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GREEN EXTRACTION TECHNIQUES FOR PLANT DERIVED BIOACTIVE COMPOUNDS

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ABSTRACT

Combating with food insecurity, malnutrition and achieving zero waste for the sustainable environment, there has been a surging interest in exploration of alternative techniques for extracting bioactive compounds derived from plants. Green extraction techniques have demonstrated effectiveness and economic affordability in improving the extraction of different plant derived bioactive compounds. In recent years, emphasis on good health and mental well-being has sparked a rising interest in bioactive compounds in food industries. These bioactive compounds have gained much more popularity due to their numerous health benefits. Due to this reason, the demand for these compounds has also increased, whether it extracted from edible or non-edible sources. This paper examined advanced techniques utilized in the extraction process, with a focus on their effectiveness, and specificity such as ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), enzyme assisted extraction (EAE), pulsed electric field (PEF), pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE). The concepts, benefits, and challenges of each technique have examined within the context of extracting bioactive substances, including polyphenols, alkaloids, carotenoids, flavonoids and essential oils. These approaches offered various advantages in terms of enhanced extraction efficiency, low solvent consumption and reduced processing time that would be able to meet the growing demand for sustainable decision-making in industries such as food, and pharmaceuticals. Moreover, the incorporation of green chemistry idealized to reduce the amount of solvents used and energy consumption have been emphasized, demonstrating a sustainable approach for extraction of bioactive compounds from plants. Polyphenols extracted from each technique with the specified plant source and their results have been mentioned. Lastly, this article discussed the present challenges and future recommendations in the field, to promote ongoing innovation and awareness of these methods in improving the extraction of new bioactive compounds from plant resources.

INTRODUCTION

Availing the numerous health benefits of phenolic compounds includes resistance against stress of oxidation and chronic diseases; phytochemicals has become a vital necessity **(1).** There are a number of plants that have been used to cure diseases, termed as medicinal or herbal plants, which have boost the international worth of these plants for trade in global marketplace. According to World Health Organization (WHO) reports, a major chunk of word population above 80% cured their ailments through the help of herbal plants and its economic contribution calculated in 2010 is about 60 billion dollars which is expected to rise upto 5 trillion dollars by the end of 2050. The herbal plants have gained significant importance due to its curing impacts on human health as antimicrobial, relief from inflammation, allergy as well as its antithrombotic and neuroprotective effect due to the presence of nutraceuticals **(2).**

The primary and secondary metabolites include dietary fiber protein, polysaccharides as well as phytochemicals constituting polyphenols, carotenoids, alkaloids and terpenes can be obtained from agricultural waste and food **(3).** A major portion of the agro waste has been found in the form of fruits and vegetable peel, stems, seeds and pomace which constituted about 25-30%. Due to the lack of waste management system, this waste, in spite of having beneficial bioactive compounds that have been used to fight against food insecurity and malnutrition, was being built up causing socio-economic and environmental threats. The proper system was required for this waste either utilized for extraction of nutrients or disposal so that it became harmless to humans **(4).**

According to the Food and Agriculture Organization (FAO) one-third of the food produced for human feed has been wasted following the harvest that was estimated to be about 1.3 billion tons of value 1 trillion US dollars FAO (2019, 2021). Accounting of the social and environmental impacts of this food waste would have charged about 2.6 trillion US dollars yearly. 670 million tons in industry and 630 million tons food was being wasted in the developing countries **(5).** This waste was estimated to reach upto 138 million tons by the end of 2025 causing a substantial threat on the loss of energy, portable water, agricultural production area and man labor **(6).**

Solvent extraction, maceration, percolation, decoction, digesting, and infusion are examples of extraction techniques. These conventional approaches were out of date since they often required an abundance of organic solvents, demand a long period of time, and yield minimal **(7).** It has limitations which include use of corrosive chemicals and harsh conditions such as temperature yet low product yield, devalue product quality and bioactivity **(8).** Owing to shortcomings of the various conventional methods, researchers have been striving to come up with a standardized technique swift and proficient purification and separation of phenolic compounds from plant base **(1)**. Standardized production was the ultimate goal without cutting back on the product efficacy, accessibility and reliability, in implementing the technology used to extract bioactive compounds from food sources. The Research has done for employing novel extraction techniques owing to the consumer preferences for non-toxic products as well as the sustainability concerned of these techniques for industrial sector **(9).** Kamaruddin *et al*. (2023) provided the valuable justification for novel extraction techniques include restrict organic solvent usage, least energy expenditure, brief extraction periods, considerate sourcing, production of valuable residual products and 100% organic and safe extract. The industrial production sustainability has improved by minimizing the usage of renewable resources along with the omission of pollutants and detrimental products production **(10).**

