

Study on Using Contemporary Extraction Methods to Prepare Bioactive Plant Extracts

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Abstract: *Extraction is the first and most crucial step in the creation of plant compounds. Using contemporary extraction methods may help progress the development of traditional herbal remedies. The advancement of modern sample-preparation techniques that offer significant advantages over conventional methods for the extraction and analysis of medicinal plants is likely to be a significant factor in the global effort to ensure that consumers can obtain high-quality herbal products. Sample preparation is essential for creating analytical methods for examining the components of botanical and herbal treatments. The concepts behind the operation of different extraction techniques, factors influencing the effectiveness of methods, research advancements, and benefits and drawbacks of different extraction strategies are all covered in this article. Techniques that are suitable for thermolabile phytochemicals and economical in terms of energy and solvent are emphasized*

Keywords: Extraction

I. INTRODUCTION

Humans have treated illness with plants for millennia. New plant chemicals may be employed in medicine and other sectors. Plant leaves, flowers, bark, seeds, fruits, roots, etc. contain alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids. These secondary metabolites work together to make plants medicinal (Tonthubthimthong et al., 2001). In 1985, Farnsworth et al. discovered 119 drug-like secondary plant compounds. Out of 255 WHO-classified basic and necessary drugs, 11% are plant-derived and others are created from natural precursors. Antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory, antiarthritic, and radio-protective phytochemicals are utilized medicinally. Drug resistance and side effects necessitate the search for new plant-based antimicrobials. These chemicals may have unique chemical structures and unexploited effects. Plant phytochemical analysis may comprise authentication, extraction, separation and isolation of the components of interest, chemical characterization, and quantitative evaluation (Evans, 2008).

Researchers have worked hard to develop efficient and effective extraction methods. Extract yield is efficient, whereas potency is effective (bioactivity/effect). Plant extraction is a sustainable biological component separation approach (Jadhav et al., 2009). Method optimization enhances herb extraction quality and efficiency. Extraction efficiency and antibacterial activity were positively correlated ($r = 0.96$) in plant seed extracts (Kothari, 2010). Sample preparation, quantification, statistical evaluations, etc. are key analytical steps (Pawliszyn, 1997). Different techniques on the same plant material with the same solvent may substantially affect extraction performance (Table 1). The best method must be standardized for repeatability.

A good extraction method and solvent are essential. Choose solvents using the 'like dissolves like' method. Polar solvents remove polar compounds, whereas non-polar solvents extract non-polar. The most frequent extraction method is solvent. Table 2 lists solvents for plant extractions. Hydroalcoholic solvent combinations (alcohol and water in varying concentrations) provide good extraction yields due to their broad polarity range (Table 1). Sample preparation is needed to extract chemical components for separation and characterization in herbal analysis (Huie, 2002). Medically beneficial active components are extracted using water, ethanol, chloroform, ethyl acetate, and methanol. Table 3-4 shows solvent mixtures that boost extraction.

Modern sample preparation reduces organic solvent use and sample degradation (1). They eliminate undesirable and insoluble extract components. Modern methods include MAE, UAE, SFE, SPME, Soxhwave, and others. This combines microwaves and Soxhlet. Fast microwave heating and Soxhlet simplicity. Unlike regular MAE, solvent recovery is conceivable here. However, it is not commonly utilized. Conventional techniques are extensively utilized but may be inefficient, slow, and wasteful of organic solvents, leading to deterioration of heat-labile components. Traditional methods have consistency, safety, and efficacy issues. Modern methods minimize sample clean-up and concentration before chromatographic analysis, improve extraction efficiency, and boost selectivity (Kothari et al., 2010). Standardizing crude drug extraction eliminates inert material and extracts therapeutically useful material utilizing specified solvents and technology. Herbal producers employ the latest extraction methods to generate high-quality extracts with little batch-to-batch variation to scale up extraction as global demand for herbal medicines and natural health care products rises. Standardising extraction enhances herbal medicine quality. Optimizing extraction procedures for the greatest phytochemical spectrum is needed to understand crude extract bioactivity. Processing compounds and raw materials determines the best technique to remove active components from natural sources for highest yield and purity [Kothari et al., 2009].

