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A comprehensive review of conventional and non-conventional solvent extraction techniques

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Abstract
Phytochemicals are produced by plants during primary or secondary metabolic activities. They are the bioactive compounds playing important role in the defense of the host plant against different biotic and abiotic agents and functioning in attraction and stimulation. Most of these phytochemicals are found to be having beneficial effects on the health of human beings. All Indian traditional system of medicine like Ayurveda, Unani, and Siddha uses phytochemicals in their formulations. In the Moderns era, synthetic analogs or prototypes of natural phytochemicals are used which is however not advisable. It is always recommended to extract the phytochemicals from their natural sources scientifically. The bioactive component is recovered from raw materials by dissolving them in appropriate solvents at suitable temperatures. Phytochemicals can be extracted by various techniques; classical as well as modern. The current review attempt to bring out a comparative study of all the conventional and non-conventional techniques used for the extraction of phytochemicals.

Keywords: Phytochemicals, soxhlet extraction, maceration, decoction, supercritical fluid extraction

Introduction
Plants are there on the earth long before man set his feet on it and it is said that the universe gave us a prize of them with materials for the survival of man and animals long before these creatures were there on earth. Nature has provided us with a very rich botanical wealth and a large number of diverse types of herbal plants growing wild in different parts of our country [1]. Recently there has been a shift from synthetic to herbal medicine [2], they are gaining more momentum despite the advancements in modern healthcare, which is "Return to Nature" as stated by Padhi *et al.* [3]. Medicinal plants have been known for therapies of various diseases and are highly used all over the world as a rich source of therapeutic agents for the prevention of diseases [4-7] Such phytochemicals known to have medicinal properties are considered to be "active ingredients" or "active principles" of natural medicines that are having therapeutic approaches [8].
The qualitative and quantitative analysis of bioactive compounds from plant materials is majorly based on the preference of appropriate extraction method [9, 10]. Optimization of the extraction procedure plays the most critical role in isolation of the bioactive compounds and their subsequent analysis. Plants contain many active compounds such as flavonoids, alkaloids, terpenoids, steroids, tannins, gums and mucilage, coumarins, glycosides, oils, resins, phenols, and proteins that are present in their parts such as roots, stems, barks, seeds, leaves, flowers, fruits [11]. To extract such bioactive compounds one can use either conventional (Traditional methods) or non-conventional (Modern and recently developed) extraction techniques. During the extraction process, the solvents move into the finely graded solid plant materials and solubilize the phytochemicals having similar polarity [12]. It should be noted that the choice of appropriate solvent is also important along with the choice of an appropriate extraction method. For the selection of solvents 'like dissolves like' principle can be used as polar solvents will extract polar substances while non-polar substances will extract non-polar solvents [13].

Extraction
From the phenomenological standpoint, extraction is a mass transfer process of one or more components from one phase to another one [14]. When using the natural products, in most cases the sample to be extracted is a solid material, and in some cases liquid samples are used. The extracting solvent is usually a liquid, but it can also be a solid or a supercritical fluid [9, 15]. In order to understand how any extraction technique works, both the target compounds and the extraction solvent must be considered [13, 16]. Therefore, knowledge of the properties of the solute is important in order to sympathize with the extraction process.

The extraction is related to one or more important properties that include high yield, high selectivity/purity, high sensitivity, and low limit of detection/quantification [16, 17]. The fundamental interaction between solute and solvent is determined by the solubility of the solute in the solvent, the vapor pressure of the solute, the hydrophobicity, molecular weight, and the acid/base properties of both matter and solvent [18, 19]. Some of the properties of solvent also may affect the extraction such as selectivity, reactivity chemical, and thermal stability, viscosity, boiling point, flammability, toxicity, and regulatory, economic aspects [20].

The compatibility between solvent and solute is based on assessing the polarity of the molecular structure to assume their solubility and miscibility. For example, water dissolves sugar due to the attraction between the partially negatively charged atom of the sugar molecule is attracted to the partially positively charged atom of the water molecule similar to the partially positively charged atom of the sugar molecule to the partially negatively charged atom of the water molecule[21]. Some of these extracts may be ready to use as medicinal agents without any formulation, in the form of tinctures and fluid extracts but some need further processing and formulation for easy applicability [22].

