plants is the preparation of plant samples to preserve the biomolecules in the plants prior to extraction. Plants samples such as leaves, barks, roots, fruits and flowers can be extracted from fresh or dried plants material. Other pre-preparation of plant materials such as grinding and drying also influences the preservation of phytochemicals in the final extracts.

Fresh *vs.* dried samples: Both fresh and dried sample is used in medicinal plants studies. In most cases, dried sample is preferred considering the time needed for experimental design. Sulaiman et al limit the interval between harvest and experimental work at the maximum period of 3 hours to maintain freshness of samples, as fresh samples are fragile and tend to deteriorate faster than dried samples. Comparison between fresh and dried *Moringa oliefera* leaves showed no significant effect in total phenolics but with higher flavonoids content in dried sample [5].

Grinded *vs.* powdered samples: Lowering particle size increases surface contact between samples and extraction solvents. Grinding resulted in coarse smaller samples; meanwhile, powdered samples have a more homogenized and smaller particle, leading to better surface contact with extraction solvents. This particular pre-preparation is important, as for efficient extraction to occur, the solvent must make contact with the target analytes and particle size smaller than 0.5 mm is ideal for efficient extraction [6]. This particular size of particle was mentioned in Sulaiman et al, preparing vegetable samples that was ground to 400 µm (0.4 mm) in size. Conventional mortar and pestle or electric blenders and mills are commonly used to reduce particle size of sample. Investigation of nanoparticles powder of *Centella*

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asiatica produced by Planetary Ball Mill (PBM) showed 82.09% higher yield compared to micro powder using maceration technique in 90% methanol for 3 days [7]. Particle size was a major factor when using enzyme-assisted extraction. Use of pectinolytic and cell wall polysaccharide degrading enzyme in sample preparation was influenced majorly by the particle size as smaller particle enhances enzyme action.

Air-drying, microwave-drying, oven-drying and freeze-drying (lyophilisation) of plants samples: Air-drying usually takes from 3-7 days to months and up to a year depending on the types of samples dried (eg. leaves or seed). Plant samples, usually plants leaves with stem were tied together and hang to expose the plant to air at ambient temperature. This drying method does not force dried plant materials using high temperature; hence, heat-labile compounds is preserved. However, air-drying take longer time in comparison to microwave-drying and freeze drying and may be subjected to contamination at unstable temperature condition.

Microwave-drying uses electromagnetic radiation that possesses both electric and magnetic fields. The electric field causes simultaneous heating through dipolar rotation; alignment on the electric field of the molecules possessing a permanent or induced dipole moment (e.g. solvents or samples), and ionic induction, that produce oscillation of the molecules [8]. Oscillation causes collisions between molecules and resulted in fast heating of the samples simultaneously. This method can shorten drying time but sometimes causes degradation of phytochemicals.

Oven-drying is another pre-extraction method that uses thermal energy to remove moisture from the samples. This sample preparation is considered as one of the easiest and rapid thermal processing that can preserved phytochemicals. Oven-drying at 44.5°C for 4 hours using 80% methanol resulted in highest antioxidants activities in *Cosmos caudatus* extracts and similar result were observed in optimized 80% methanol extracts at 44.12°C for 4.05 hours [9]. Shorter period of extraction time was obtained using this method. However, effect of drying on *Orthosiphon stamineus* showed no significant effect on the antioxidant activity but the bioactive phytochemicals; such as sinensetin and rosmarinic acid content were affected by the oven- and sunlight-drying, suggesting the sensitivity of the compounds to temperature [10].

Freeze-drying is a method base on the principle of sublimation. Sublimation is a process when a solid is changed into gas phase without entering the liquid phase. Sample is frozen at -80°C to -20°C prior to lyophilisation to solidify any liquid (eg. solvent, moisture) in the samples. After an overnight (12 h) freezing, sample is immediately lyophilized to avoid the frozen liquid in the sample from melting. Mouth of the test tube or any container holding the sample is wrapped with needle-poked-parafilm to avoid loss of sample during the process. Most of the time, sample was lost by splattering out into the freeze-flask (Figure 1a and 1b).Freeze-drying yielded to higher level of phenolic contents compared to air-dying as most of the phytochemicals are preserved using this method. However, freeze-drying is a complex and expensive methods of drying compared to regular air drying and microwave-drying. Thus, the use is restricted to delicate, heat-sensitive materials of high value.

