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Mini Review

A Review on Extraction of Bioactive Compounds from *Moringa oleifera* Leaves: Their Principle, Advantages, and Disadvantages

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ABSTRACT

Moringa oleifera, belongs to Moringaceae family, is an indigenous plant and native to North India region. It has been used for centuries as traditional medicine and nutritional supplement. *Moringa olefera* leaves contain high phenolics and flavonoids compounds as major constituents such as kaempferol, quercetin, caffeoylquinic acid, coumaroylquinic acid, and feruloylquinic acid. The extraction techniques play a critical role in the extraction outcome such as crude extracts yield, type and quantity of compound extracted. To date, there is a wide range of technologies for crude plant extraction such as ultrasound-assisted extraction, microwave-assisted extraction Soxhlet extraction, and dipping (maceration) technique. These extraction techniques employ various types of solvent which could enhance the efficiency of extraction and quality of compound extracted. Hence, this review aims to describe and compare the conventional and novel extraction techniques methods of *Moringa oleifera* leaves based on the total phenolics content, flavonoids content, and antioxidant activity. The difference based on the extraction process principle, advantages, and disadvantages were further evaluated to show the suitability, environmentally friendly, the economic feasibility of the various extraction methods. From this review, Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) has minimized the processing time which is useful to extract thermolabile compounds, such as phenol compounds. In conclusion, novel extraction techniques could be effectively enhancing the total phenolic compound, flavonoid content, and antioxidant activities of crude extracts, which provides a theoretical basis for upgrading to a large-scale application in the future.

Keywords: *Moringa oleifera*; Extraction; Ultrasound-assisted extraction; Antioxidant

INTRODUCTION

Moringa oleifera (Moringaceae) has been used as traditional medicine in many tropical countries such as Malaysia, Cambodia, and the Philippines [1,2]. Every part of the *Moringa oleifera* plant has it specific medicinal benefits. It is versatile in terms of its usage in combating malnutrition and medicinal properties, for instance, anti- microbial and anti-inflammatory properties. Hence it has been commercialized as a nutritional food and medicinal remedy. The presence of phenolic compounds (flavonoids and phenolic acids) in the leaves makes the plant as potential source of natural antioxidant and anti-diabetic properties, as well as anticancer agent [3,4].

The study of any medicinal starts with the pre-extraction and extraction procedures is a major step in the processing of the bioactive constituents from the plant materials. Solvent extraction is the process of removing bioactive constituents from the plant material using an appropriate solvent. There are several factors that can affect solid-liquid extraction, such as solvent type, temperature, and agitation [5]. Temperature can increase the solubility of the bioactive component and lower viscosity of the solvents which were used in the extraction method. Previously, *Moringa oleifera* crude has been extracted using few techniques such as maceration, Soxhlet, microwave-assisted extraction, and ultrasound-assisted extraction. Traditional methods such as maceration and Soxhlet extraction are commonly used in the

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small research setting; however, significant advances have been made in modern extraction methods which are microwave-assisted extraction and ultrasound-assisted extraction. Careful evaluation needs to be considered, especially the selection of proper extraction method [6]. This review describes the principle, advantages, and disadvantages of extraction technique which help in the selection of proper technique for easy, feasible, and fast extraction of bioactive compounds from *Moringa oleifera* leaves.

LITERATURE REVIEW

Pre-extraction preparation of plant sample

Preparation of sample before extraction is important to preserve the biomolecules integrity in the plant leaves. The preparation is including washing, drying, grinding, and sieving which influences the preservation of phytochemicals in the final extracts and it is depending on the extraction procedures and the purpose of the extraction. Fresh samples are fragile and tend to deteriorate faster than dried samples. However, a comparison between fresh and dried *Moringa oleifera* leaves showed no significant effect in total phenolics contents detected, however, high flavonoids content was detected in the dried sample [2]. Primarily, the *Moringa oleifera* leaves were cleaned and dried under the shade. The dried sample was ground and sieved (20 mesh) to become powder. The powdered form is kept in a sealed container such as a desiccator to prevent moisture trapped in the samples until it is used for the extraction. The presence of moisture could promote the growth of unwanted fungal [7].

Maceration

Maceration is previously used in wine-making techniques and has become widely used in plant extraction research. The plant materials (coarse or powdered) were soaked in a solvent such as methanol, acetone, and ethanol at room temperature for a minimum of three days with frequent agitation [8]. The maceration technique is based on the diffusion and osmosis phenomena. This process assists the release of phytochemicals from the softened plant's cell wall. After three days, the mixture was strained by filtration. Previously, Vongsak et al., has used the maceration technique on *Moringa oleifera* leaves, in which the dried powdered leaves were macerated with 70% ethanol (1:40, w/v) for 72 hours at room temperature with occasional shaking [2]. The extract was filtered, and the marc (the remains of extraction) was re-extracted by the same process and solvent until the extraction was exhausted. This maceration technique has produced the highest yield of the extract (40.50%, w/w) with the maximum contents of total phenolics of 13.23 g CAE/ 100 g extract and total flavonoids 6.20 g IQE/ 100 g extract, respectively. This extract also exhibited high DPPH scavenging activity at effective concentration, EC50 of 62.94 µg/mL. The maceration technique requires longer extraction duration to obtain a high yield of total phenolic content. Although maceration is one of the traditional techniques, this method appears simple and easy to handle [9]. Suitable solvent type and strength can help to enhance the extraction efficiency to produce high yield of crude extract. Besides, high amount of solvent used in the extraction process also requires proper management of waste.