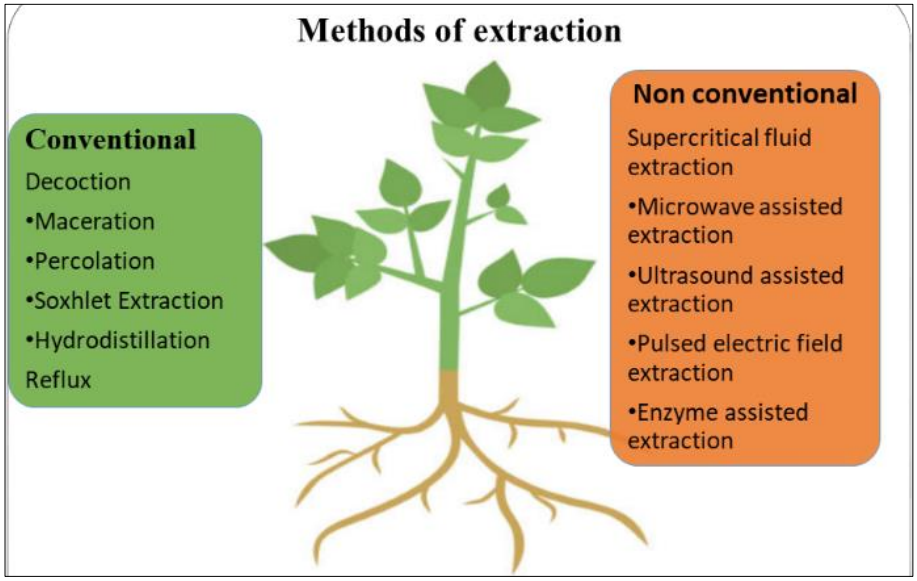

Fig 1: List of conventional and non-conventional extraction methods

The conventional extraction methods, including Maceration, Percolation, Reflux extraction, decoction, Hydro distillation, and Soxhlet extraction, usually use organic solvents or water and require a large volume of solvents and a long extraction time [8, 23-25]. While Pressurized liquid Extraction, Supercritical fluid Extraction, Ultrasound-assisted Extraction, Microwave-assisted Extraction, Pulsed electric field Extraction, Enzyme assisted extraction are included under the non-conventional techniques for the extraction that require less time and solvents [26-30].

Table 1: Comparison of some common conventional and non-conventional extraction techniques

Sr. No.	Name of the technique	Solvent	Temperature	Time	Pressure	Volume of solvent consumed	Polarity extracted chemicals	Disadvantage over other methods	Reference
1.	Decoction	Aqueous	Higher temperature	Few hours	14-15Psi	None	Polar	The extract contain large amount of water soluble impurities; Cannot use for the extraction of heat sensitive constituents.	[8]
2.	Maceration or soaking	Aqueous and non-aqueous solvents	Room temperature or sometime at higher temperature	Few hours up to several weeks	14-15Psi	Large	Depends on solvent	The time-consuming and labor intensive operation leading to large volume of hazardous solvents and further clean-up and concentration steps required; potential loss of metabolites and/or plant material. Furthermore, some compounds may not be extracted efficiently if they are poorly soluble at room temperature; the mass transfer rate decrease with time because the solvent is continuously enriched with solutes.	[31-33]
3.	Percolation	Aqueous and non-aqueous solvents	Room temperature or sometime at higher temperature	Few days	14-15Psi	Large	Depends on solvent	Requires more time than soxhlation; Skilled person is required; plant with mucilage can't use as it can clog the percolator; Special attention should be paid on particle size of material and throughout process; solvent recirculated is not fresh, so that the possibility of dissolving more solutes into The liquid phase decreases with time.	[13, 16, 34, 35]
4.	Soxhlet	Organic solvents	Heated at the boiling point	Few hours up to several days	14-15Psi	Large	Depends on solvent	Increase the possibilities of thermal Degradation; Agitation is not possible in the Soxhlet device; The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time.	[36,37]
5.	Reflux extraction	aqueous and non-aqueous solvents	Under heat	few hours up to several days	14-15Psi	Large	Depends on solvent	Long extraction times at high temperatures and large volumes of frequently toxic solvents; degradation of the bioactive compounds and not suitable for human consumption.	[38]
6.	Supercritical fluid extraction	Supercritical fluid (usually S-CO_2), sometimes	40–100 °C	Few minutes upto one hour	2100–6000 Psi	None or very small	Non polar to slightly polar	sophisticated and expensive (the capital cost is substantially higher (at least 50%) than a conventional extraction) high pressure equipment and technology required	[39,40]