Extraction methods

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures [11]. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue).The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but

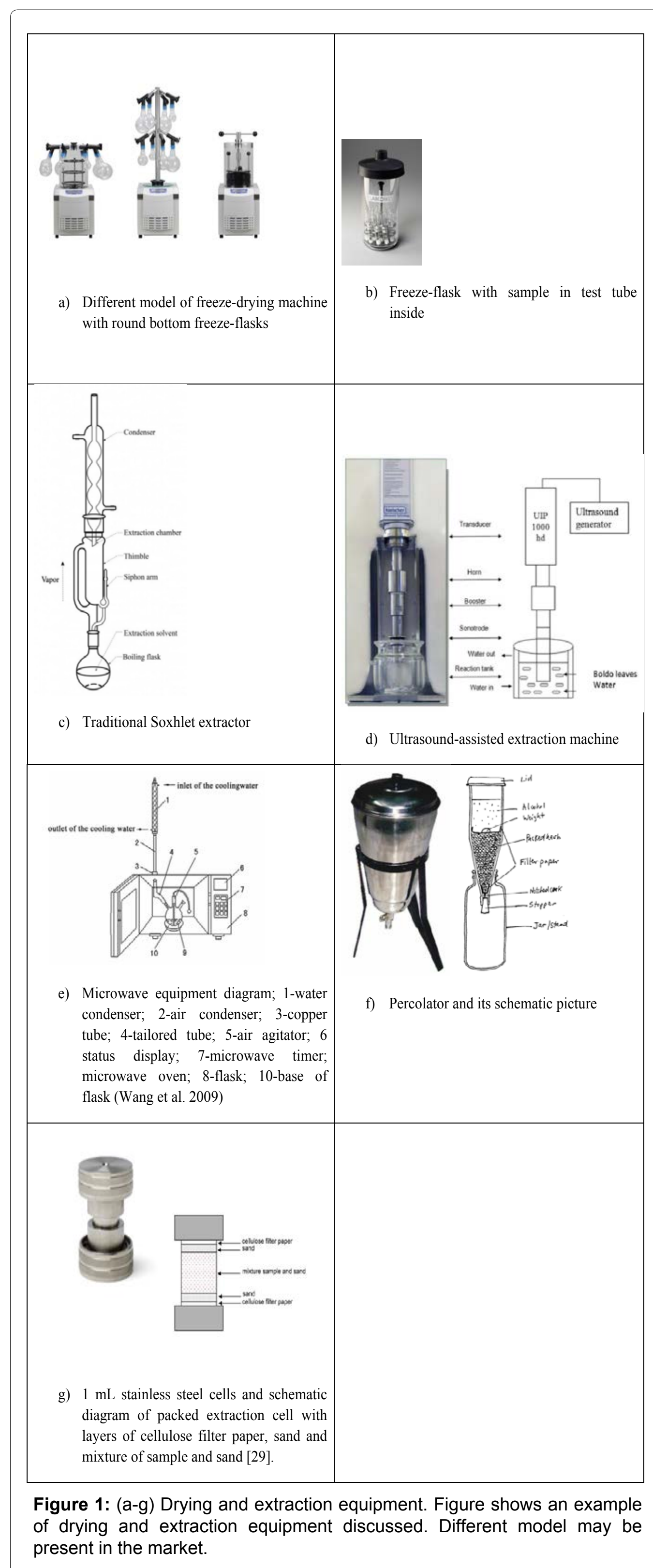

Figure 1: (a-g) Drying and extraction equipment. Figure shows an example of drying and extraction equipment discussed. Different model may be present in the market.

some need further processing. Several of the commonly used extraction methods are discussed below:

Maceration, infusion, percolation and decoction: Maceration is a technique use in wine making and has been adopted and widely used in medicinal plants research. Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation [11]. The processed intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from the samples. Infusion and decoction uses the same principle as maceration; both are soaked in cold or boiled water. However, the maceration period for infusion is shorter and the sample is boiled in specified volume of water (eg. 1:4 or 1:16) for a defined time for decoction [11]. Decoction is only suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) and usually resulted in more oil-soluble compounds compared to maceration and infusion. Unique equipment called percolator (Figure 1c and 1d) is used in percolation, another method that shares similar fundamental principle. Dried powdered samples are packed in the percolator, added with boiling water and macerated for 2 hours. The percolation process is usually done at moderate rate (e.g. 6 drops /min) until the extraction is completed before evaporation to get a concentrated extracts [12].

