



Review

A Review of Emerging Technologies for the Extraction of Bioactive Compounds from Berries (Phalsa Berries)

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Abstract: Berries have been gaining in popularity among consumers and producers due to their natural bioactive compounds that have beneficial effects on human health. This review aimed to identify effective techniques for the extraction of bioactive compounds from berries, consolidate the findings of recent studies using various extraction technologies, and provide a global perspective on the research trends in this field. These extraction techniques include pulsed electric field, ultrasound-assisted extraction, pressurized liquid extraction, microwave-assisted extraction, and supercritical CO₂ extraction. The solid waste generated during the industrial berry juice production process is assumed to be a less expensive source of raw materials for the natural extraction of bioactive compounds. The main aim of modern techniques is to produce more of the desired compound and find a method to extract bioactive compounds from berries without the use of hazardous solvents. These include flavonoids, phenols, anthocyanins, and antioxidants. Regarding the characterization of the bioactive compounds that are isolated from berries, aspects such as scanning electron microscopy, X-ray powder diffraction, Fourier transform infrared spectroscopy, and nuclear magnetic resonance were reviewed.

Keywords: phalsa berries; bioactive profile; solvent extraction; antioxidant; non-thermal processing



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1. Introduction

Among all vegetables and fruits, berry fruits are one of the biggest suppliers of phytochemicals and antioxidants, like ascorbic acid, carotenoids, flavonoids, tocopherols, and phenolic acids, and organic acid, alcohols, and some other inflammable substances have been found in several species [1,2]. Due to their numerous properties that promote health and wellness, including their enormous anticancer, anti-aging, antioxidant, and anti-cardiovascular disease properties [3,4], fruits are being utilized in dietary supplements and cosmetics in addition to being consumed fresh, which is another reason for the notable growth in their consumption globally. Different parts of the berry plant (stem, roots, leaves, fruit) are used as a medicinal product for the treatment of various diseases, including diabetes, fevers, anemia, dysentery, diarrhea, ulcers, smallpox, and itching [5,6]. Polyphenolic substances such as anthocyanins have been connected to these advantageous effects [6,7]. Extraction must be performed to acquire a high concentration of antioxidants from berries to benefit from these compounds in nutraceuticals and lower oxidative stress.

The process of isolating phenolic compounds from plant materials with antioxidants is the first stage in the analysis of polyphenols. These attributes play an important role in the hindrance of chronic illnesses, including heart problems and cancer [8,9]. According to data, phytochemicals with antioxidant capabilities are linked to a lower risk of death [10,11]. During berry processing, chemical recovery from the solid residue has been found to be possible using non-thermal procedures, including supercritical fluid extraction (SFE) [12], ultrasound-assisted alcoholic extraction (UAE) [13], pressurized liquid extraction (PLE), pulsed electric field (PEF), and microwave extraction (MAE).

The bioactive compounds present in phalsa berries offer a range of potential health benefits, making them valuable additions to the human diet. Phalsa berries are rich in antioxidants such as anthocyanins, flavonoids, and phenolic acids. These compounds help neutralize harmful free radicals in the body, reducing oxidative stress and protecting cells from damage. Antioxidants play a crucial role in preventing chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. For phalsa berries, despite their high nutritional, economic, and medicinal value, their availability, and commercial use is restricted because of their large seed size, perishable nature, and the inconsistent ripening of the fruit [14]. When compared to other growing fruit plants, these undesirable features also prevent it from being eye-catching. Moreover, no well-characterized cultivar of *G. subinequalis* exists. In India, basically, species of phalsa are divided into two different genotypes known as sharbati and dwarf [15]. Phalsa (*Grewia asiatica* L.) is a tropical bush from the *Tiliaceae* family and contains about 150 species of small to medium shrubs. It is primarily found in tropical and subtropical parts of the world [3,16]. Phalsa is a little berry-bearing flowering plant that blooms in warm climates and sheds leaves in the winter. It is well liked for its reviving and delicious flavor, eye-catching color, and remarkable nutritional and therapeutic value. From a horticultural perspective, it is regarded as a minor fruit crop, but it is important in folk medicine. It is freshly consumed, utilized in sweets, and condensed into stimulating and refreshing drinks, juice, jam, and squash, among other uses for ripe fruits. Also, the phalsa fruit is exceedingly perishable; it is challenging to store it in its raw form [17].

Phalsa berries are generally low in calories but high in sugars (glucose, fructose). In contrast, they contain a high level of organic acids (malic, oxalic, fumaric, citric, and tartaric acids), dietary fiber (hemicellulose, cellulose, and pectin), and, in trace amounts, certain minerals, phytochemicals (phenolic acid), and vitamins (folic acid and ascorbic acid) [18]. However, berries also contain a considerable amount of oil, up to 5% free fatty acids, including oleic acid, stearic acid, palmitic acid, linolic acid, and unsaponifiable material (13.5%, 11.0%, 8%, 64.5%, and 3%) [8,19]. The majority of berry pomace contains a seed, which provides a valuable source of oil for the food and pharmaceutical industries. Oil possesses a high level of free fatty acids with an ideal amount of omega-3 and omega-6 fatty acids. Sterols, carotenoids, tocopherols, and phenolic compounds have additional beneficial components of oil [19,20]. Because of its increased level of bioactive compounds, such as anthocyanins, flavonoids, tannins, phenolics, and flavonoids, phalsa has recently been regarded as healthy in human diets [17,21]. Berries are a fruits that have been demonstrated to have several positive effects on human health and are relevant in nutrition and medicine [22]. Studies have been conducted over the past few years to examine the medicinal and dietary advantages of the phalsa fruit plant [23,24]. A few illnesses, including inflammatory problems as well as cardiac, respiratory, and blood diseases, have been treated using phalsa fruit as a folk remedy. It was discovered that several polyphenolic elements of the phalsa plant exhibit strong antioxidant action (anthocyanins, phenolic acids, and flavanols). The effectiveness of drug use is changing, with synthetic drugs being replaced increasingly frequently by natural plant-based remedies as a result of consumers' rising disapproval of the use of drugs to address medical conditions [25]. There has been a rise in demand by customers for wholesome, safe, and nutrient-rich foods. As a result, rather than using thermal processing techniques, processors are experimenting with unique non-thermal methods [26,27]. Thermal treatments increase the storage life of food and ensure

food safety. Also, they help reduce a product’s heat-sensitive nutrients [28]. Non-thermal methods have been studied recently [9,27]. However, enzymes cannot be inactivated by these non-thermal technologies alone [29,30]. These non-thermal techniques help with this problem in combination with improving other qualitative parameters of drinks [31]. One of the cutting-edge methods of food processing to improve the quality, storage, safety, and stability of fruit and vegetable juices is the integration of non-thermal processing methods [32]. Based on this, this review discusses the various bioactive components found in berries, including flavonoids, phenols, anthocyanins, and antioxidants. Scanning electron microscopy, X-ray powder diffraction, Fourier transform infrared spectroscopy, and nuclear magnetic resonance are all used for the characterization of bioactive compounds extracted from berries. Non-thermal approaches implemented to extract bioactive components from berries, such as supercritical CO₂ extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric field, and pressurized liquid extraction, are described in this review. The search strategy is presented in Table 1. A word plot of important keywords is presented in Figure 1.