Novel extraction techniques which were more sustainable, economical and effective have been discovered by technologists keeping in view the underutilization of these phenolic compounds of plant based **(1).** Sustainably developed technology termed as the "green technologies" that favored both the economic and social well-being **(10).** These novel techniques were profitable in terms of minimizing the usage of toxic solvents, shorten the extraction time, automation for recurrent extraction process whilst using least energy consumption and increased recoverable extraction yield **(11).**

Novel extraction techniques included ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), electro technologies-assisted extraction like pulsed-electric fields (PEF) and pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and enzyme-assisted extraction (EAE) were used to separated and purify these polyphenols **(12).** Novel extraction techniques were more favorable, because of its properties like least solvent, time and energy consumption along with higher yield with less functioning expenses for the separation and purification of temperature sensitive polyphenols, over strenuous and lingering conventional techniques. By equipping ourselves with advance knowledge to better the novel extraction methodologies, futuristic study was desired to eradicate procedural obstructions of industries **(13).** Solvent has been an important component because of the combustible and toxicity issues, so always pay heed in selecting the right solvent as medium. Proficiency, bio enosis and cost of extraction process should have also been considered **(14).**

This review has comprehensively focused on the novel extraction techniques, comparison to the conventional techniques, their benefits, applications for purification of phenolic compounds from plants and the limitations of these techniques. The main aim was to describe these techniques like UAE, MAE, EAE, PLE, SFE and PEF for the extraction of different bioactive compounds, solvent and conditions used for the desired extraction results to reduce the agro waste and their utilization for the welfare of mankind.

Comparison between conventional and novel extraction techniques

Techniques like Soxhlet extraction, percolation, maceration, decoction and hydro-distillation were termed as the conventional methods have been used to obtain bioactive compounds from agro waste but these techniques were not eco-friendly while novel extraction techniques were more reliable in terms of solvent, energy and temperature usage **(15).**

Advantages	Novel VS Conventional	Disadvantages	Ref
Reduced extraction time, low solvent use, increased mass transfer, temperature control, preservation of heat sensitive compounds, ecological and inexpensive	UAE VS Con.	Invasive process, intensive labor and attention, limited extraction efficiency filtration required difficult to scale up	**(16)**
Rapid, selective and uniform heating, low organic solvent, high effectiveness, accelerated extraction process, wide range of applications	MAE VS Con.	Chemical modification phenolic compounds by high temperature and pressure application, clean up mandatory, filtration required, expensive	**(16)**
Non thermal, increased cell permeability, preservation of heat sensitive compounds, selective extraction, low organic solvent use, accelerated extraction process, inactivation of microorganisms	PEF VS Con.	High equipment cost, require significant amount of electrical energy, limited application range, cell membrane can be reversible and irreversible during electroporation mechanism	**(16)**
Extraction rate can be enhanced by increasing solubility and mass transfer rate which is due to decreased viscosity and surface tension.	PLE VS Con.	Large amounts of solvents used, longer time duration and low extraction yield	**(9)**
It is mostly used for low polar compounds at low temperature results in higher extraction yield	SFE VS Con	Conventional techniques are usually thermal with lower extraction yield results in downgrading the extracted compounds	**(9)**
It facilitates extraction from plant tissue with higher efficiency	EAE VS Con	Thermal destruction of extracted compounds with longer time duration and lower yield	**(9)**

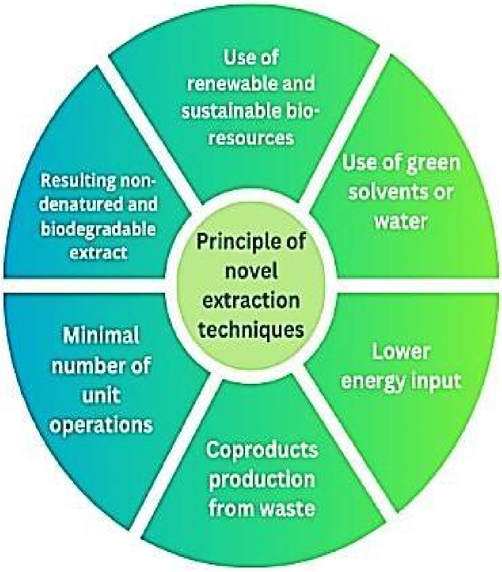
Table 1: Comparison between novel and conventional techniques