Soxhlet extraction

Soxhlet extraction has been a standard technique for extraction for over a century [10]. In this technique, the ground sample is placed in a thimble which was filled with solvent for extraction purpose. A siphon aspirates it from the thimble and unloads it back into the distillation flask with extracted phytochemical when the liquid reaches the overflow level. This is a continuous technique thus the operation will be repeated until complete extraction is achieved. Moreover, when the sample is repeatedly brought into contact with fresh portions of the extractants, thereby helping to displace the mass transfer equilibrium. *Moringa oleifera* crude has been previously extracted using the Soxhlet technique where the dried leaves were placed on thimble and extracted with 70% ethanol and solvent ratio 1:50, w/v [2]. The extraction was repeated for five times until exhaustion. For the final part, the combined extract from each extraction method is filtered and the filtrates were dried under reduced pressure at 50°C. The crude yield obtained from the Soxhlet method is 35.87% w/w, which is lower than the maceration method. The total phenolics contents and total flavonoids contents using the Soxhlet technique were 12.47 g CAE/100 g and 6.71 g IQE/100 g respectively [2]. Soxhlet method requires a smaller quantity of solvent compared to maceration [11]. Similar with maceration technique, Soxhlet extraction requires a longer extraction duration which is 16 to 20 hours for extraction and also produces a high volume of solvent as wastes which can cause environmental problems if not treated properly before discharge. The ideal sample for Soxhlet extraction is also limited to a dry and finely divided solid. Additionally, temperature, solvent-sample ratio, and agitation speed were among the important factors influencing the Soxhlet extraction efficiency [12].

Microwave-Assisted Extraction (MAE)

Microwave-Assisted Extraction (MAE) is a modern technique that has become an interest to researchers for its capability. The MAE uses microwave energy to ease the partition of analytes from the plant material into the solvent [13]. MAE increases the kinetics of extraction and reduces solvent consumption for efficient extraction [14]. Figure 1 shows the extraction mechanism of MAE in which the solute in the plant matrix undergoes desorption under high pressure and temperature condition. Subsequently, the solutes will separate from the plant matrix and diffuse in the solvent. The transfer of the analytes from the matrix to solvent is achieved by the diffusion and convection processes. Previously, phenolics compounds have been extracted from *Moringa oleifera* leaves and the microwave oven operates at a frequency of 2.45 Hz with a wavelength of 12.2 cm [15]. There were several parameters investigated such as temperature, time, sample-to-solvent ratio, ethanol concentration, and microwave power. After the irradiation of the microwave, the mixture of the extract was cooled before the filtration. The ethanolic extract at optimum conditions was concentrated and then lyophilized to dryness before quantifying the antioxidant content and phenolic content of the compounds. Under the optimum conditions with 35% ethanol solvent, the total phenolic content was 16.5 mg GAE per g of the dry *Moringa oleifera* leaves [16]. In another research by Rodríguez-Pérez the optimum MAE conditions were at temperature of 158°C, solvent concentration 42% ethanol, and 20 min extraction, has produced 25.75% crude yield, and total phenolic content of 86 ± 4 (mg Eq GAE/g dry leaf) respectively [17] . They concluded, extraction temperature and the solvent-sample ratio play an important role in the extraction of polyphenols using MAE. MAE has several advantages over conventional technique in term of higher extraction rates, automatization, and a resource to simultaneously produce different samples [15]. By contrast, MAE may also cause high pressure and localized heating, thus may lead to an explosion risk and a limited number of samples in the microwave space.

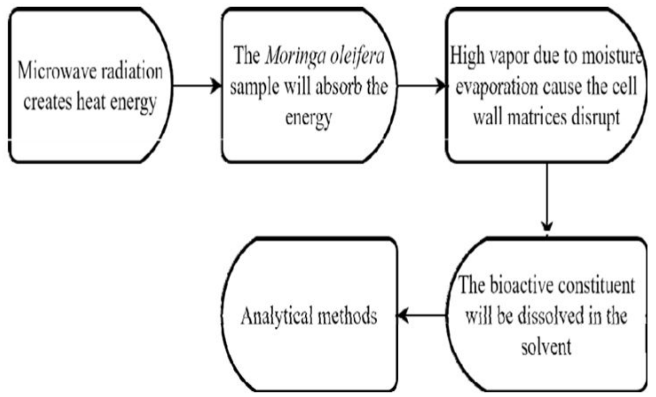

Figure 1: Mechanism of the microwave-assisted method.