		with modifier							
7.	Microwave assisted extraction	Aqueous and non-aqueous solvents	Room temperature	Few minutes	14-15Psi	Very small	Depends on solvent	Extraction solvent Must be absorbs microwaves; high maintenance cost in commercial-scale settings; need to cool down vessel every single time to avoid solvent evaporation.	[41,42]
8.	Ultrasound assisted extraction	aqueous and non-aqueous solvents	Room temperature or under heat	Few minutes	50-1000 Psi	Moderate	Depends on solvent	Poor purity and low efficiency; Lack of uniformity in the distribution of ultra sound frequency; Not suitable for heat labile compound as ultrasound increase temperature with prolong treatment.	[43]
9.	Pulsed electric field extraction	Aqueous and non-aqueous solvents	Room temperature or under heat	Few minutes	14-15 Psi	Moderate	Depends on solvent	Depends on electric field strength and electrode gap; Dielectric breakdown can be affected by the existing of air bubbles in the treatment chamber and causing the PEF treatment to become less uniformity; The cell membranes can be reversible or irreversible during electroporation mechanism;	[44]
10.	Enzyme assisted extraction	Aqueous and non-aqueous solvents	Room temperature or under heat	Few minutes upto one hour	14-15 Psi	Moderate	Depends on solvent	Enzyme cannot break down the plant cell walls completely; enzymes behave was limited by environmental conditions	[45]

Decoction

This procedure is suitable for extracting water-soluble, heat-stable constituents [46]. In the process of decoction, The plant material is generally broken into small pieces or Powdered and boiled with water. In the Ayurvedic method, traditionally known as *kwatha*, the crude drug in the form of *yavakuta* (small pieces) is placed in earthen pots or tinned copper vessels with clay on the outside. Water is added and the pot is heated on a fire. If the material is soft, 1:4 ratio of plant: water was used; if the drug is moderately hard, 1:8 ratio was used and if the drug is very hard, 1:16 ratio is recommended. The mixture is then boiled on low flame until it is reduced to 1/4th starting volume, in the case of soft drugs, and 1/8th in the case of moderate or very hard drugs. The extract is then cooled and strained and the filtrate is collected [13]. It uses the same principle as maceration [22].

High extractive yield of Saponin, tannin, total phenols, alkaloids, flavonoids, terpenoids, and steroids using decoction method was reported from six different plants *Manilkara zapota*, *Rubia cordifolia*, *Hemidesmus indicus*, *Tinospora cordifolia*, *Psoralea corylifolia*, *Azadirachta indica* [47, 48]. Similar results were observed, in some plants of *Zingiberaceae* [49]. However in some cases decoction method did not yield a significant amount of phyto-constituents [50]. As per observation of Sadeer *et al.*, the fruit extract of *Bruguiera gymnorhiza* (L.) Lam. prepared via maceration was found to be richer in phenolic, flavanol, tannin, and triterpenoid contents [50].

