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Extraction methods for plant materials containing antimicrobial properties and microencapsulation: An overview

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Abstract
Medicinal plants are attaining a large amount interest recently because of their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. Study on medicinal plants started with extraction procedures that play a critical role to the extraction outcomes and also to the consequent assays performed. A huge 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. The majority of the phytochemicals from plant sources such as phenolic and flavonoids have been reported to have positive impact on health and cancer prevention. Extraction is the key process for natural dyes and secondary metabolites for making eco-friendly dyed and finished fabric in global textile industries. In recent days their demands are increasing towards ecofriendly textiles especially in terms of dying, printing and finishing. Now a day many natural dyes and pigments are available for printing and dyeing but for finishing of textile there are so many challenges for extraction of secondary metabolites such as alkaloids, flavonoids, oils, steroids and terpenoides In functional textiles the microencapsulation technique is preferred to incorporate antimicrobial property, deodorant or fragrance property, fireproof property and wound healing property.

Keywords: Extraction, property, fireproof

Introduction
The importance for functional finishes has been increasing rapidly in textile market because of competition, gaining added values and increasing market share. The consumer's demands are not only defined by aesthetic properties but also by their functional properties. Microencapsulation is now grown as an increasing area in functional finishes. Plants and plant product were traditionally used for healing of wounds, burn injuries, anti-fungal, anti-viral, anti-bacterial and anti-microbial activity against skin infections. Microorganisms are available naturally in the surrounding environment; thus they can be easily reached to our skin. The importance of antimicrobial textiles has been recognized for decade of years and ancient writing contains frequent to it. Natural antimicrobial finishes were in ancient time used for storage and preservation from microbes like *Haldi, Palash, Neem, Tulsi, Lahsun, and Kalimirch* etc. Some of them are used as dyes also such as *Haldi*, *Palash*, henna, marigold, *Parijat* etc. Traditionally the crude extracts of different parts of medical plants, including leaves, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases (Khan *et al*., 2013) [13]. In technical textiles medical textiles are a very potential sector which plays a vital role in health of mankind. They consist of textiles used in operative and post-operative tasks in and around the patient and the medical practitioners. Medical textiles are generally classified as non-implantable materials, implantable materials, extra corporeal devices, and hygiene products, protective and health care textiles. Health care disposable and non-disposable hygiene products mainly used by health workers. The use of such products helps to reduce the probability for contamination by biological toxins and infectious pathogens. In recent decades the growing demand for herbal products has led to the idea of developing healthcare textile products. The aim of paper is to describe various extraction methods in detail.

The study of medicinal plants begins with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Conventional methods such as maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Significant 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. Besides, modifications on the methods are continuously developed. With such variety of methods present, selection of proper extraction method needs particular evaluation. This review describes the principle, strength and drawback of the commonly used methods with examples in recent years to assist in the selection of proper procedures.

Plant extracts extraction methods for the application of antimicrobial finishes

Collection of plant materials

The first step of extraction is the collection of plant material or raw material. If extraction material is fresh leaves, flowers, roots and barks, it's necessary to clean or wash the plant with running water for removing the dust particles. After washing the plant material requires drying at room temperature and after drying the plant material should be crushed in small particle for better results in extraction process.

Pre-extraction preparation of plant samples

In the study of medicinal and aromatic plants, the preparation of plant samples for the behaviour of bio molecules in the plant, prior to the extraction process. The Plant samples such as leaves, bark, roots, fruits, and flowers-that can be extracted from the fresh or dried plant material. Other pre-treatment of agricultural by-products, such as cut-and-dry, also has an impact on the behaviour of the phytochemicals in the final extracts.

Fresh and dried samples

Medicinal plant research makes use of both fresh and dried samples. In most cases, a dried-up specimen is recommended that, in view of the time needed for the pilot project. Sulaiman *et al*. (2011) [21] the limit of the period of time between the harvest and of the experimental work, up to a maximum period of 3 hours in order to maintain the freshness of the samples, because of the fresh specimens are fragile and have a tendency to deteriorate faster than the dried samples. The comparison of fresh and dried *Moringa oliefera* leaves did not have a significant effect on the total phenolic content, however, due to the high flavonoids content in the dry sample.