Table 1. Search strategy.

S. No.	Search Term	Descriptor
1.	Berries	“Berries” OR “Phalsa” OR “Fruits”
2.	Bioactive compounds	“Phenol” OR “Flavonoid” OR “Antioxidant” OR “Nutraceuticals”
3.	Extraction	“Thermal Processing” OR “Non-thermal Processing”
4.	Combination	1 AND 2 AND 3

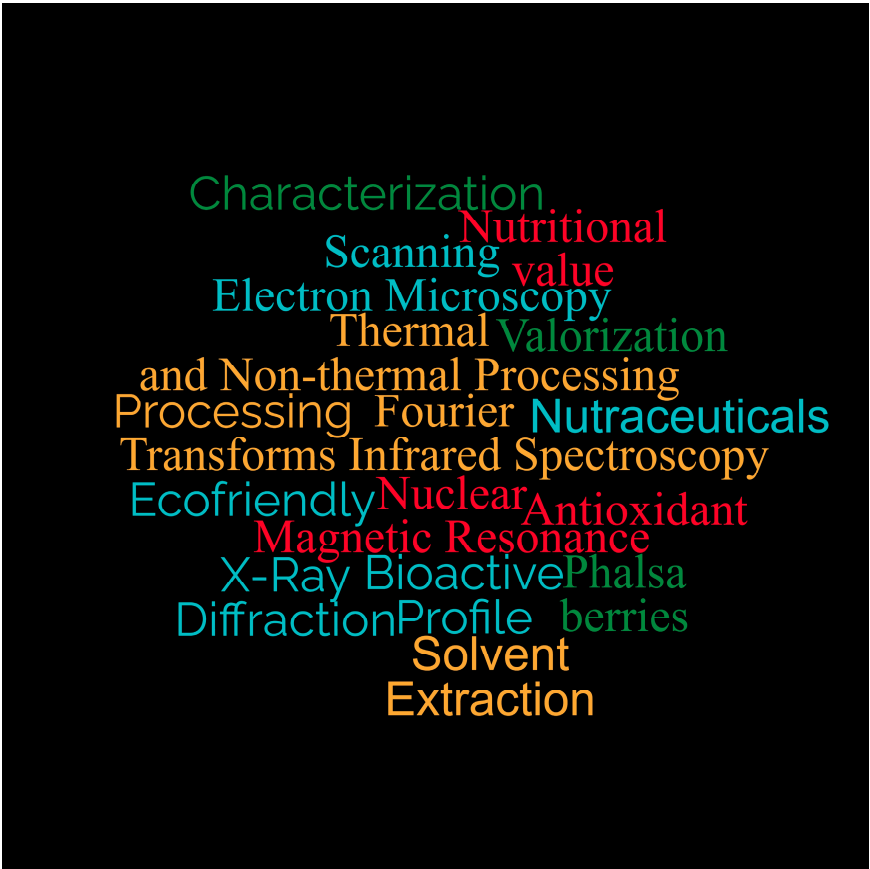


Figure 1. Word plot of important keywords.

2. Various Bioactive Compounds in Berries

Phalsa berries contain a high concentration of polyphenolic compounds, such as flavonoids and phenolic acids. The presence of these chemicals in phalsa berries enhances their antioxidant qualities by effectively counteracting detrimental free radicals and minimizing oxidative stress in the body. Anthocyanins, a type of flavonoid pigment, are responsible for the purple color of Phalsa berries. Anthocyanins have been linked to a range of health advantages, such as reducing inflammation and protecting the heart. Phalsa berries are a rich source of vitamin C, a vital component that possesses antioxidant characteristics. Vitamin C enhances immunological function, promotes collagen formation, and contributes to general health. Phalsa berries may contain tannins, which are polyphenolic compounds known for their astringent qualities. Tannins have been investigated for their possible contribution to improving digestive health and exerting antibacterial properties. Phalsa berries, although not as abundant as other fruits, may contain carotenoid compounds like beta-carotene, which has antioxidant characteristics and promotes eye health. Phalsa berries may contain a variety of phenolic acids, such as gallic acid and ellagic acid, that possess antioxidant and anti-inflammatory properties. Phalsa berries, like other fruits, are rich in dietary fiber. This fiber promotes healthy digestion, aids in controlling blood sugar levels, and potentially assists with weight management. Incorporating phalsa berries into a varied fruit consumption might enhance general health and well-being. The details of the various bioactive compound present in Phalsa berries are presented below.

2.1. Flavonoids

Flavonoids, members of the polyphenol family, are secondary metabolites found in almost all fruits and vegetables. One heterocyclic ring and two phenyl rings make up the 15 carbon atoms characterizing flavonoids [33]. Flavonoids are virtually always present in plants in free glycoside and aglycone linkages. The glycosidic bond found is the most prevalent in flavanol and flavone consumed in the human diet [34]. The highest amounts of flavonoids are found in phalsa's stem, callus, leaves, bark, etc. Anthocyanins, isoflavones, flavones, flavan-3-ols, flavanones, and flavonols are the six main families of flavonoids. Research was conducted on the flavonoid concentrations in various phalsa extract components taken from stems and leaves using in vivo and in vitro methods [35]. Phalsa pomace's flavonoid concentration was estimated to be 12.42056 CE mg/g [36,37]; it was found that the rind/bark contained 39.114 ± 65 mg of flavonoids. The results of another investigation showed that the fruit's solvent extract's total flavonoid concentration and phenolic content were, respectively, 0.13 and 5.25 GAE/mL [38]. The bioactive potential of various parts of berry trees is given in detail in Table 2.

Table 2. The bioactive potential of phalsa fruit.