Ultrasound-Assisted Extraction (UAE)

In recent years, ultrasound-assisted extraction (UAE) has been used in extracting bioactive compounds from plants on a laboratory scale and industrial scale. UAE involves the use of ultrasound ranging from 20 kHz to 2000 kHz [11]. Numerous bioactive compounds have been extracted by UAE with water and ethanol-water as solvent. Mechanic effect of acoustic cavitation from ultrasound in the mechanism of UAE methods displayed in Figure 2 increases the area of contact between solvents and the plant sample. The mechanical energy will form cavities in the liquid. The expansion of bubbles by energy absorption causes collapsing between cells and bubbles leads to disruption of biological cell walls hence facilitates the release of the compounds and enhancing mass transport of the solvents into the plant cells. A study by Rodríguez-Pérez reported that the extraction of crude *Moringa oleifera* extracts using 25 mL of solvents for 15 minutes extraction at room temperature producing higher phenolic content using UAE technique compared to the maceration technique [18]. Besides, Lin et al., reported 52% of ethanol was used as a solvent and obtained higher flavonoid content values which were 47.04 mg QE/ g MOLs dried weight [19]. Similar to MAE, the UAE technique has successfully obtained higher phenolic content and flavonoid content with shorter extraction duration and less amount of solvent [15]. Patist et al., reviewed the current examples of ultrasonic applications in industry and provided a significant economic potential of this technique [20,21]. Ultrasound-assisted extraction appears as low cost on small and large-scale applications. However, the ultrasound power should not exceed 20 kHz as it will induce the formation of free radicals, thus affecting the active polyphenols available in the crude extracts (Table 1) [22,23].

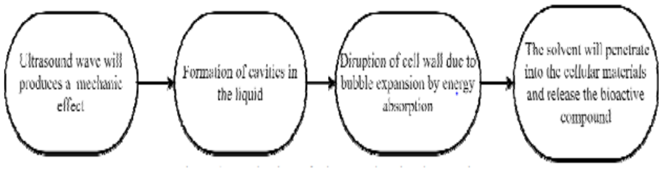

Figure 2: Mechanism of Ultrasound-assisted extraction.

Characteristic	Maceration	Microwave-assisted extraction	Soxhlet extraction	Ultrasound-assisted extraction
Driving force	Solvent contact	Microwave power	Heat	Acoustic cavitation
Extraction time	Several hours	3-30 min	6-24 hours	10-60 min
Sample size	1-30 g	1-10 g	1-30 g	1-30 g
Solvent amount	Large volume	10-40 mL	150-500 mL	50-200 mL
Power amount	High	High	High	Moderate
Advantages	• Not use of sophisticated equipment.	• Fast	• Not use of sophisticated equipment	• Easy to handle
	• Simple and cheap	• Easy to handle		• Safe (atmospheric and ambient temperature)
		• Moderate use of solvent		• Moderate use of solvent
				• Cheap
				• Rapid if a probe is used
Disadvantages	• Risk of spills and exposure to organic vapors	• Risk of explosion (solvent must absorb microwave power)	• Exposure risk to organic vapors	• Required filtration Possible degradation of the compound at high frequencies
	• Required filtration step	• Expensive • Required filtration step	• Degradation of thermo labile compounds	
		• Possible degradation of thermolabile compounds (higher pressure)		

		• Sample process is limited		
Compounds extracted	• Phenolic compound	• Phenolic compound	• Phenolic compound	• Phenolic compound
	•Flavonoid compound	•Flavonoid compound	•Flavonoid compound	•Flavonoid compound

Table 1: Comparison of various solid-liquid extraction techniques for extraction of bioactive compound from plants.

DISCUSSION AND CONCLUSION

Comparing the extraction techniques of polyphenols from leaves and considering their advantages the ultrasound-assisted extraction and microwave-assisted extraction techniques appears as the most promising technique in term of yield and compound extracted. Besides, solvent type is important factor in all extraction techniques reviewed. There is also no significant effect caused by the solvent volume used in the four methods. This study has only considered the assessment of total phenolic content, flavonoid content, and total yield as a comparison. Ultrasound-assisted extraction and microwave-assisted extraction are more applicable and could require less cost for small and large-scale application. Large volume of chemical waste produced using the maceration technique has been major issue compared to MAE and UAE technique which is known as the "Green extraction method". Other than that, parameters such as solvent types, solvent strength, extraction time, agitation speed, sample-solvent ratio, and temperature investigated using factorial design experiments; solvent strength using 50% ethanol is the most influential factor in *Moringa oleifera* extraction. On the other hand, 70% ethanol solvent is the suitable extraction for maceration and Soxhlet extraction. Draw to close, the solvent types and strength give a significant effect on the extraction methods. The temperature, solvents, and agitation also need to be considered at the same time because these influences have possibilities to enhance the extraction. Thus, extraction with few influential factors can be a better extraction method.

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