Grinded and 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 are-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. This particular size of particle was mentioned in Suleiman *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 Centellaasiatica produced by Planetary Ball Mill (PBM) showed 82.09% higher yield compared to micro powder using maceration technique in 90% methanol for 3 days. Particle size was a major factor when using enzyme-assisted extraction. Use of pectinolytic and cell wall polysaccharide degrading enzyme in simple preparation was influenced majorly by the particle size as smaller particle enhances enzyme action.

Various drying processes

Air-drying

The air-drying process usually takes 3-7 days to a few months to a year, depending on the type of samples to be dried, such as leaves or seeds. The Plant samples, and usually the leaves and stems were tied together and hung exposure of the plants to the air at room temperature. This drying method is not that of the dried plant material, the use of a high-temperature. However, air-drying will take longer than the microwave drying and freeze-drying, and may be exposed to contaminants in an unstable operating temperature range

Microwave-drying

The microwave-drying uses electromagnetic radiations that possess the 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. 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

Oven drying is another pre-extraction method that uses hot energy to remove moisture from the samples. This sample preparation is considered to be one of the simplest and fastest thermal substances that can store phytochemicals. Drying in the oven at 44.5 °C for four hours using 80% methanol led to very high antioxidants activity in extracts of *Cosmos caudatus* and a similar effect was observed in the well-formed concentration of 80% methanol at 44.12 °C in 4.05 hours. Short-term extraction time was obtained using this method. However, the drying effect on *Orthosiphon stamineus* showed no significant effect on antioxidant activity but on bioactive phytochemicals; such as sinensetin and rosmarinic acid content are affected by oven and solar drying, which raises chemical sensitivity to heat.

Freeze-drying (lyophilisation)

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. 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.

Solvents for extraction

There are many medium for extraction such as aqueous medium, acidic medium, alkaline medium and alcoholic medium. In aqueous medium distilled water use to extract any plant material, in acid medium of extraction with acetic acid (CH_2COOH) and Sodium carbonate used as alkaline medium for extraction. In alcoholic medium of extraction there are various solvents such as methanol (MeOH), ethanol (EtOH), dichloromethane (CH_2Cl_2), acetone (Me_2CO).

Aqueous extraction

Distilled water act as an aqueous extraction medium and plant material is required to add in distilled water and maintain the temperature between 60˚C - 80˚C for 30 minutes. Then left to cool and extract must be filter in a container and evaporate the extra amount of water. The extract was collected and kept in the bottles stored at 4˚C for further use (Shafei etal 2018) [20].

Acidic medium

In acidic medium acetic acid is add in to water to maintain pH (3-4), plant material is added into solution provide heat with maintaining temperature between 60 ˚C-80 ˚C for 30 minutes. Left to cool the extract afterwards filter the extract into a container and evaporate the extra amount of water till required concentration, collect the extract in sterilized bottle and store at 4 ˚C for further use.

Alkaline medium

In alkaline medium sodium carbonate is add in to water to maintain pH (11-12), plant material is added into solution provide heat with maintaining temperature between 60 ˚C – 80 ˚C for 30 minutes. Left to cool the extract afterwards filter the extract into a container and evaporate the extra amount of water till required concentration, collect the extract in sterilized bottle and store at 4 ˚C for further use.

Alcoholic medium

Collected leaves of the plant were allowed to Shadow dry in room temperature to reduce the moisture content. The dried leaves were ground into fine powder by mechanical shearing, and sieving. The fine powder obtained after grinding was used for extraction. The dried leaf powder was weighed and added into different flasks containing solvents (Methanol, Ethanol, Ethyl acetate, Chloroform, Benzene) each and was kept in a shaker for 48 hours, the extracts were then filtered using Whatman no 1 filter paper. The residue obtained was mixed with Dimethyl sulfoxide (DMSO). The extracts were then used for the determination of bacterial susceptibility testing and minimal inhibitory concentration.

Properties of solvent of extractions

Water: It is the most polar solvent and is used in the extraction of a wide range of polar compounds. It dissolves a wide range of substances; it is cheap, nontoxic, non-flammable, and highly polar. It promotes bacterial and mould growth; it may cause hydrolysis, and a large amount of heat is required to concentrate the extract.