Assays	Fruit	Leaves	Root	Reference
Flavonoid (mg QE/g DW)	116.95	9.90	16.37	[34]
Phenols (mg GAE/g DW)	294.353	30.20	11.00	[39,40]
Anthocyanin ($\mu\text{g/g}$)	1193.8	-	-	[41]
Antioxidant IC ₅₀ ($\mu\text{g/mL}$)	4.88	23.9	-	[42]
DPPH IC ₅₀ ($\mu\text{g/mL}$)	257.66	16.3	82.5	[43]
FRAP IC ₅₀ ($\mu\text{g/mL}$)	4.14	18.3	82.53	[43,44]
ABTS IC ₅₀ ($\mu\text{g/mL}$)	134.22	-	96.41	[45]

2.2. Phenols

Phenolic compounds are important natural antioxidants with potential health advantages that are found in most plants, grains, fruits, vegetables, and other seeds. Other uses include preserving nutritional value, pharmaceuticals, extending the shelf life of foods,

reducing, or preventing lipid oxidation in food products, and postponing the generation of harmful oxidation products. Polyphenols, often called phenolic compounds, are a class of hydroxylated molecules that are highly oxidation sensitive. According to various studies, they possess various biological qualities, including antimicrobial, antiproliferative, antidiabetic, anti-cancer, antiviral, and anti-inflammatory [39]. Although their structures vary, an aromatic ring is generally present with one or more hydroxyl groups. Because of the stability of the electron due to the displacement on the aromatic ring of the phenolic compounds, the radical-scavenging activity of phenolic antioxidant molecules depends upon their capability of transforming into radicals, which are more stable compared to many free radical species. The most significant phenolic acids and anthocyanidins, a subclass of flavonoids, are included in the classification of phenolic antioxidants. Hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives are the two main subgroups of phenolic acids. The first category contains molecules like gallic, hydroxybenzoic, ellagic, and vanillic acids. Hydroxycinnamic acid, ferulic acid, *p*-coumaric acid, caffeic acid, and chlorogenic acid are all present in the second group. Berries contain a variety of these compounds, and every type of fruit has phenolic molecules with a unique profile [46]. The total phenolic content varies depending on the extraction solvent and varies depending on the location, such as the peel, seed, and pulp [40]. Phalsa was found to have 67 to 288 mg/100 g of total phenols in various extracts [47]. In another study, the highest phenolic contents were measured by 70% acetone extraction, and the results showed that the seeds, peel, and pulp each contained 1020 mg gallic acid equivalent GAE/100 g, 5080 mg GAE/100 g, and 2060 mg GAE/100 g, respectively [40].

2.3. Anthocyanins

Anthocyanin is a member of the flavonoid family and is a pigment found in water-soluble vacuoles in plants, such as vegetables and fruits. They provide plants and plant parts with their distinctive hues, such as purple, blue, and red hues of varied botanical origin, based on pH [48]. Anthocyanin is accessible as a glycoside that has been glycosylated with the appropriate glycone and its derivative. There are just a few anthocyanins, and only six of them, peonidin, delphinidin, pelargonidin, malvidin, petunidin, and cyanidin, are common [41]. A recent study of phalsa proved that it is a promising native fruit rich in a total of seven anthocyanins, including cyanidin-3-O-(6''-acetyl glucoside), which accounts for 44–63% of the fruit's total anthocyanin content; peonidin-3-O-glucoside and pelargonidin-3-O-(6''-acetylglucoside), which account for 8–14% and 3–30%; peonidin-3-O-(6''-acetyl glucoside); malvidin-3-O-glucoside pyruvic acid; pelargonidin-3-O-malonyl glucoside; and delphinidin-3-O-glucoside [21].

In addition, total anthocyanin analysis revealed that phalsa fruit extract had a high anthocyanin content and powerful antibacterial properties compared to four different Gram-positive and Gram-negative species. Despite having a wide range of uses, the plant in question has received an incredible lack of attention, as evidenced by the paucity of literature on it. To increase the commercialization of phalsa fruit products, it was deemed critical to continue the research into fruit pigment [41].

2.4. Antioxidant

Antioxidants' properties give them the capability to stop oxidative damage like lipid peroxidation. Antioxidants play several roles in biological systems, including protection against oxidative damage and involvement in the cell, with major impacts on signaling networks. Cells' primary usage of antioxidants is to delay the harm that reactive oxygen species can inflict. The considerable variation in the antioxidant activity of fruits, berries, and the products they produce, documented in several studies, is partly attributable to the use of various oxidation systems and techniques to analyze antioxidant components. The total antioxidant capacity of some foods has evolved into a selling point for those in the food industry due to the possible synergistic action of all these bioactive chemicals found in food [49]. It has been found that there are various factors that affect antioxidant activity in phalsa plants, such as