Maceration

This simple, but still widely used, the procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container at room temperature. Soaking procedures can be carried out more rapidly when using high extraction temperatures rather than room temperature [51]. Occasional or constant stirring of the preparation can increase the speed of the extraction. The process is intended to soften and break the plant's cell wall to release the soluble phytochemicals [22, 23]. When the concentration of metabolites in the extract and that in the plant material are equal, the extraction ultimately comes to an end. After extraction, the residual plant material (marc) has to be separated from the solvent. This involves a rough clarification by decanting, which is usually followed by a filtration step. Centrifugation may be necessary if the powder is too fine to be filtered [34]. The remaining solid material at the end of the process is pressed and it is usually re-extracted.

When Sruthi and Indira compare the maceration and soxhlation method for the leaves of *Nephelium lappaceum.* L. that suggests, that petroleum ether extracts is showing the presence of steroids in maceration while tannin in soxhlation extraction but results of other extracts such as ethyl acetate, methanol, and aqueous shows the presence of similar phytochemicals [52]. Ćujić *et al.* achieved high yields of total phenols and total anthocyanins from chokeberry fruit at an optimized condition with 50% ethanol, a solid–solvent ratio of 1:20, that resulted in the experimental total phenolics were 27.8 mg GAE/g, and total anthocyanins were 0.27%, which suggested that maceration was a simple and effective method for the extraction of phenolic and anthocyanins compounds from chokeberry fruit [53]. Comparison of various extraction techniques for *Medicago sativa* shows that supercritical fluid extract was rich in saponins [622.2±30.3 mg oleanolic acid equivalents (OAE)/g DM]. However, maceration extracts from stems had the lowest chemical content as reported by a previous study [54].

Senizza *et al.*, reported the methanolic macerate of tubers of *Colchicum triphyllum* to possess higher content of alkaloids, Flavones, Phenolic acids and lignans as compared to that extracted by Soxhlet method using similar solvent [55]. However the catechin percentages were found to be significantly lower in the macerated extracts of *Camelia sinensis*, especially the quantity of EGCG decreases by 4 to 5 fold after the process. While the high concentration of GA In the case of methanolic macerate of tubers of *Colchicum triphyllum* alkaloids, Flavones, phenolic acids and lignans were analyzed by Senizza, *et al.*, that are comparatively higher than the soxhlation extract with similar solvent[55]. Barreira, *et al.*, check the Effect of extraction methodology on the phytochemical composition on powdered Tea extracts of and as a result, they obtain that (gallic acids) [56].

Percolation

Percolation literally means "pass a liquid through a solid material drop by drop." In percolation, a percolator (a narrow, cone-shaped vessel open at both ends) is generally used where the matrix is placed inside a thimble holder, like in Soxhlet, and the solvent is allowed to percolate through it. The solvent extract mixture is recirculated in the system using a pump [57]. All of these steps are conducted at room temperature. It works on the same principle as maceration and infusion [58].

Devgun, *et al.*, compare the conventional and non-conventional phytochemical extraction methods from Heartwood of *Pterocarpus Marsupium roxb* which resulted in the highest extraction yield in the case of percolation extract compared to other conventional extraction methods [59]. At the same time they reported that the active compound pterostilbene is present in a higher percentage in MAE (0.667%) (Non-conventional extraction method) compare to the percolation (0.176%). While Urango *et al.*, design Thermosonication Process for Recovery of Bioactive

Compounds and during their analysis they extracts phytochemical compounds from *Foeniculum vulgare* as a results they observe that percolation extraction gives higher global yield and extraction yield of solids than thermo-sonication method but when they measure antioxidant activity by TEAC (Trolox Equivalent Antioxidant Capacity) that extract obtained by thermo-sonication has higher antioxidant activity with higher phenol content than the extract obtained by percolation [60]. A Study on *Onosma gmelinii* (Boraginaceae) indicated that the percolation extracts had the highest antiviral activity against influenza virus strain A/H1N1 followed by the ultrasound-assisted extraction and supercritical CO_2 extraction. Percolation also has the strongest effect on S*taphylococci*: *Staphylococcus aureus* ATCC 6538-P, *Staphylococcus aureus* ATCC BAA-39, *Staphylococcus epidermidis* ATCC 51625, and *Staphylococcus epidermidis* ATCC 12228 [61].