Alcohol

It is also polar in nature, miscible with water, and could extract polar secondary metabolites. It is self-preservative at a concentration above 20%. It is nontoxic at low concentration, and as small amount of heat is required for concentrating the extract. It does not dissolve fats, gums, and wax; it is flammable and volatile.

Chloroform

It is a non-polar solvent and is useful in the extraction of compounds such as terpenoides, flavonoids, fats, and oils. It is colourless, has a sweet smell, and is soluble in alcohols. It is also well absorbed and metabolized in the body. It has sedative and carcinogenic property.

Ether

It is a non-polar solvent and is useful in the extraction of compounds such as alkaloids, terpenoides, coumarins, and fatty acids. It is miscible with water, has low boiling point, and is tasteless in nature. It is also a very stable compound and does not react with acids, bases, and metals. It is highly volatile and flammable in nature.

Ionic liquid (green solvent*)*

This is a unique solvent of extraction and is highly polar and extremely heat stable. It can remain in a liquid state even at 3,000 ˚C and usable where high temperature is applicable. It has extreme miscibility with water and other solvent and is very suitable in the extraction of polar compounds. It has excellent solvent that attracts and transmit microwave, and hence it is suitable for microwave-assisted extraction. It is non-flammable and is useful for liquid-liquid extraction and highly polar. It is not ideal for preparation of tinctures.

Extraction methods

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. The reason of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue).The initial crud extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, Phenolics, terpenoides 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 some need further processing

Methanol extraction

The following procedure was used for the methanol extraction of the selected herbs. Herbal powder was mixing thoroughly with methanol and water 80/20ml and was kept in airtight conical flask. The conical flask was incubated for 24 h in the room temperature. The supernatant was filtered and the filtrate was dried then the methanol was evaporated in the room temperature when exposed to the air. The filtrate was collected and kept in an airtight bottle at 4 ˚C for further use (Shafei *et. al* 2018) [20]. A known quantity of leaf powder was mixed in 70% ethanol, 70% methanol and distilled water and incubated for 24 hours at room temperature. The extract was centrifuged at room temperature for 10,000 rpm and supernatant was separated. The extract obtained was filtered using Whatman filter paper and measured. Extracts were stored at 8 ˚C for further analysis within 7 days (Vastrad *et al.* 2015) [24].

Ultrasound-assisted extraction (UAE) or sonication extraction

Ultrasonic assisted extraction (UAE) method has been considered according to its simplicity to extract the

responsible chemical compounds, easy handling, low cost, high yield or efficiency, lower organic extraction solvent consumption, reduced time for extraction, and reliable procedure in an extensive range of organic solvents for various phenolic compounds in large-scale level and industry. Air-dried leaf sample weighted and immersed into water and 97.5% ethanol and extracted using ultrasonic extraction techniques for 30-90 extraction minutes at 45–50 °C. The extracted ingredient was filtered through a filter paper (Whatman No. 1) which impregnated with the same solvents. The ethanol concentrated to near dryness under reduced pressure below 50 °C using a rotary evaporator machine. The amount of the concentrated ingredient extracted was noted down and stored in air tight glass bottles in a refrigerator until further use. UAE involves the use of ultrasound ranging from 20 kHz to 2000 kHz. The mechanic effect of acoustic cavitations 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. The procedure is simple and relatively low cost technology that can be used in both small and larger scale of phytochemical extraction.

Strength and weaknesses

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.

Supercritical fluid extraction (SFE)

Supercritical fluid (SF) or also called as dense-gas is a material 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 non-polar analytes and CO2 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 ºC temperature, 10% modifier concentration and 90 minute extraction time. A major drawback of this method is the initial cost of the equipment is very high.

Conclusion

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. However, the finding is limited to assessment of phenolic, flavonoids content and total yield as comparison.

Vongsak *et al*. have been suggested that Maceration 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”. Although, all these extraction methods resulted in crude extracts containing a mixture of metabolites, the efficacy of that crude extracts using nano-encapsulated processing in *Centella asiatica* showed to have similar efficacy as those purified. 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. The all phases of extractions, from the pre-extraction and extraction are equally essential 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 finally have an effect on the final extracts. It can be concluded that, there are no universal extraction methods is the ideal method and each extraction procedures is unique to the plants. Formerly optimized methods can be used to lead in the selection of suitable methods.

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