the growing conditions of the plant (including soil and climatic conditions), the plant variety, and genetic orientation of the plant. Additionally, the plant parts under extraction (such as the fruit, peel, pulp, stem, bark, leaves, and roots), extraction technique and temperature used, fractionation method, solvents used, and solute–solvent ratio can significantly affect antioxidant activity [9,44,50]. According to [40] phalsa contains high concentrations of antioxidants such as anthocyanins, ascorbic acid, flavonoids, total phenolics, and tannins, which they determined using the DPPH method. Anthocyanins (fraction II), flavanols (fraction Ia), phenolic acid (fraction Ic), and flavanols are the different fractions' radical scavengers (fraction Ib). Flavanols > phenolic acid > anthocyanins > flavanols represents the levels of antioxidant action in ascending order. Fresh phalsa samples have powerful antioxidants that play a part in cardiac disorders like hepatitis and diabetes. In phalsa, it is indicated that the total phenolic content is higher in fraction Ib and low in fraction Ia, ranging between 89% and 58% [47]. According to recent research, freeze-dried fruit has a stronger antioxidant content than fresh fruit and higher flavonoid and total phenolic contents (11,695 mg gallic acid equivalent/g and 294,353 mg gallic acid equivalent/g, respectively) [51]. According to a study by [42], methanol extracts with greater levels of flavonoid concentration (4.608 QE mg/g), total phenolic acid (14,411 mg GAE/g), and anthocyanins content (4.882 mg/kg) than other solvents have higher antioxidant activity. The antioxidant capabilities of phalsa plants vary between plant parts. The antioxidant activity of fruit that has been stored significantly declines; the seed size goes from 49.0 to 19.3 mol TEAC/g to 19.3 mol TEAC/g for the peel and 56.1 to 25.8 mol TEAC/g for the pulp. Therefore, researchers contend that the anthocyanin, total flavonoid, total phenolic, and total tannin of phalsa (and other factors including maturity, fertilizer, season, post-harvest conditions, storage conditions, and extraction solvent) affect the plant's antioxidant activity [44,50,52,53]. The bark and flower of the phalsa plant, as well as other parts, "exhibit high antioxidants" [37,44]. Meanwhile, the roots have a high antioxidant concentration (FRAP (82.53%), DPPH (82.5%), and ABTS (96.41%)) [43]. It has been reported that treatments of 0.1% sodium benzoate (T3) and salicylic acid 2 mM + calcium chloride 1% (T2) can effectively enhance antioxidants and bioactive compounds such as total anthocyanins and ascorbic acid. These treatments can also increase total phenolic-inhibited polyphenol oxidase activity and decrease the microbial load in phalsa fruit [54]. In the study by [55], it was found that the most effective pure solvent for extracting antioxidants was methanol followed by acetone, ethanol, and water. This was due to the interlinkage (hydrogen bonds) between solvents and the polar sides of antioxidant molecules, which improves the solvents' ability to dissolve the antioxidant components that are found in berries. Additionally, methanol is more efficient for the extraction of antioxidants compared to ethanol, although they have equal polarity. This could be because of the low solvation that is provided by ethanol, as a result of the longer ethyl radical than the methyl radical, resulting in the lower solvation of antioxidant molecules [56]. Since acetone molecules are solely proton acceptors, while the other solvents (methanol, ethanol, and water) are solely proton donors [56], acetone had the lowest recovery of antioxidant molecules due to their reduced solvation efficiency [56,57]. Additionally, the bioassay-guided fractionation of phalsa fruit extracts proved to be promising for obtaining bioactive fractions bearing significant in vitro antioxidant activity, suggesting that chlorogenic acid, caffeic acid, gallic acid, and morin represent the key components responsible for the cytotoxic effect on lung, breast, and laryngeal cancer cells [58]. The methodology followed for inclusion and exclusion is presented in Figure 2.

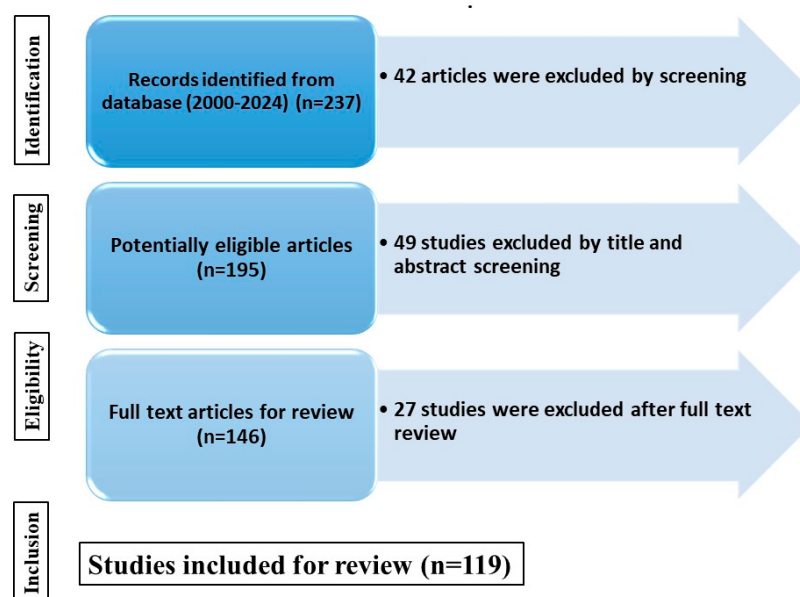


Figure 2. The methodology followed for inclusion and exclusion.

3. Effect of Solvent on Extraction Techniques

The solvent composition and extraction time, length, temperature, storage conditions, and solvent-to-solid ratio were the primary areas concentrated on in order to optimize the extraction process. The quantity and makeup of the extract, as well as the antioxidant capacity as evaluated, are significantly influenced by these factors. The bulk of the phenolics must be removed, and this must be ensured. To extract the antioxidant chemicals from various plant sources, a variety of solvent types are employed. Acetone, methanol, and their water mixtures, whether acidified or not, are the most often used solvents for extracting phenolic compounds from fresh food at various concentrations [59,60]. Antioxidant capacity and the total phenolic content were examined, and these values were considerably impacted by the extraction solvents' characteristics. Phenols are frequently extracted from plant sources using mixtures of water and ethanol [61,62]. This is because aqueous ethanol mixtures can dissolve a wide variety of phenols. Moreover, ethanolic mixtures can be used in models for human ingestion. However, for the extraction of polar antioxidants, acetone–water combinations make effective solvent systems [63,64]. Methanol, followed by water, acetone, and ethanol, was the most effective pure solvent for extracting antioxidant chemicals. The antioxidant qualities of fruits and vegetables are often primarily due to phenolic substances [65]. Most of these substances fall within the category of hydrophilic antioxidants [66]. This was confirmed by [67], who found that certain fruits, particularly berries, had higher hydrophilic values than others. Even though their polarities were identical, ethanol proved less effective than methanol at extracting antioxidant chemicals. This may be because ethanol has a poor solvation capacity, likely because of the longer ethyl radical than the methyl radical present in methanol, which results in the reduced solvation of antioxidant molecules. Acetone molecules are solely proton acceptors, but other solvents like methanol, ethanol, and water are also proton donors. As a result of their reduced effectiveness in solvation, acetone molecules result in the lowest recovery of antioxidant chemicals.