Strength and limitation: This technique is the easiest and simple method. However, organic waste come into an issue as large volume of solvents is used and proper management of the waste is needed. Alteration in temperature and choice of solvents enhance the extraction process, reduce the volume needed for extraction and can be introduced in the maceration technique, when such alteration is not objectionable. Boiling *Centella asiatica* at 90°C showed to increase phenolics content and antioxidant activities, but jeopardized the pH of the extracts with increase extraction time [13]. In this method, solvents used in the soaking process play a critical role.

Studies: Extraction of *Psidium guajava* L. leaves using ethanolic and hydroalcohol extracts (4:1 v/v) resulted in highest extraction yield with maximum presence of phytoconstituents (alkaloids, saponins, carbohydrates, tannins and flavonoids) compared to the other solvents such as petroleum ether, chloroform and water [14]. Non-polar solvents such as petroleum ether and chloroform showed no active compounds preserved and very little tannins presence respectively in the extracts. Water showed to have similar efficiency as ethanol except no trace of alkaloids was presence in water extracts [14]. Polar solvents are more effective in the extraction of bioactive molecules from *Psidium guajava*. Methanol extracts of *Garnicia atriviridis* (1:10 w/v) exhibited higher antioxidant activities compared to the aqueous (1:10 w/v) extracts, but the aqueous showed higher anti-hyperlipidemic activity [15]. Effect of different solvents using maceration at 1:10 w/v sample to solvent ratio for 1 hour showed 70% acetone as efficient solvent for *Portucala oleracea* based on total phenolics, and 70% methanol as efficient solvent for flavonoids in *Cosmos caudatus* [16]. In the case of *Moringa oliefera* , maceration with 70% ethanol powdered dried samples at 1:40 w/v exhibited highest phenolics and flavonoids content compared to Soxhlet extraction and percolation using similar solvent [5].

Soxhlet extraction or hot continuous extraction: In this method, finely ground sample is placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which is place, is in thimble chamber of the Soxhlet apparatus (Figure 1c). Extraction solvents is heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm (Figure 1c), the liquid contents emptied into the bottom flask again and the process is continued.

Strength and limitation: This method requires a smaller quantity of solvent compared to maceration [11]. However, the Soxhlet extraction comes with disadvantage such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Solvents used in the extraction system need to be of high-purity that might add to cost. This procedure is considered not environmental friendly and may contribute to pollution problem compared to advance extraction method such as supercritical fluid extraction (SFE) [17]. The ideal sample for Soxhlet extraction is also limited to a dry and finely divided solid [6] and many factors such as temperature, solvent-sample ratio and agitation speed need to be considered for this method [18].

Studies: Extraction of *Azadirachta indica* (Neem) leaf powder in methanol (~1:5 w:v) shows numerous phytochemicals were retrieved using Soxhlet extraction; mostly nonpolar compounds [19]. Evaluation of Soxhlet extraction for *Moringa oliefera* leaves resulted in lower yield, phenolics and flavonoids content [5]. Optimization of *Centella asiatica* extraction using Soxhlet extraction showed to achieve optimum metal chelating activities at the temperature of 25°C, sample-solvent ratio of 1:45, at 200 rpm agitation speed and for 1.5 hour [18]. Soxhlet extraction has been used to remove lypodial materials from powdered *Clitorea ternate* flowers using petroleum ether at 60°-80°C, resulted in 2.2% yield w/w [20]. Further extraction of the marc with ethanol ascertained the presence of alkaloids and saponins [20], but the major component of *Clitorea ternate* flowers, the anthocyanin was absence, suggesting oxidation and degradation had occurred.

Microwave assisted extraction (MAE): MAE utilizes microwave energy to facilitate partition of analytes from the sample matrix into the solvent [21]. Microwave radiation interacts with dipoles of polar and polarizable materials (e.g. solvents and sample) causes heating near the surface of the materials and heat is transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic disrupts hydrogen bonding; enhancing the migration of dissolved ions and promotes solvent penetration into the matrix [8]. In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only [11]. MAE can be considered as selective methods that favour polar molecules and solvents with high dielectric constant (Table 1).