II. EXTRACTION TECHNOLOGY

(Handa et al., 2008) Common extraction procedures include:

- Plant gathering, authentication, drying
- Reduced size
- Extraction
- Filtration
- Concentration
- Drying and rehydrating

Plant components, solvent, extraction method, plant material:solvent ratio, etc. affect extract quality. All extraction parameters are controlled from lab to pilot scale. Selective solvent extraction separates plant metabolites.

III. ADVANCED EXTRACTION METHODS

MAE: Microwave-Assisted Extraction

Heat is created via microwave absorption. At 2450 MHz (2.45 GHz), the most frequent frequency, commercial microwave equipment produces 600-700 W (Jain et al., 2009). MAE efficiently, sustainably, and inexpensively recovers plant physiologically active chemicals (Hemwimon et al., 2007). In 1975, Samra et al. detected metals in biological samples using microwave home ovens (Letellier and Budzinski, 1999). Ganzler and colleagues introduced MAE to plants in 1986 (Kaufmann and Christen, 2002).

Electric and magnetic fields in microwaves are perpendicular. Ionic conduction and dipolar rotation warm electrical field. In liquid and solid samples, dipolar rotation occurs when molecules having dipole moments align on the electric field. By colliding with nearby molecules, this oscillation warms the medium. At 2.45 GHz, the process speeds by 4.9×10^9 times, causing substantial heating. Higher solvent dielectric constants heat faster (Table 4). Microwaves heat the whole sample, unlike conductive heating. Molecular dipole rotation during microwave heating disrupts weak hydrogen bonds, enabling extraction (Kaufmann and Christen, 2002).

Sample dielectric constants absorb microwave radiation (Ahuja and Diehl, 2006). Plant material is directly heated by microwave radiation without a microwave transparent solvent, instantly heating moisture. High vapour pressure breaches substrate cell walls and releases content into solvent during heating. Most MAE techniques use solvents with high dielectric constants and microwave energy absorption, however solvent combinations may impact extraction selectivity and microwave interaction. Camel (2001) lists common binary solvent combinations with one microwave-absorbing solvent in Table 3. The 'broken cell-wall theory' prefers microwave-transparent solvents over microwave-absorbing ones (Kothari, 2009). Polar solvents are typically better (Jagetia et al., 2005; Kothari, 2010; Proestos and Komaitis, 2007). Solvents with water may boost yields. The microwave-transparent solvent acetone extracted phenolic compounds successfully (Proestos and Komaitis, 2007). High methanol:chloroform dissipation ratio enhances heating efficiency. Low polarity renders

chloroform transparent (Kothari et al., 2009; Proestos and Komaitis, 2007). In microwaves, hexane extracts thermolabile components (Mandal et al., 2008).

MAE may be done in closed containers at increased pressure and temperature or open vessels at atmospheric pressure. Chemat and Esveld (2001) describe microwave-assisted PMAE and FMAE extractions. Closed containers may heat solvents over atmospheric boiling. Extracting faster and better (Kaufmann and Christen, 2002). Correct pressure may raise closed container temperatures. Closed containers help volatile chemicals. Open vessel system maximum temperature depends on solvent boiling points (Camel, 2001). Open cells extract bigger, safer samples than closed vessels (Kaufmann and Christen, 2002). Many extraction tubes are in open cells. AGITATION improves mass transfer in both MAE modes (Mitra, 2003; Sarker, 2006). Due to homogenous superheating, microwave techniques are unlikely to thermally damage phytoconstituents (Chemat and Esveld, 2001).

The microwave heats solid material enough to extract essential oils. This produces less volatile essential oils than hydro distillation (Handa et al., 2008). Moderate MAE extracts good. MAE extracts faster, uses less solvent, is purer, cheaper, and yields more than Soxhlet. It may replace traditional approaches (Ahuja and Diehl, 2006; Chemat and Esveld, 2001). Not microwaves, moisture destroyed plant-based compounds like ascorbic acid (Sasaki et al., 1998). MAE removes phenols quicker than reflux.