4. Characterization of Extracted Bioactive Compounds

The typical functional groups present in bioactive substances that are vital for biological activity include amino ($-\text{NH}_2$), hydroxyl ($-\text{OH}$), carbonyl ($\text{C}=\text{O}$), carboxyl ($-\text{COOH}$), sulfhydryl ($-\text{SH}$), phosphate ($-\text{PO}_4$), ester ($-\text{COO}$), epoxides, amines, and nitriles. The molecular and atomic arrangement of individual molecules, as well as their interactions, are referred to as the microstructure of bioactive substances. The microstructure of bioac-

tive chemicals is important for their biological activity because it specifies how molecules interact with biological systems. Depending on their chemical structure and how they are produced, bioactive substances can have a range of microstructures. The microstructure of bioactive substances influences their solubility, stability, and bioavailability. Bioactive substances that are highly soluble in water, for example, are more easily absorbed by the body, whereas those that are insoluble or weakly soluble may need to be modified to improve their bioavailability. The microstructure of bioactive compounds influences their biological activity, and knowing the microstructure of these compounds is vital for designing novel medications and cures. The process for determining the crystal structure of bioactive compounds involves exposing a sample to an X-ray beam and observing the diffraction pattern that results. The diffraction pattern exposes details about the crystal structure of the sample, including the angles between atom planes, interatomic distances, and crystal lattice symmetry. A bioactive substance’s crystal structure may be determined using XRD, which is essential for comprehending its physical and chemical properties and biological activity. For instance, the crystal structure of a drug may affect its solubility, stability, and bioavailability. Understanding the crystal structure might help formulators create more potent dosage forms. The microstructure, crystallinity, and presence of functional groups in various bioactive compounds is presented in Table 3.

Table 3. Microstructure, crystallinity, and presence of functional groups in various bioactive compounds.

Characterization Techniques	Key Findings	References
SEM	The morphological structure of nanoparticles at different resolutions at 0.5, 1, and 5 μm scales was observed in berry extract prepared using powder under different concentrations and temperatures.	[68]
XRD	Crystallinity of fiber in raw and defatted powder under different temperatures, conditions, and concentrations shows a broad peak at an angle of 18° .	[69]
FTIR	FTIR was used for identifying the functional groups in different spectra of $4000\text{--}400\text{ cm}^{-1}$ with a resolution of 2 cm^{-1} . The starch film in berries created a hydrogen bond and was displayed under an absorption band.	[70]
NMR	NMR revealed the proton environment and produced a graph of the correlation between intensity and absorption frequency. Different acids were observed in fruit berries (caffeic acid, kaempferol, 5-hydroxyferulic acid, 3,4,5-trihydroxycinnamic).	[71]

4.1. SEM (Scanning Electron Microscopy)

SEM analysis studied the size of the produced nanoparticles at 5, 1, and 0.5 μm scales to determine their surface and shape morphology (at different resolutions). This may be due to the fact that certain nanoparticles grouped together and created larger nanoparticles when solvent evaporated from them. In a study on a JEOL Model JSM-6390LV SEM, an analysis of SEM data was performed. Images were then obtained at various magnifications after a sample drop was dried on a copper grid covered with carbon for analysis. The JSM-7600F from JEOL was used to analyze the FESEM at 50,000 $\times$ magnification and 10 kV of accelerating voltage [67]. The powder was made under various circumstances with varying concentrations and inlet temperatures. Many changes in the morphology of the particles were seen. The aggregates did not vanish as the inlet temperature rose, but the particles had a smooth surface, and their shape was amorphous [68].

4.2. XRD (X-ray Diffraction)

In a study, tests using X-ray diffraction were conducted to examine the fiber crystallinity index present in the raw and defatted powder of berries. A BRUKER AXS D8 ADVANCE (BRUKER KAPPA APEX II) machine, operating at a 30 mA current and a 40 kV voltage, was used to conduct the XRD analysis. Cu, K radiation was applied to the sample at a speed of 5°/min in order to produce two data [72]. The crystalline plane of face-centered cubic silver was clearly visible in the XRD pattern of the synthetic fruit extract of the berries. Diffractograms of berry powder were made at various temperatures, concentrations, and inlet conditions. The diffractograms showed a broad peak of low intensity at a diffraction angle of about 18°. This could be attributed to the number of neutral polysaccharides having just one sharp acidic polysaccharide possessing three peaks. The sample had a single peak around 19°, and after defatting, more peaks at 12°, 19°, and 24° were seen (defatted berry powder) [69].

4.3. FTIR (Fourier Transform Infrared Spectroscopy)

Fourier transform infrared spectroscopy is a crucial instrument for quickly and efficiently identifying the functional groups contained in organic compounds using only a limited number of samples. In the FTIR spectrum, many functional groups, such as hydroxyl, oxirane, alkoxyl, carbonyls, carboxyl, and many others, display their absorption bands [70]. Information on inter- and intramolecular hydrogen bonds, as well as details about the nature and different types of amines, such as primary or secondary, are also provided by FTIR spectrum analysis. According to Sharifi-Rad and Pohl explanation, FT-IR spectra were scanned between 4000 and 400 cm^{-1} in transmittance mode with a resolution of 4 cm^{-1} [73]. FTIR measurements on berry extracts were made using a Nicolet 6700 (Thermo Scientific Co. Waltham, MA, USA) in the mid-infrared range (4000–650 cm^{-1}) to determine each sample's chemical composition. Moreover, when added to starch films, natural plant extracts like berry extract can create new hydrogen bonds, changing their interior structure. When natural plant extracts are added to starch films, changes in the hydrogen bonds and intermolecular interactions can be seen in the FTIR spectra [74].

4.4. NMR (Nuclear Magnetic Resonance)

Nuclear magnetic resonance (NMR) reveals an object's physical, chemical, and biological properties. A sample can absorb radiofrequency electromagnetic radiation under the right magnetic field conditions with the spectroscopy method. Some molecules' nuclei play a role in absorption. Specific markers and H-NMR spectra enable us to measure the proportion of amino acids for the analysis of a sample. Lactic acid is formed after malo-lactic acid fermentation, succinic acid is a subordinate product of ethanolic fermentation, acetic acid induces the acetic fermentation process, and tartaric acid makes salt with minimum solvability [74]. An NMR spectrum is a graph of peak intensities versus absorption frequencies. It is an effective method for resolving the proton environment in molecules and can also identify nuclei such as ^{13}C , ^{15}N , ^{17}O , and ^{19}F . Caffeic acid,

kaempferol, 5-hydroxyferulic acid, and 3,4,5-trihydroxycinnamic acid were discovered by the NMR-based analysis of fruit berries, in addition to relatively trace levels of quercetin, *p*-coumaric acid, chlorogenic, and myricetin acid. The residues’ protons between C-2 and C-6 were given NMR spectra. The NMR spectrum of a plant or fruit extract can be divided into three main regions: (a) the region from H 0.5 to H 3.2 ppm, where the majority of signals come from amino acids and organic acids; (b) the region from H 3.2 to H 5.5 ppm, where the signals of carbohydrates are located; and (c) the region from H 5.5 to H 10.0 ppm, also known as the phenolic region [71].