Soxhlet Extraction

Soxhlet is having similar assembly as well as it also works on the same principle of condensation as reflux extraction. soxhlet extractor that has three part system: percolator, thimble, and siphon [62,63]. The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the setup is heated under reflux [34, 64]. Condensed solvent is sucked into the flask below when it reaches a specified level in the thimble. The fact that Soxhlet extraction is a continuous process is its fundamental benefit. As the solvent (saturated in solubilize metabolites) empties into the flask, the fresh solvent is re-condensed and extracts the material in the thimble continuously [61, 65]. Solvents used in the extraction system need to be of high purity which might add to cost. This procedure is considered not environmentally friendly and may contribute to pollution problems compared to advanced extraction methods such as supercritical fluid extraction (SFE) [66].

As a result revealed by Bimakr *et al.,* soxhlet extraction had a higher crude extract yield compared to supercritical CO_2 extraction [67]. But having more main flavonoid compounds with high concentration compared to the 70% ethanol soxhlet extraction. They show that the highest extraction yield was obtained with methanol solvent, which has seven flavonoid compounds including catechin, epicatechin, rutin, myricetin, luteolin, apigenin, and naringenin. However, the concentrations of Myricetin and Naringenin were low and apigenin had the highest concentration from spearmint (*Mentha spicata* L.) leaves. While results of Vongsak *et al.,* indicated that the soxhlatation extract with 70% and 50% ethanol is almost having similar or slightly lower phenolics and flavonoids content when analyzed in the leaves of *Moringa oleifera* [68]. Furthermore study of Nayak *et al.,* indicates that the Flavonoids and tannins were present in the soxhlet ethanol extract that may be responsible for the antimicrobial activity [69]. The comparison of UAE and two conventional extraction methods (maceration and Soxhlet extraction) shows that the UAE is much more effective for extracting antioxidants from the flower of *Jatropha integerrima* [70].

Reflux

Grinded samples were dissolved in solvents. The dissolution time was evaluated in a reflux system at constant temperature and with agitation. Up till the point of total dissolution. The agitation process was maintained for another few minutes until the whole solution cooled down and the mixture was then filtered on a vacuum system [71]. Reflux is an analytical apparatus that works based on the principle of condensation [72, 73]. The Major difference of reflux from soxhlet is that it cannot contain the soxhlet extractor [63].

The study of Zhang compared the extraction efficiency of active phytoconstituents such as baicalin and puerarin from seven different TCM (Traditional Chinese medicine) with two different methods, decoction, and reflux. In that study, the reflux method was found to be more effective than the decoction method as the highest yields were obtained from the reflux method with 60% ethanol [74]. While results of Yasir *et al.,* on optimization of extraction method for *Clerodendrum paniculatum* L. shows that the reflux extract is having highest amounts of bioactive phyto-constituents in-compare to other methods such as MAE and maceration [75]. Ma *et al.,* Optimized the reflux extraction method for the polyphenol extraction and to obtain the highest antioxidant activity that resulted in an optimized ethanol concentration of 75%, a 20:1 liquid to solid ratio, and an extraction time of 2.1 h [76]. The number of polyphenols was 7.216 mg/g. But the study of Alonso-Carrillo *et al.,* In Extraction of phenolic compounds from *Satureja macrostema* Microwave-ultrasound assisted technique showed advantages over the reflux extraction [38].

Supercritical fluid extraction

SFE works on the basic principle that the favorable transport properties of fluids near their critical points allow deeper penetration into solid plant parts and more efficient and faster extraction than with conventional organic solvents [77]. An SFE process is basically composed of the feed, containing the substance of interest that comes in contact with supercritical fluid, at suitable temperature and pressure (Low pressure cold extraction: 35-55°F, 800-1500 psi; Supercritical extraction: 87°F, 6000-10000 psi), in an extraction device in batch or continuous manner [15, 78, 79]. In this simple process, the component is selectively extracted and must be recovered from the supercritical solution. Product recovery occurs in the separation section [80]. If the temperature is kept constant, product separation is achieved by depressurization, and mechanical energy has to be provided to the system to raise the CO_2 pressure from the separator to the extractor conditions [79]. To develop an SFE process with CO_2 (possibly assisted by ethanol or water), we need to know and optimize: The solubility of the substance of interest, The selectivity of this substance, The extraction profiles, The way to separate the substance of interest from the total extract. Supercritical fluid technology is now recognized as an effective analytical technique with efficiency comparable to existing chemical analysis methods [23]. SFE is favorably applicable for the qualitative and quantitative identification of constituents of natural products, including heat-labile compounds [81].