MAE extracts curcumin faster and better (Mandal et al., 2007). MAE quickly and easily extracts *Salvia miltiorrhiza* Bunge root tanshinones (Pan et al., 2002). Solvent composition, volume, extraction temperature, and matrix characteristics in rosemary and peppermint. MAE found that microwave-transparent, pure solvents like hexane may rapidly extract essential oils from water-containing sample matrices. Direct microwaves bursting the cell released essential oil into hexane by interacting with free water molecules. Heat rosemary and peppermint leaves in the microwave by optimising plant material:solvent ratio (Huie, 2002). MAE extracts tomato antioxidant phenolics better at higher microwave temperatures and shorter times (Li et al., 2012). MAE extract is most phenolic, tanninic, and scavenging. MAE absorbed phenolic and tannins better than UAE, increasing antioxidant activity 20%. Microwave and sonication extraction increased phenols, tannins, and antioxidants (Thomas et al., 2012). Upadhyay et al. (2012) found that MAE recovered chlorogenic acids from green coffee beans and had strong radical scavenging activity. MAE produced the most alkaloid fraction, whereas UAE extract of *Mitragyna speciosa* leaves contained the most mitragynine (Orio et al., 2012).

Manlikara zapota, *Annona squamosa*, *Tamarindus indica*, *Phoenix sylvestris*, *Citrus limon*, *Carica papaya*, and *Tricosanthes dioica* seeds extracted with MAE show 2-15% extraction efficiency and antibacterial and antioxidant activity. *A. squamosa* seeds were extracted 17% effectively in chloroform-methanol with 50 s microwave irradiation (Kothari et al., 2009). MAE extracted paclitaxel from Iranian yew trees in methanol-water (Ahuja and Diehl, 2006). MAE developed plant extracts for antibacterial and antioxidant research.

Ultrasonication Assisted Extraction (UAE)

UAE uses high-intensity, high-frequency sound waves to interact with materials. UAE seems promising since it requires few tools (Fig 1) and is cheap. Small- and large-scale application are possible (Dai and Mumper, 2010). UAE uses ultrasonic acoustic cavitations. Ultrasonic action vibrates and accelerates solid and liquid particles, causing solute to diffuse fast from solid to solvent (Cares et al., 2009). Cell rupture, penetration, swelling, capillary effect, and hydration are possible ultrasonic extraction augmentation processes (Huaneng et al., 2007). Cavitation occurs when ultrasonic intensity exceeds the intramolecular forces that can keep the molecular structure together, causing it to break down and form bubbles (Baig et al., 2010). Bubble collapse can disrupt biological membranes to release extractable compounds, increase solvent penetration into cellular materials, and improve mass transfer (Cares et al., 2009; Metherel, 2009). Sound waves help extraction by forming and asymmetrically collapsing microcavities near cell walls, generating microjets that rupture cells. Acoustic streaming from bubble pulsation prevents the solvent layer around plant tissue from becoming saturated, improving mass transfer rate and convection (Kadkhodae and Kakhki: http://confbank.um.ac.ir/modules/conf_display/saffron/pdf/p55.pdf). Sonication may readily break the thin skin of plant cell wall exterior glands, releasing essential oil contents into the extraction solvent and speeding up extraction (Huie, 2002). Ultrasound improved tea solid extraction yield by 20% from dried leaves with water. UAE improved carnosic acid extraction with ethanol, ethyl acetate, and butanone and decreased extraction time (Baig et al., 2010). One study found that UAE increased extraction rate and yield of total isoflavones from the stem of *Pueraria lobata* (Willd.) for all solvents. The