5. Different Non-Thermal Methods Used for Extraction of Bioactive Compounds

Non-thermal methods are used to extract bioactive components from food sources, including plants, fruits, and vegetables. The bioactivity and stability of the extracted compounds are preserved using these methods, making them superior to thermal processing. There are several different kinds of non-thermal extraction methods, including ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, enzyme-assisted extraction, and pressurized liquid extraction. Ultrasound-assisted extraction uses high-frequency sound waves to create cavitation bubbles in the extraction solvent, which enhances mass transfer and facilitates the removal of bioactive compounds. Bioactive components such as phenolic compounds, flavonoids, and essential oils are extracted using ultrasound-assisted extraction. Carbon dioxide and other supercritical fluids are used in the supercritical fluid extraction method because of their exceptional solvating properties, which allow for the highly selective and efficient extraction of bioactive compounds. Lipids, carotenoids, and terpenoids are examples of bioactive compounds extracted with supercritical fluid extraction. The extraction of bioactive compounds may be sped up by microwave heating of the extraction solvent. Bioactive compounds including polyphenols, alkaloids, and essential oils are extracted via microwave-assisted extraction. Bioactive chemicals may be extracted more efficiently by using enzymes to speed up the breakdown of cell walls and other barriers in the natural source. Polysaccharides, proteins, and peptides are some of the compounds that may be extracted with enzyme-assisted extraction. The pressurized liquid extraction method enhances solubility and extraction efficiency by employing high-pressure solvents to remove bioactive compounds from their natural sources. Flavonoids, terpenoids, and alkaloids are some of the bioactive compounds that can be extracted with pressurized liquid extraction. Shorter processing times, higher extraction efficiencies, and enhanced bioactivity and stability of the extracted compounds are the advantages of non-thermal extraction techniques over traditional thermal extraction methods. The roles of various non-thermal techniques in the extraction of bioactive compounds are stated in Table 4.

Table 4. Roles of non-thermal techniques in the extraction of bioactive compounds.

Non-Thermal Techniques	Bioactive Component Techniques	Mode	Advantages	Reference
Supercritical CO ₂ extraction	Extraction of phenolic compounds and fatty acid from berries using CO ₂ as a solvent and ethanol as a co-solvent	The extraction process produces value-added products with low critical temperature and pressure (31.1 °C, 7.39 MPa). Volatile secondary metabolites are used to extract polyphenol compounds.	Environmentally safe Inexpensive Low toxicity Low polarity	[75,76]
Microwave assisted extraction	Polysaccharides, methoxyl group of pectin, extraction of polyphenolic compounds using acetone and ethanol as solvents.	It employs electromagnetic waves with frequencies between 300 MHz and 300 GHz with wavelengths of 1 cm to 1 m to dehydrate berries with strong antioxidant capacity.	Homogenous Less solvent consumed Improved extraction yield	[77–79]

Table 4. Cont.

Non-Thermal Techniques	Bioactive Component Techniques	Mode	Advantages	Reference
Ultrasound-assisted extraction	Extraction of polysaccharides and phenolic compounds from fruit peel using acetone and ethanol as solvents by the UAE method	The ability to extract solvents using ultrasonic waves (20–100 kHz) is considered a rapid process for the extraction, crystallization, emulsification, homogenization, and enzyme inactivation of berries with higher phenolic and sugar levels	Improved efficiency Versatile Environmentally friendly Increased extraction yield	[80,81]
Pulsed electric field	Phenolic compounds and antioxidants extracted using aqueous extract from fruit parts	It involves two electrodes, and an electric field is applied in a pulsed manner with pulse amplitudes ranging from 10 to 80 kV/cm. PEF treatments resulted in an increase in anthocyanin content due to enhanced extraction, improved quality and microbial inactivation in sour cherry juice.	Less energy required Non-toxic Less solvent required Improved productivity Environmentally friendly	[82–84]
Pressurized liquid extraction	Polyphenolic compounds extracted from berries using ethanol and water as solvent	To remove the biological components, high pressure (3.3–20.3 MPa) and high temperature (40 °C–200 °C) requires low quantity of solvent.	High yield Less solvent consumed Reliable	[77,85]

5.1. Supercritical CO₂ Extraction

Supercritical fluid, often referred to as dense gas, is a material that, at its critical point, possesses the physical characteristics of both a gas and a liquid. Temperature and pressure are the two basic factors that push a material into its critical area [86]. Also, because CO₂ is readily available and inexpensive and has comparatively low toxicity, supercritical CO₂ is the best solvent for non-polar compounds. Its extraction from polar substances is facilitated by the addition of tiny amounts of ethanol and methanol [87]. Moreover, these solvents are used to extract phenolic compounds and oil from berries. Adjusting the temperature and pressure or adding modifiers allows the S-solvent strength to be altered, eventually shortening the extraction time [86]. Supercritical carbon dioxide is the most used SFE due to its low critical temperature and pressure (31.1 °C, 7.39 MPa) and its non-toxic, noncorrosive, and non-flammable nature [88]. The Food and Drug Administration considers it to be widely recognized as safe. Because CO₂ may be recycled and added to the process and oxidation is also prevented during operations, its usage for human consumption is safe, secure, and environmentally benign. The temperature of the process, the fluid's pressure, the supercritical CO₂ flux, and the length of the extract's supercritical extraction time all impact efficiency [75,89]. In a previous study, the CO₂ flow rate was maintained at 10 g/min. The extraction time was kept constant at 60 min. SC-CO₂ technology has been suggested as a viable non-thermal method to produce fruit and vegetable juices with high-value-added chemicals. Beetroot juice has been stabilized in many natural beverages, including strawberry juice [76]. A diagrammatic representation of supercritical fluid extraction is presented in Figure 3.