Novel extraction techniques

The extraction of bioactive chemicals from plant materials has been a crucial step in several industries, including food, pharmaceuticals, and cosmetics. Conventional methods such as maceration and Soxhlet extraction have been used for a long time; however novel techniques have been developed to improve efficiency and selectivity **(17).** Advanced extraction techniques such as microwave-assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction, and enzyme-assisted extraction offer improved efficiency and reduced extraction times, overcoming the difficulties caused by the insolubility of certain bioactive compounds **(18).** Incorporating ultrasound generation with standard methods have shown a potential to enhance extraction efficacy by facilitating mass transfer and cell rupture, ultimately leading to increased yield and improved characteristics of isolated chemicals **(19).**

The extracted polyphenols were generally recognized as safe (GRAS) and found its applications as nutraceuticals, preservatives, food additives, texture enhancers, flavor and coloring agents in functional foods, food industry, snack industry, meat and dairy products etc. Novel extraction techniques were contemporary solution to the problems of scientist, researchers and academia people that were working with the sustainable development goals (SDGs) to conserve environment, resources and provided feasibility to community **(4).**

Principles of Novel extraction techniques explained as



The emergence of advanced extraction methods, such as the utilization of deep eutectic solvents (DES) and herbal deep eutectic solvents (NADES), demonstrated as increasing focus on environmentally friendly and sustainable extraction processes. This progress enabled more efficient extraction of bioactive compounds from plant sources **(19).**

Ultrasound assisted extraction (UAE)

UAE was the novel extraction techniques that operate at high frequency (intensity) sound waves to separate and purify polyphenols from plants. These generated physical forces broke the plant cell wall and caused the discharge of phytochemicals into the medium by augmenting the transfer of mass within shorter time period **(20).** Cavitation bubbles were created due to high frequency around typical range of 20 kHz to 100 MHz, rapidly expanded and shrunk, breaking down the plant cells by localization of heating and cooling that caused the release of phytochemicals **(21).** UAE at low intensity having high frequency range 5-10MHz using low power of 1W/cm^2 was more compatible for the purification of food compounds rather than UAE at high intensity using low frequency ranges of about 20 to 100 kHz at power usage of 10 to 1000 W/cm^2 operated by cavitation methods for chemical reactions used for extraction, emulsification and crushing. UAE was a potential technique and found its applications in vast fields like medical testing, non-destructive testing, distance measurement, welding, cleaning, and food technology **(22).** UAE has emerged as a novel green technology and more economical to extract desirable bioactive compounds from plants than conventional methods but it has its limitation as well as factors affecting its overall product yield **(23).**

Table 2: extraction of bioactive compounds from plant sources

Under observation plant	Compounds Extracted	Treatment	Results and conclusion	Ref
Red cabbage	Anthocyanins	Using an ultrasound bath operating at 37 kHz frequency , extraction time (5–75 min), temperature (40–80 ◦C), Ethanol (5–75%) + water + formic acid (1%)	Anthocyanins about 58.67 mg/L obtaine with the optimized UAE method	**(19)**
Parsley Leaves	Flavonoids Apigesnin (9.48 ± 0.11)	30 minutes at 40 °C particle size: 0.25 mm ultrasonic power: 90%, frequency: 80 kHz Ethanol 80%	Flavonoids Apigenin obtained with 51.22% extraction efficiency	**(19)**

Blueberry	Phenolic, Flavonoid, and Anthocyanin	This extraction is done by UAE bath method at required power and frequency of 64 W, 35 kHz runs at temperature 20,40,60 °C for 30, 40, 60 and 90 minutes, Water and ethanol is used as solvent	High yield of TPC (22.33) mg/g, TFC (19.41 mg/g) and TAC (31.32 mg/g) obtained	**(24)**
Lime, orange, and tangerine Peels	Phenolic compounds	Ultrasonic bath works frequency at 60 kHz takes extraction time of about 30-90 minutes, Water content of peel (0 and 75%)	Total phenolic content obtained was 74.80 ± 1.90, 66.36 ± 0.75 and 58.68 ± 4.01 mg for Lime, orange and tangerine peel respectively	**(25)**
Strawberry-guava leaves	Phenolic compounds	Probe operates at frequency of 20 kHz coupled with a titanium tip of 4 mm diameter at 40, 50, and 60 °C, having power of 100, 300, and 500 W, Solvent ratio (1:10, 1:15, and 1:20 g/mL)	Solvent ratio and power dynamics greatly effects the UAE yield of phenolic compounds	**(23)**