extraction yield increased with electrical power input between 0 and 650 W (Huaneng et al., 2007). UAE extracts vanillin faster than Soxhlet for various solvents (Jadhav et al., 2009). Ultrasounding the pre-leached mixture for a short time may make commercial ultrasound treatment reliable and straightforward (Jadhav et al., 2009). Grape resveratrol UAE was effective. Within a particular extraction time, UAE may not degrade grape resveratrol (Cho et al., 2006). UAE retains protein and pectin, improving tea taste. Xia et al. (2006) found UAE suitable for aroma compound and glycosidic aroma precursor extraction. UAE extracted soybean, rapeseed, and *Monopterus albus* oil (Li et al., 2004; Ibiari et al., 2010; Abdullah, 2010). UAE can improve extraction efficiency and processing time, and ultrasound did not affect oil composition during processing. Studies on solvents and their mixtures, solvent volume, sonication power, and sonication time showed that UAE can improve extraction efficiency and reduce processing time. UAE extracts flavonoids such as tectoridin, iristectorin B, iristectorin A, tectorigenin, iris-tectorigenin A, and total isoflavones faster than maceration and Soxhlet extraction (Sun et al., 2011). UAE derived beneficial grape seed components. Grape seed UAE of total phenolics, antioxidants, and anthocyanins is highly influenced by extraction duration and temperature (Ghafoor et al., 2009). Ultrasound-assisted extraction of ginsenosides (saponins) from different ginseng was shown to be easier and more successful than standard extraction procedures. Sonicated ginseng saponin extraction was three times quicker than standard extraction. Ultrasonic extraction was faster and easier for active component recovery and purification. Sonication-assisted extraction is better for thermally unstable chemicals at lower temperatures (Wu et al., 2001). Sonication effectively extracted phytochemicals like hypericin, pseudohypericin, hyperoside, rutin, quercitrin, and hyperforin from *Hypericum perforatum* L., outperforming conventional maceration, indirect sonication, Soxhlet extraction, and accelerated solvent extraction (ASE) (Smelcerovic et al., 2006). UAE greatly increased nicotine extraction from pharmaceutical and plant preparations. Conventional cold extraction takes up to 24 h each stage, however UAE takes less than 20 min and uses less ecologically damaging organic solvent.

Ultrasound's key benefits include quicker reaction/preparation time, little material use, low solvent costs, and increased sample throughput. It is helpful for bioactive principle separation and purification (Ishtiaq et al., 2009). In rare cases, ultrasonic radiation (> 20 kHz) may damage medicinal plant active components by forming free radicals and altering drug molecules (Handa et al., 2008).

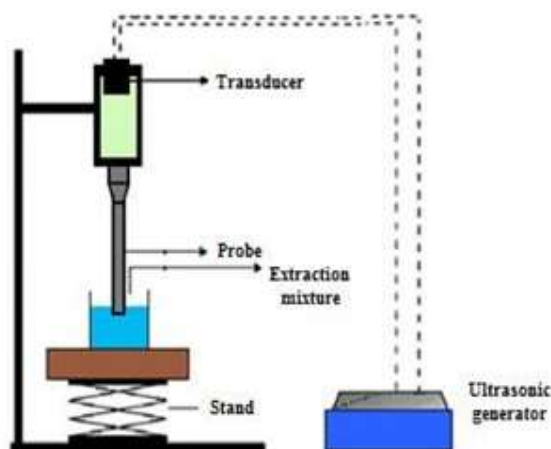


Fig 1. Schematic Representation of UAE Setup [adapted from Jadhav et al., 2009] Supercritical Fluid Extraction (SFE)

SFE can extract plant components around ambient temperature without thermal denaturation. SFE is an ancient solvent extraction method, but its commercial use was delayed owing to the costly high-pressure apparatus and technologies needed (Tonthubthimthong et al., 2001). Since its design and operation conditions are well recognized, SFE is a well-established extraction and separation process (Li et al., 2010). Fluids at their critical points penetrate solid plant matrix deeper and extract quicker than standard organic solvents due to their favorable transport qualities. High-pressure apparatus extracts batch or continuously. Both methods include supercritical solvent interaction with the substance to be

separated. Sample preparation usually uses cylindrical extraction tubes (Handa et al., 2008). In batch processing, solid is put in extraction vessel and supercritical solvent is added until desired extraction conditions are obtained. Semi-batch processing uses one or more separation stages to precipitate solute from supercritical solution given continuously via a high-pressure pump at a set flow rate. Recently, supercritical fluid technology has been shown to be as efficient as chemical analysis. SFE is useful for qualitative and quantitative identification of natural product ingredients, including heat-labile chemicals (Mohameda and Mansoor, 2002).

Aroma chemicals like essential oils and caffeine are extracted from plants using SFE. SFE extraction involves temperature, pressure, sample volume, cosolvent addition, and flow and pressure control. Supercritical fluids that are slightly beyond the critical temperature and pressure for a material are commonly used and have qualities between liquid and gaseous phases (Evans, 2008). With changes in pressure and temperature, the fluid's characteristics may be altered between gaseous and liquid states (Kroon and Raynie, 2010). Any fluid may become supercritical under certain circumstances. Density determines whether supercritical fluids may be extraction solvents. A dense gas, a supercritical fluid is above its critical temperature (TC) and critical pressure (PC). The decreased temperature T_r (T/TC) must not exceed 1.2 or 1.3, whereas the reduced pressure P_r (P/PC) may be as high as technical limitations to be supercritical (Handa et al., 2008). Water has critical temperature and pressure values of 374°C and 220 atm, whereas carbon dioxide has 30.9°C and 73.8 atm (Evans W.C., 2008).