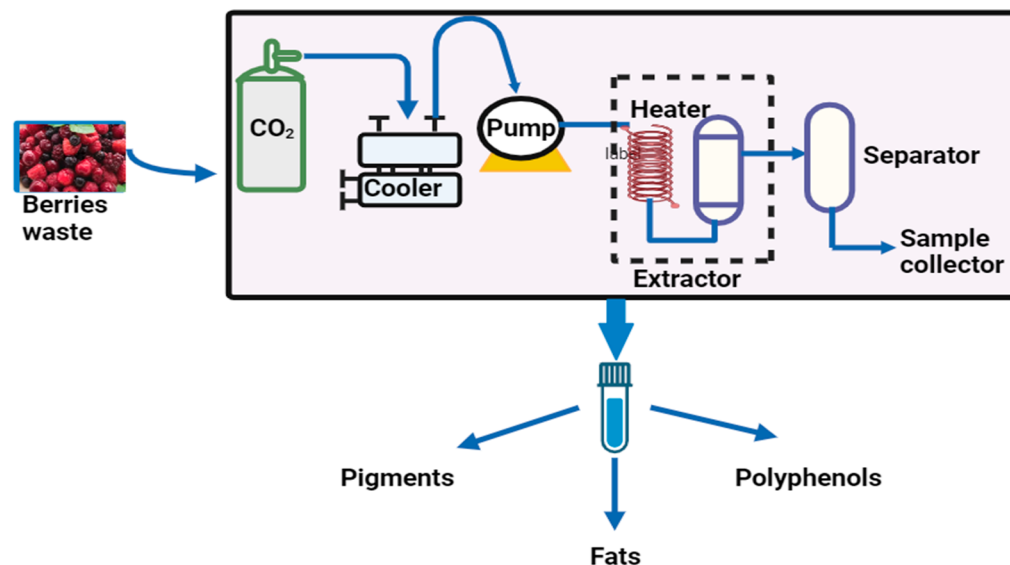


Figure 3. Mechanism of supercritical fluid extraction method for extraction of bioactive compounds.

5.2. Microwave-Assisted Extraction (MAE)

The microwave extraction method has been found to be particularly helpful in the field of green chemistry for extracting value-added chemicals. Moreover, it is a form of non-thermal technology that employs electromagnetic waves with frequencies between 300 MHz and 300 GHz produced by both electric and magnetic fields [77]. As a result of microwave energy heating polar food components through dipole rotation and ionic conduction, the rate of extraction is directly correlated with the solvent's dielectric susceptibility and matrix in food. It is possible to think of MAE as a selective technique that favors polar molecules and liquids with high dielectric constants [90]. Using the natural moisture found in fruit, mass and heat transfer events occur from the berry matrix to the liquid medium in the same direction. Intracellular material is discharged into the medium as a result of cell injury [91]. The most notable benefit of MAE is quick heating with less equipment [91]. As a result, less solvent is used during the extraction process, which automatically minimizes the amount of CO₂ released into the atmosphere [92]. Methanol and ethanol are solvents that have been effectively used to extract polyphenols, methoxyl groups of pectin, and polysaccharides from a variety of berries in an economical and environmentally friendly manner [83]. According to [83], microwave energy absorbed by food materials is turned into volumetric heat for microwave drying, which instantly elevates the temperature to accelerate the diffusion of moisture from inside to outside evaporation. In order to dehydrate berries, microwave technology has been successfully used [93]. Because of the lack of air during dehydration, a vacuum may hasten evaporation and stop polyphenol oxidation. According to [78], freeze-drying led to similar retention of anthocyanins and antioxidant activity that was relatively higher than that obtained from cranberries that were dried by hot air. SC-CO₂ leads to increased quality of the product, maximum anthocyanin content, and strong antioxidant capacity when paired with heat, and microwave-assisted drying has succeeded in dehydrating berries [79,93].

5.3. Ultrasound-Assisted Extraction (UAE)

The most extensively used non-thermal approach in the industry is ultrasound-assisted extraction, which has several characteristics and is appropriate for a variety of industrial applications, including the food industry [80]. The UAE method benefits from the ability to extract solvents using ultrasonic waves (20–1000 kHz). Due to its rapid extraction period, power ultrasound has a wide range of applications in the extraction of bioactive chemicals from plants [94]. This approach has received attention because it is easy to use, requires little equipment, and is more efficient than solvent extraction due to lower heat

and solvent costs [91,95]. It is presented as a more environmentally friendly extraction technique by utilizing various solvents (methanol, ethanol, and acetone) [96]. Additionally, the use of these solvents yields the maximum number of polysaccharides, phenolic content of polysaccharides, phenolic compounds, antioxidants, and anthocyanin content with a blend of solvents such as acetone, ethanol, water, and alcohol from whole berry fruits [97]. Because of these characteristics, ultrasound is a useful technique for the extraction of berries and the crystallization, homogenization, emulsification, and enzyme inactivation processes [81]. As an example, grape juice, red grape juice, and berry juice all had higher phenolic and sugar levels, as well as superior color density, thanks to ultrasound [52]. In comparison to conventional solvent extraction, optimized ultrasonic-assisted extraction produced higher yields of total phenolics and anthocyanins from berry wine pomace [98].

5.4. Pulsed Electric Field (PEF)

Pulsed electric field is a non-thermal method of extraction in food processing that emits brief electric pulses for a very short time. The electric field is applied between two electrodes in a pulsed manner with a repeating cycle, with pulse amplitudes ranging from 10 to 80 kV/cm [82]. Electroporation is the process of creating reversible or irreversible pores in a substance by applying pulses through electrodes that may cause tissue softening and the destruction of cell integrity [98]. This pore-formation mechanism improves the mass transfer of intracellular components by increasing the efficiency of solvent diffusion in sample tissues [99]. Moreover, these solvents are used to extract phenolic and antioxidant compounds from berry parts, which improves productivity and is considered environmentally friendly, and are non-toxic in nature [84]. PEF has thus been used in a variety of berry processes, including grape wine pre-treatment and sterilization [100], and berry juice [91]. Several studies have demonstrated the effectiveness of PEF in preserving fluids, and most of them have found no discernible effect on anthocyanin levels. While effects were found in sour cherries [84], no effects were demonstrated in berries [91]. These findings suggest that when applied to a complex matrix like fruit juice, PEF treatment has no negative effects on the stability of anthocyanins. In one study, if the juice matrix has a protective impact on anthocyanins, the field strength applied was significantly lower; however, when PEF was applied to berry mash, berry juice showed an increase in anthocyanin content due to enhanced extraction [83]. The pulsed electric field extraction process is illustrated in Figure 4.