Strength and limitation: This technique reduced extraction time and solvent volume as compared to conventional method (maceration & Soxhlet extraction). Improved recoveries of analytes and reproducibility were observed in MAE method but with caution of using proper conditions to avoid thermal degradation [8]. However, this method is limited to small-molecule phenolic compounds such as

Solvent	Dielectric constant (20°C)
Hexane	1.89
Toluene	2.4
Dichloromethane	8.9
Acetone	20.7
Ethanol	24.3
Methanol	32.6
Water	78.5

Table 1: Dielectric constant of some commonly used solvents, adapted from Kauffman and Christen, 2012.

phenolic acids (gallic acid and ellagic acid), quacertin, isoflavin and trans-resveratrol because these molecules were stable under microwave heating conditions up to 100°C for 20 minutes. Additional cycles of MAE (e.g. from 2 × 10 s to 3 × 10 s) resulted in drastic decrease in the yield of phenolics and flavanones, mainly caused by the oxidation of compounds [21]. Tannins and anthocyanins may not be suitable for MAE as they were potentially subjected to degradation at high temperature.

Studies: Evaluation on MAE as new method to extract triterpene from *Centella asiatica* showed an increase in yield, twice of Soxhlet extraction with extraction condition; absolute ethanol as solvent, at 75°C and irradiation power at 600 W for four cycles [22]. Enzymolysis (e.g. cellulase) has been combined with MAE to improved extraction, and optimum condition of sample-solvent ratio at 1:36, temperature of 45°C for 30 minutes enzyme pre-treatment, with irradiation at 650 W for 110 s resulted in 27.10% yield [23]. However, the effect on the phytochemical degradation by the MAE was not evaluated as observed by Trusheva et al. with additional MAE cycle. MAE with 100 W for 20 minute on *Dioscorea hispida* yielded highest extraction using 85% ethanol at 1:12.5 sample-solvent ratios [24]. Decreased in yield was observed when the optimum value of each parameters on *Dioscorea hispida* MAE is exceeded. Extraction time and irradiation power is as critical as solvents type in MAE. Effect of 119.7 W and 39.9 W reached their optimum yield at 5 minure and 17.5 minute respectively in *Andrographis paniculata* extraction [25].

Ultrasound-assisted extraction (UAE) or sonication extraction

UAE involves the use of ultrasound ranging from 20 kHz to 2000 kHz [11]. The mechanic effect of acoustic cavitation from the ultrasound increases the surface contact between solvents and samples and permeability of cell walls. Physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall; facilitating release of compounds and enhancing mass transport of the solvents into the plant cells [26]. The procedure is simple and relatively low cost technology that can be used in both small and larger scale of phytochemical extraction.

Strength and limitation: The benefits of UAE is mainly due reduction in extraction time and solvent consumption. However, use of ultrasound energy more than 20 kHz may have an effect on the active phytochemicals through the formation of free radicals [8,11].

Studies: UAE was shown to be the most effective methods in propolis extraction based on high yield, extraction time (10-30 min) and high selectivity [21]. UAE was employed in extraction of thermo-labile compounds, such as anthocyanin from flower parts, to reduce extraction time and avoid exposure to high temperature [27]. UAE of *Withania somnifera* by water solvent at 15 minute showed maximum yield, 11.85% compared to ethanol and water-ethanol at different 5, 15 and 20 minute extraction period [26].Higher efficacy on phenolics was observed in *Cratoxylum formosum* extraction by ultrasound at 45 kHz, 50.33% ethanol v/v, at 65°C for 15 minute [28]. However, formation of free radicals at irradiation higher than 20 kHz might need to be considered.

Other extraction methods

Other methods such as accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) are also being used in the extraction of plant materials. These methods are less popular due to high cost despite the efficiency of the methods.

Accelerated solvent extraction (ASE)

ASE is an efficient form of liquid solvent extraction compared to maceration and Soxhlet extraction as the method use minimal amount of solvent. Sample is packed with inert material such as sand in the stainless steel extraction cell (Figure 1e-1g) to prevent sample from aggregating and block the system tubing [6,29]. Packed ASE cell includes layers of sand-sample mixture in between cellulose filter paper and sand layers (Figure 1g). This automated extraction technology is able to control temperature and pressure for each individual samples and requires less than an hour for extraction. Similar to other solvent technique, ASE also critically depend on the solvent types. Cyclohexane-acetone solution at the ratio of 6:4 v/v with 5 minute heating (50°C) showed to yield highest bixin from *Bixa orellana* with 68.16% purity [29]. High recoveries (~94%) of flavonoids from *Rheum palmatun* were observed using 80% aqueous methanol by ASE, suggesting the suitability of this method for quality control evaluation [30].