SFE solvents include hexane, pentane, butane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons (Reverchon and Marco, 2006). CO₂ is the most popular SFE extraction solvent (Handa et al., 2008). Adding a co-solvent or modification to CO₂ improves its extraction capacity and selectivity. After extraction, co-solvent is easy to remove. Due to its low critical temperature of 304 K, CO₂ is the best SFE solvent for heat-labile chemical extraction. CO₂ is also an inert, safe (non-flammable, non-explosive), inexpensive, noncorrosive, odourless, colorless, clean solvent that leaves no solvent residue in the product, non-toxic, and widely accepted as a harmless ingredient in pharmaceuticals and food. It can be easily removed from the extracted oil by expansion. Carbon dioxide is an appealing supercritical solvent due to its low surface tension, viscosity, and diffusivity (Handa et al., 2008; Tonthubthimthong, 2001). Regulations consider carbon dioxide safe, affordable, and environmentally favorable. The phase equilibrium scenario, which is sensitive to operating circumstances, is crucial to supercritical solvent process design. Thus, phase equilibrium engineering is crucial to these processes' synthesis and design (Herrero et al., 2009).

Argon is cheaper and more inert than CO₂, hence it's sometimes utilized instead. Component recovery rates rise with pressure or temperature. The maximum recovery rate for argon occurred at 500 atm and 150°C. When modifiers (co-solvents) interact with matrix, plant matrix swells, improving extraction recovery.

SFE has certain advantages over traditional approaches (Handa et al., 2008; Ahuja and Diehl, 2006):

- Low-temperature ingredient extraction prevents heat damage.
- Free of solvents
- Environment-friendly
- Improved diffusivity
- Low supercritical fluid viscosity allows specific extractions
- Quick extraction

SFE settings may be adjusted to selectively extract an antioxidant fraction from rosemary with little scent. CO₂ may selectively extract essential oils and diterpene glycosides from medicinal plants at subcritical temperature and pressure (Huie, 2002). Supercritical CO₂ acts as a lipophilic solvent but has the benefit over liquid solvents that its selectivity and solvent power may be adjusted from gas-like to liquid form. SFE produces high-quality products without artifacts and greater flavor or scent retention. For essential oil extraction, low CO₂ density (0.25-0.50 g cm³) is best. The extraction of non-oil substances may employ high CO₂ density [58]. CO₂ (methanol co-solvent) is used to extract nimbin in a high-pressure HPLC tube extractor. The effect of CO₂ flow rate, pressure, extractor temperature, and neem sample weight during extraction was studied. Supercritical CO₂ extraction yielded 0.35 kg nimbin/kg of seeds. Estimated extraction yield was 0.175 mg nimbin/g of neem seeds, with an expected 0.5 mg/g. The optimal extraction conditions were 308 K, 23 MPa pressure, and 1.24 ml/min. A variety of samples showed no correlation between sample size and extraction rate (Tonthubthimthong et al., 2001).

When optimizing SFE conditions for Curcuma zedoaria active ingredient extraction, CO₂ density and fluid volume passing through the plant matrix were found to be the most important factors, while temperature had little effect (Huie, 2002). The rate of SFE extraction of volatile active components like essential oils from matrices seems to be governed by analyte-matrix interaction rather than bulk solubility in pure CO₂. Adding an organic modifier greatly increased the extraction rate.

SFE with CO₂ extracts grape seed oil with improved quality and efficiency. The oil recovered by extraction with supercritical carbon dioxide is devoid of organic solvent, processing time was also reduced than during traditional solvent extraction. Complete elimination of organic solvents from the extract, otherwise is a long and energy intensive procedure in present-day oil technology (Aleksovski et al., 1998). Supercritical CO₂ extraction has been employed for obtaining seed oils from fennel seeds also (Reverchon and Marco, 2006).