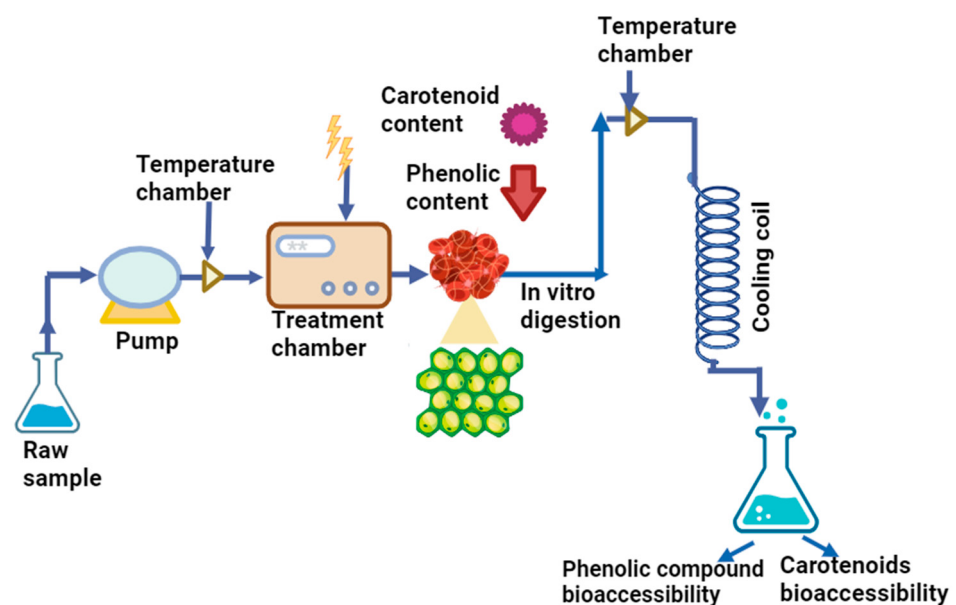


Figure 4. Principle of pulsed electric field extraction for extraction of bioactive compounds.

5.5. Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction, also called accelerated solvent extraction (ASE), integrates the thermal solvent extraction liquid and pressure application [101]. When using water as the solvent, this procedure is also referred to as rapid solvent extraction, high-pressure solvent extraction, pressurized hot water extraction, or subcritical water extraction [102]. The strategy aims to extract a wide variety of natural chemicals by using solvents with various polarities. In order to boost the solubility and mass transfer qualities, it uses an organic solvent at high temperatures (over the boiling point) and increased pressure. As a result, the least amount of solvent is needed to properly penetrate the solid sample without compromising the bioactive component. Compared to traditional extraction techniques, this method is quick and requires less solvent. The effectiveness of liquid extraction under pressure depends on the solvent's viscosity, the forms of the matrix, and the parameters, including the extraction temperature and time [103]. High temperature (40 °C–200 °C) and high pressure (3.3–20.3 MPa) are used to remove the biological components. A low quantity of solvent requires a combination of temperature and pressure. This technique can benefit from the field of extracting thermolabile chemicals [77]. PLE is a useful alternative to extract phenolic compounds from berries using ethanol and water as the solvents and results in a higher yield with less consumption of the solvent [85]. An accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA), outfitted with a solvent controller, was used for PLE in berry fruits. In accordance with the experimental strategy indicated in the next section, extractions were conducted at various extraction temperatures and using different green solvent mixtures (ethanol/water). The constant extraction parameters were 20 min and 1500 psi, respectively [85].

6. Future Scope and Limitations

Prospects for extracting bioactive components from phalsa berries include investigating novel methodologies and addressing current challenges to improve effectiveness, sustainability, and practicality. Optimizing process parameters such as ultrasonic frequency and intensity, microwave power and duration, and enzyme concentration can enhance extraction efficiency while maintaining bioactivity. Investigating ecologically conscious and sustainable extraction techniques, such as employing non-toxic solvents, green solvents, or solvent-free processes, can effectively reduce the negative effects on the environment. An exploration of innovative methods, such as subcritical water extraction or pressurized hot water extraction, can be carried out to extract bioactive compounds from phalsa berries. An exploration of combined or sequential extraction strategies that utilize a variety of extraction techniques (such as ultrasound-assisted extraction followed by microwave-assisted extraction or enzyme-assisted ultrasound-assisted extraction) can effectively increase the amount of extracted material and enhance its biological activity. Thorough analyses and the identification of certain biologically active substances found in phalsa berries can be conducted through the utilization of advanced analytical methods like HPLC-MS/MS, GC-MS, or NMR spectroscopy. An investigation needs to be conducted into the utilization of encapsulating methods, such as nanoencapsulation and microencapsulation, to enhance the durability, absorption rate, and regulated discharge of bioactive substances derived from phalsa berries. Further, there is a need to investigate the application of biotechnological methods, such as metabolic engineering or fermentation, to improve the synthesis of bioactive substances in phalsa berries. Genetic modification or breeding efforts can be conducted to enhance the levels of specific bioactive chemicals while also preserving favorable agronomic characteristics. There is need to expand scaled-up extraction procedures to produce phalsa berry extracts for commercial usage in functional foods, dietary supplements, cosmetics, and pharmaceuticals. Exploring these prospects will help harness the complete potential of phalsa berries as a valuable reservoir of bioactive chemicals with varied applications in the culinary, health, and pharmaceutical sectors.

There are various constraints associated with the process of extracting bioactive components from phalsa berries, which can impede effectiveness, productivity, and the mainte-

nance of bioactivity. Phalsa berries possess a resilient outer peel and seeds, which impede the extraction process and inhibit the release of bioactive compounds. Conventional extraction techniques may not effectively disintegrate cell membranes and liberate intracellular constituents, leading to suboptimal extraction outputs. During the process of extraction, the bioactive compounds found in phalsa berries may experience deterioration because of being exposed to heat, light, oxygen, or enzymatic activity. Ensuring the ideal extraction conditions to avoid degradation while optimizing yield is crucial but can be challenging to accomplish. Although specific extraction methods may yield positive results in a controlled laboratory environment, the process of expanding these procedures to a larger commercial scale can present difficulties. For the large-scale manufacture of phalsa berry extracts, it is important to consider factors such as the cost of equipment, the ability to scale the process, and the consistency of product quality.

7. Conclusions

Phalsa (*Grewia asiatica*) fruit has been employed as a stomachic, a cooling agent, and an astringent in traditional folklore medicine. Several industrial businesses are already generating various “antioxidant” concentrates as interest in functional foods and other items with potential health impacts grows. Industrial businesses include established juice manufacturers, large companies specializing in natural colors and flavors, and emerging businesses focusing on health-promoting nutrients. Various effective strategies have been developed to extract antioxidants from such plant parts creatively. The most popular method for extracting antioxidant chemicals from red berries (fruits) on an industrial scale is conventional solvent extraction, although this process uses a lot of resources. To reduce the energy and solvent requirements, new non-traditional technologies have been revealed to be ecologically friendly alternatives to the earlier methods, like ultrasound, pressure-assisted extraction, and microwave applications.

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