SFE is widely used for extraction and fractionation of carbohydrates, extraction of lipids and lipophilic components, extraction of bioactive compounds from plant materials in order to characterize compounds responsible for an specific functional activity [82]. Such as isocoumarins and coumarins from *Coriandrum sativum* [83] and *Helichrysum italicum (Roth)* G., *Citrus maximum Don, Ruta graveolens L., Salvia officinalis* L., *Melilotus officinalis* L., *Angelica archangelica* L., and *Lavandula officinalis* L. respectively [84, 85]. Optimization and extraction rate analysis of carotenoids at 80 ºC were analysed from rosehip by Machmudah *et al.* [86]. Studies included tocopherols [87-90], fatty acids [91-93],

phytosterols [94] and terpenes [95, 96], among other compounds extracted using same method to screen various activity such as blood-thinning, anti-fungicidal and anti-tumor activities associated with respected phyto-constituents. SFE can be also use in pharmaceutics, enantiomeric separations, solvent removal and new drug delivery formulations, food toxicology and ecotoxicology and metals recovery [82].

Microwave assisted extraction

Microwave-assisted extraction consists of heating the solvents in contact with the sample with microwave energy. The partitioning of the analytes of interest from the sample matrix to the solvent depends on the temperature and the nature of the extractant. For a proper understanding of the technique, the effects of microwaves on the sample and solvent mixture must be understood. As the microwave has an electromagnetic nature, it possesses electric and magnetic fields. The electric field causes heating via two mechanisms, dipolar rotation and ionic conduction [97-99]. This oscillation produces collisions with surrounding molecules and thus the liberation of thermal energy into the medium [99-102] and the resulting heating is very fast. It must be realized that, unlike classical heating, microwaves heat all the samples simultaneously without heating the vessel. Therefore, the solution reaches its boiling point very rapidly, leading to very short extraction times. There are two different types of microwaves instruments (closed vessel and open vessel microwaves) [103]. In a closed vessel system, extraction is carried out under controlled temperature and pressure, while in an open vessel system it is under atmospheric pressure [104]. Polycyclic aromatic hydrocarbons, Polychlorobiphenyls, Pesticides, Phenols, Organometallic, Dioxins, and Phthalate esters were extracted by Camel, V. using the MAE method [105]. Kaufmann and Christen also reported the application of MAE in the extraction of natural products such as pyrimidine glycosides, Gossypol, Alkaloids, Terpenes, Essential oils, Carotenoids, Steroids, Taxanes [106]. While Dhanani *et al.* has reported the effect of soxhlet, UAE and MAE methods on yield, and phytochemical constituents of *Withania somnifera* [107]. Their results shows that the highest % yield was obtained in the MAE extracts compare to the other two methods while the same prevalence was observed in the estimation of total phenolic content (higher in ethanolic MAE extract) while Dahmoune *et al.* optimized the parameters of MAE for the phenol extraction [108]. Nisca *et al.* also optimize Conditions to Improve Phenolic Content in *Quercus cerris* Bark Extracts [109].

Ultrasound assisted extraction

In ultrasound-assisted extraction method, Plant sample, and extraction solvent is placed in a vessel then sonotrode is placed in contact with them [110]. This sonication results in cavitation and generation of small vacuum bubbles in the solvent, which implode at the solid sample resulting in localized high temperatures (about 4500 °C-5000 °C) and pressures (about 30-50 MPa). These forces produce effects such as sonolysis, destruction of cell membranes, and the extraction of intracellular material [111-113].