SFE has been proved to be more successful than other standard approaches for the extraction of antimicrobial chemicals. A prominent trend in food and natural products analysis, as well as in other domains of analytical research, is the hyphenation of sample treatment techniques with analytical equipment in order to generate efficient on-line couplings. Some of these couplings have required the usage of SFE (Herrero et al., 2009).

We recently completed a comparative research of several techniques for extraction of antioxidant and antibacterial chemicals from plant seeds (Kothari et al., 2012). Extracts from seeds of five different plants- Annona squamosa (Annonaceae), Manilkara zapota (Sapotaceae), Phoenix sylvestris (Palmae), Syzygium cumini (Myrtaceae), and Tamarindus indica (Cesalpiniaceae)- were prepared in water, methanol, and ethanol by employing five different methods of extraction viz. Soxhlet method, Ultrasonication, extraction by continuous shaking at room temperature, and microwave assisted extraction- with and without intermittent cooling. All these extracts were compared with regard to extraction efficiency, total phenol content, total flavonoid content, antioxidant capacity, and antibacterial activity. Soxhlet technique demonstrated optimal in terms of high extraction efficiency, and extraction of phenolic chemicals. Microwave aided extraction with intermittent cooling (MAE), room temperature extraction by shaking (ERT), and ultrasonication assisted extraction (UAE) showed excellent in extracting antibacterial chemicals from plant seeds. Latter also proven useful for extracting antioxidant chemicals. Methanol found most acceptable solvent for extraction of flavonoids. Following these research, we have also been able to extract antifungal (especially against Malassezia furfur) phytochemicals from plant seeds using MAE (Ramanuj et al., 2012).

IV. CONCLUSION

Medicinal plants are essential for the discovery and synthesis of new therapeutic compounds. The extraction process is important when identifying and isolating different phytochemicals from herbs and looking for novel leads in plant extracts. Conventional methods are more labor-intensive and need more power, time, sample, solvent, and power than their modern counterparts (Table 5). An improved method may improve the extract's overall quality, stability, and recovery. With the use of contemporary, enhanced techniques, a particular compound (or class of plant metabolites) may be extracted, and the extract can be directly applied to high pressure liquid chromatography (HPLC) or gas chromatography (GC). It has been argued that MAE is a more appropriate approach to utilize with subsequent separation and characterization processes among the current techniques. One such instance is the use of high-performance liquid chromatographic separation, naphthoquinones in Zicao and lignans in Wuweizi, together with on-line continuous sampling dynamic microwave-assisted extraction (on-line CSDMAE) (Gao et al., 2012). Compared to conventional extraction methods like Soxhlet extraction, ultrasound assisted extraction, and off-line continuous microwave assisted extraction, this strategy is said to be quicker and more effective. Improvement of microwave-assisted extraction for phenolic component identification in olive leaves using high- Additionally, new studies on the combination of electrospray time-of-flight mass spectrometry (ESI-TOF-MS) and electrospray ion trap tandem mass spectrometry (ESI-IT-MS2) with performance liquid chromatography (HPLC) have been published (Taamalli et al., 2012). It is shown that MAE is a better alternative to the conventional method for characterizing phenolic compounds from olive leaves because of its efficiency and speed.

Modern methods have the potential to be highly automated and to manage several elements at once. Selecting the optimal strategy also results in a reduction of sample and solvent usage. In comparison to material generated using a traditional method, a high-quality extraction may be obtained in less time, and the extracted material may have a greater yield and

quality. Methods such as SFE, MAE, and UAE The enhanced selectivity, efficiency, and specificity of the former make them more suitable for industrial use. Further innovations such as a Soxhwave under reduced pressure, which combines advantages like rapid heating due to microwave, fast boiling of solvents due to reduced pressure, and scope of solvent recovery, will make the extraction of thermolabile plant constituents much more efficient and quick in the future. However, doing so will require overcoming challenges related to creative engineering and production. There aren't enough reasonably priced choices available right now to allow such a device to be utilized extensively worldwide. Currently, open- Comparing vessel MAE to techniques like ultrasonication or SFE, it appears to be the most practical, user-friendly, and quick way to extract thermolabile phytoconstituents because it just requires a basic microwave oven and no additional complex equipment.

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