UAE was optimized technique for the extraction of components like anthocyanins from red cabbage **(19),** Flavonoids Apigenin from parsley leaves **(19),** phenolic, flavonoid, and anthocyanin from blueberry **(24),** flavonoids from Lime, orange, and tangerine peels **(25)** as well as from strawberry-guava leaves **(23)** under the desired conditions as described in **Table 2.**

UAE was a novel green technology and more economical to extract desirable bioactive compounds from plants than conventional methods but it has its limitation as well as factors affecting its overall product yield **(23).**

Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) has been a relatively current, sustainable, and scalable technique for recovering highly valuable natural chemicals. It may have proved to be a promising option for extracting macro algal antioxidants or hybrid carrageenan. MAE enabled quick and consistent extraction, little equipment; rapid startup, reduced solvent and energy consumption, and ionic conduction and rotation of dipoles were two ways that microwaves affected molecules.

Higher extraction yields have been obtained by studying the environmentally friendly process of microwave-assisted water extraction to produce hybrid carrageenan, carbohydrate, protein, and antioxidant substances from Mastocarpus stellatus red algae. The characteristics of the retrieved soluble extracts have been demonstrated to be significantly impacted by microwave temperature, with the highest protein values appearing at 150 °C. Kappa carrageenan can create hard, strong, or brittle gels, whereas iota carrageenan frequently made soft, weak gels. As a result, hybridization has made it possible to create a sturdy elastic gel. There were numerous applications for kappa/iota hybrid carrageenan gels over various sectors. Among many other applications, the dairy industry could use it to make cakes, the cosmetics company to produce lotions or creams, the pharmaceutical industry to make semi-synthetic antibiotics, the chemical industry to form paints or shoe polish, the experimental medicine field to test anti-inflammatory medications, or the biotechnology sector to inhibit cells or enzymes **(26)**.

Microwaves frequencies ranging from 300MHz-300GHz and heated solvent were used to extract the phytochemicals perceived as the novel extraction technique. Microwaves interacted with polar molecules of tissue which has increased the temperature. Lower viscosity has eased the scattering and dissolution of charge particles. When cell wall of algae irradiated with microwaves, its cellular anatomy has dissociated that resulted in the release of organic compounds in the medium **(27).** MAE was beneficial technique for its quicker results, low consumption of solvent, economically efficient and higher extraction yield but the only drawback could be exhibited that was used low molecular phenols, quercetines and isoflavines with their stability lies within the range of microwave temperature **(28).** Microwaves were insensitive to the non-polar molecules having low dielectric constant like hexane than the polar molecules which have high dielectric constant like water and ethanol resulting in increasing the temperature of the system. Radiation duration time and power can also be used to control the temperature of the system **(29).**

Table 3: Applications of MAE for extraction of bioactive compounds from plant

Under observation plant	Bioactive compounds extracted	Treatment	Results and conclusions	Ref
Red cabbage	Anthocyanins	Extraction time (30 s, 1, 2, and 4 min), and microwave power (100, 300, 400, 600, and 800 W), Ethanol (0, 20, 40, 60, 80, and 100%)	Anthocyanin content of about 73.89mg/L obtained with optimized MAE	**(30)**
Olive leaves	Oleuropein Hydroxytyrosol VerbascosideEpigenin-7-glucosideLuteoline-7-glucoside	Temperature (80◦C),time(2min), Polypropylene glycol(PPG),water and lactic acid(LA)	Majority in the first 20 min, and to 80 min, there was a rise of 17.80% in TP,9.90% inoleuropein and 9.70% inverbascoside concen-trations; after 80min,the same numbers rose to 19.30% in TP,11.60%inoleuropein and 6.60% inverbascoside.	**(31)**
Carrots (juice waste)	Carotenoids	Operates at Microwave power of 165 W require extraction time of 9.39 min and oil to waste ratio of 8.06:1 g/g	MAE is suitable for extraction of carotenoids from carrot juice residue.	**(28)**
Broccoli leaves & Stalks	Phenolics & β-Carotenoids	15.04 mg/100g (Phenolics from stalk), 3.02 mg/100g (β-	MAE is suitable for extraction of phenolics and β-carotenoids with	**(32)**