Altemimi *et al*. determined that the best conditions for extracting total phenolics from pumpkins were a temperature of 41.45 °C, a power of 44.60%, and a period of 25.67 min. They extracted the phenolic compounds from peaches and pumpkins using UAE and also optimised it by response surface methodology (RSM) [114]. However, an extraction temperature of 40.99 °C, power of 56.01%, and for peach extracts were an extraction temperature of 41.53°C, power of 43.99%, and time of 27.86 min for total phenolics. Similarly, *Moringa oleifera L.* leaves were also used by Wu *et al.* for total phenol extraction and optimization and resulted in optimized 37% water content, 144 W ultrasonic power, and 40 °C ultrasonic temperature [115]. Cai *et al.* use UAE for anthocyanins, total phenol and flavonoids extraction from purple sweet potatoes and compare it with accelerated-solvent extraction and conventional extraction methods and conclude UAE as more convenient then other conventional methods [116]. Zhen *et al.* optimize extraction conditions and as a result they obtained an ultrasonication power of 490 W, a liquid-to-solid ratio of 30 mL/g, and an ultrasonication time of 6.5 min with 83.93% flavonoid yield [117].

Pulsed electric field extraction

Pulsed electric field (PEF) processing is an efficient non-thermal extraction technique using short, high-voltage pulses. These pulses induce the poration of plant cells, leading to cell disintegration [118, 119]. The equipment of PEF consists of three subunits: a hammer mill, a thermomalaxer, and a centrifuge [120]. A hammer mill is used to grind the plant parts into a fine paste. Then that paste was subjected to malaxation. The malaxation was conducted at 15 to 40°C for a few min [121]. After malaxation, the paste was centrifuged and collected. Because heat is minimized in PEF, products have a longer shelf life and maintain better nutritional value compared with conventional techniques [122,123]. There are two major types of it: one is the speeding of chemical-based reactions from various compounds to enhance the solubility of solvent, and the other is the electroporation process [124]. Salee *et al.* Optimized the pulse electric field assisted extraction of black rice grain and obtained 5 kV/cm, 3000 pulses and 0.5 g/mL leading to achieving higher yield [125].

Enzyme assisted extraction

The structure of the cell membrane and cell wall, micelles formed by macromolecules such polysaccharides and protein, and the coagulation and denaturation of proteins at high temperatures during extraction are the main barriers to the extraction of natural products [8]. Due to the hydrolytic action of the enzymes on the components of the cell wall and membrane as well as the macromolecules inside the cell that promote the release of the natural product, EAE will increase the efficiency of extraction. In EAE, cellulose, -amylase, and pectinase are typically used [8, 126].

phenolics from citrus peels using enzyme-assisted extraction method was carried out by Silva Martins *et al.* and got the highest recovery in the enzyme-assisted extraction process (Celluzyme MX) was up to 65.5% [127]. While Macedo *et al.* use Integrated microwave and enzyme-assisted extraction of phenolic 2 compounds from olive pomace and also got higher extractive yield [128]. And Vardakas *et al.* also use it for extraction of polyphenols and they have optimized the parameters for extraction of polyphenols and anthocyanin and observed the negative effects of the higher enzyme dose on anthocyanin, positive effects of incubation time on the total polyphenol yield and concluded as yields of total anthocyanins and total polyphenols will increase when the incubation time or enzyme dose increases up to a certain point, after which they begin to decrease [129].

Conclusion

Extraction of the bioactive constituent is the most critical and challenging task considering that all the classical and modern

techniques usually adopted have their own merits and demerits. The non-Conventional methods are gaining attention now a days for being eco-friendly, rapid with improved extraction yield. But the methods require high capital cost and elaborate optimization. The conventional methods are easy and less costly but are time consuming with high solvent requirement. As discussed in the current review it was found that Taking into account the merits and demerits of all the methods discussed in the current review, the most suitable extraction method to be adopted to have maximum output for subsequent Fractionation and purification.

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