Supercritical fluid extraction (SFE)

Supercritical fluid (SF) or also called as dense-gas is a substance that shares the physical properties of both gas and liquid at its critical point. Factors such as temperature and pressure are the determinants that push a substance into its critical region. SF behaves more like a gas but have the solvating characteristic of a liquid. An example of SF is CO_2 that become SF at above 31.1°C and 7380 kPa. Interest in Supercritical-CO_2 (SC-CO_2) extraction due to excellent solvent for nonpolar analytes and CO_2 is readily available at low cost and has low toxicity. Even though SC-CO_2 has poor solubility for polar compounds, modification such as adding small amount of ethanol and methanol enable it to extracts polar compounds. SC-CO_2 also produces analytes at concentrate form as CO_2 vaporizes at ambient temperature. SC-solvents strength can be easily altered by changing the temperature, pressure or by adding modifiers that lead to reduce extraction time. Optimization of SC-CO_2 on *Wadelia calendulacea* achieved its optimum yield at 25 MPa, 25 °C temperature, 10% modifier concentration and 90 minute extraction time [31]. A major drawback of this method is the initial cost of the equipment is very high [17].

Discussion

All the methods that employ solvents in the procedures (maceration, MAE, UAE and ASE) are critically influenced by the solvents types. However, no significant effect caused by the solvent volume used using three methods (maceration, MAE and UAE) on the biologically active compounds in the poplar type propolis at ratio (1:10 w:v), suggesting use of solvents at greater ratio is unnecessary [21]. However, the finding is limited to assessment of phenolic, flavonoid content and total yield as comparison.

Maceration have been suggested by Vongsak et al as more applicable, convenient and less costly method for small and medium enterprises (SMEs) compared to other modern extraction methods. However, chemical waste is a major issue in maceration technique as compared to MAE and UAE, which is known as the "Green method" [26]. Although, all these extraction methods resulted in crude extracts containing a mixture of metabolites, the efficacy of those crude extracts using nano-encapsulated processing in *Centella asiatica* showed to have similar efficacy as those purified [32]. This particular fact suggests that further isolation and purification of extracts, which is rather complex and time consuming is not necessary if proper preparation and extraction are done.

Suitable conditions for each extraction methods are also important.

Certain factors such as temperature and light need to be evaluated to extracts thermo-labile compounds. Slightly acidic solvent (0.1% HCl-methanol v/v) was used to extract anthocyanin from the red and blue flowers, pointed the effect of pH in extraction procedures [33]. Hydrochloric acid in ethanol system was found to be more efficient than acetic acid in extraction of anthocyanin [34]. Among parameters such as solvent types, solvent strength, extraction time, agitation speed, sample-solvent ratio and temperature investigated using factorial design experiment; solvent strength, which is 70% ethanol, is the most influential factors in *Curcuma longa* extraction [35]. Similar observation of 70% ethanol as the most influential parameters was seen in triterpenoids extraction from *Jatropha curcas* leaves [36] and phenolic extractions from *Moringa oliefera* [5].

Among those optimization studies, the most influential parameter in almost all method is solvent types and strength. However, solvent-sample ratio is reported to have no significance effect, suggesting unnecessary large volume of solvents can be avoided. Each optimized method is unique to the plants. All the influential factors (temperature, solvents, agitation speed and etc.) might have the ability to enhance extraction, but without proper judgment may cause degradation of compounds. Thus, considering methods that has least influential factors might be a wise selection step in choosing suitable methods. However, in the case of purity is a concern, advanced extraction technology such as ASE should be considered.

Conclusion

All stages of extractions, from the pre-extraction and extraction are equally important in the study of medicinal plants. The sample preparation such as grinding and drying affected the efficiency and phytochemical constituents of the final extractions; that eventually have an effect on the final extracts. It can be concluded that, no universal extraction methods is the ideal method and each extraction procedures is unique to the plants. Previously optimized methods can be used to lead in the selection of suitable methods. However, evaluation and selection of pre-extraction preparation and extraction methods are depending on the study objectives, samples, and target compounds.

Conflict of Interest

None.

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