		carotenoids from stalk)		
Citrus peel, rag & Seeds	Gallic Acid, Ferulic Acid, Vanillic Acid, Caffeic Acid, Naringin, Hesperidin, Neohesperidin, Luteolin, Sinensetin, Kaempferol, Lutein, Luteoxanthin, & Cryp-Tochrome	Peels dried at 70°C and extracted 500 mg portions with 10 mL of DMSO, hexane, methanol.	25.9 mg/100g (Gallic acid from peel), 206 mg/100g (Naringin from orange peel), 4117 mg/100g (Hesperidin from orange peel), 410 mg/100g (Carotenoid content from orange peel), 204 mg/100g (Carotenoid content from tangerine peel)	**(32)**
Tomatoes	Total polyphenolic content	At powers 360, 600, and 900 W at different time and temperature 40, 50 and 60 °C and 10, 20, and 30 min	TPC found in 272.58-453.08 mg/kg through MAE	**(33)**

MAE was useful because of its high extraction rate that boost the extraction yield but considered to be not no economical because of high consumption power and capital cost **(34).**

In this work, phenolic compounds from tomatoes were extracted using a novel technique called microwave-assisted extraction (MAE), which was contrasted with traditional solvent extraction. The phenolic components of the tomato were extracted using 80% methanol (Sigma) and 1% HCl (Honeywell, Germany). To determine the optimal conditions for tomato extraction, a multi-level factorial design was employed. For this, different powers (360, 600, and 900 W) and timings (30, 60, and 90 s) were used for MAE, while different temperatures (40, 50, and 60 °C) and times (10, 20, and 30 min) were used for CSE. The phenolic compounds in tomato extracts were identified using Fourier transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC). Because of their health benefits, which include intestinal anti-inflammatory activity, tumor suppression, and cancer prevention, bioactive compounds (flavonoids, anthocyanins, and polyphenols) were utilized in the food and pharmaceutical industries **(33)**.

MAE was one of the optimized novel techniques for the extraction of bioactive compounds (anthocyanins, oils, phenolic compounds) from different plant sources like red cabbage **(30)**, olive leaves **(31)**, carrots **(28)**, broccoli leaves & stalks **(32)**, citrus peel, rag & seeds **(32)** as well as from tomatoes **(33)** under the described conditions mentioned in **Table 3.**

Supercritical Fluid Extraction (SFE)

Supercritical fluid was formed when the physical properties of carbon dioxide was changed, after compressing and subjecting the gas to temperature, the ability of gas to be dispersive and the dissolution property of liquid was increased. Superfluid fluid extraction (SFE) was advanced extraction technique when the under observation parts of plants interact with supercritical fluid and extract the phytochemicals based on the solubilization differences. SFE was more preferable over conventional techniques because of their no need of organic solvent except CO2; a nontoxic gas, quick and rapid pre-treatment and extraction process, an automatic process, low temperature requirement and protect the sample from oxidation and was an operational extraction technique for industry. This techniques has some shortcomings e.g. worked at high pressure and rather expensive because of the equipment, high voltage utilization and coild not separate polar substances **(35).**

Low critical temperature and pression of CO_2 made it an ideal solvent as it can maintain bioactive compound integrity. Additionally it was non-toxic and non-explosive in nature with low cost and has high penetrating power to extract bioactive compounds from the plants to extract good yield of desired compounds. Other solvents like propane, methanol, cooking gas (LPG), ethane, ethene, nitrous oxide, sulfur hexafluoride, n-butene, n-pentene and water were considered as GRAS (recognized as safe) were used in the SFE. Supercritical fluid extraction was the most suitable technique to extract antioxidants from agro waste and found its application in food, pharmaceutical and cosmetic industry **(36).**

Table 4: Applications of SFE for extraction of bioactive compounds from plant

Under observation plant	Extracted bioactive compound	Treatment	Solvent used	Result & conclusion	Ref
Ginger	(Gingerols and 6-shogaol)	Time: 153 min, Pressure: 276 bar, Flowrate: 30 g min^{-1} Temperature: 40 °C, Size: 253 µm	Deionized Milli-Q water	36.97 wt % of gingerols and shogaol obtained through SFE extraction	**(37)**
Mango peel	Carotenoids	At 25.0 MPa pressure and 60 °C temperature	15% *w/w* Ethanol	Carotenoids obtained with 6.25% extraction yield through SFE	**(38)**
Onion residues	Oleoresin (Sulphur and pyruvate)	At conditions of 80 °C temperature, 400 bar pressure, 0.53 mm particle size and 60 min dynamic Time	CO_2 is used as solvent	1.012% oleoresin, 31 g sulphur content per kg of oleoresin and 10.41 µmole pyruvate per g were obtained	**(39)**
Apple	Phenolic compound like phloridzin in oil	Done at temperature 40°C, pressure24 MPa, time 140 min	CO_2 flow rate 1 L/hour	Phloridzin (2.96 ± 0.046 µg/gseed in SFE oil)	**(40)**
Mulberry	Extract and β-sitosterol	Done at 60°C, pressure 200 bar for 120 min	CO_2	Supercritical extraction resulted in the highest yield of extract about 1.08% and β-sitosterol about 1.11% wt%	**(41)**

High extraction yield of different bioactive compounds like Gingerols and 6-shogaol, carotenoids, oleoresin, phloridzin and β-sitosterol from different plant sources like ginger **(37)**, mango peel **(38)**, onion residue **(39)**, apple **(40)** and mulberry **(41)** respectively under the respective treatment conditions as described in **Table 4** with the help of novel extraction technique SFE with minimum solvent consumption.

Pulsed Electric Field (PEF)

Pores formation in cell membrane of cells through a non-thermal technique was known as Pulsed electric field. This was also termed as electroporation. The applied electric field has caused polarity in the components of cells which caused the attraction and repulsion between them. This created empty spaces called pores in the cells of observed plant. Pore formation may be reversible or irreversible depending upon the intensity of electric field. Size, type, shape of cell membrane, characteristics, process conditions determined the electroporation process **(42).**

Table 5: Applications of PEF for extraction of bioactive compounds from plant

Under observation plant	Extracted bioactive compound	Treatment	Solvent used	Result & conclusion	Ref
Red cabbage	Anthocyanins	22 ◦C, 4 h, 2.5 kV/cm Electric field strength,15 µs pulse width, 50 pulses, 15.63 J/g specific energy	Water	44 to 889 µg/Ml yield of anthocyanin obtained through PEF	**(31)**
Saffron (pomace and stigma)	Crocin, picrocrocin and safranal levels	5 kV of electric power, 35 of pulse number, and 100 µs of pulse width	----	Extraction yield results: pomace (5.76, 5.9 and 7.5%) and stigma (14, 10.2 and 15.5%) contain these compounds like Crocin, picrocrocin and safranal levels.	**(43)**
Potato peels	Phenolic compounds	Extraction done at time = 230 min, T = 50 °C	Ethanol = 52%,	PEF improved the extraction yield of phenolic content (10%) and antioxidant activity (9%) than the untreated sample	**(44)**
Custard apple leaves	Phenolic compounds (Purpureacin 2 and rutin)	Done at electric field strength 6kV/cm, 300 pulses, specific energies 142 kJ/kg for 5 min	Ethanol = 70	PEF improved the extraction yields of phenolic compounds (+5.2%) and the antioxidant activity than the untreated sample	**(45)**

The optimal conditions were used for the extraction of bioactive compounds from different plant sources e.g. red cabbage **(31)**, saffron **(43)**, potato peels **(44)**, custard apple leaves **(45)** using PEF have described in **Table 5.**

PEF was globally recognized for its advantages like non-thermal technology, preservation of thermolabile compounds, reduced processing time increased mass transfer, improved extraction yield but it has its limitations which included difficult to use with conductive materials, liquid medium is required, needed to be combined with heat to achieve higher extraction efficiency **(46)**.

Pressurized Liquid Extraction (PLE)

Liquefied solvents at high temperature and pressure were used for rapid and efficient extraction through pressurized liquid extraction (PLE). The solubility and bulk transfer properties of polarizable solvents like water and ethanol was increased when used at temperature above than the boiling point. PLE operated at high temperature and involved shorter duration for extraction than MAE but it may have resulted into the detrimental compounds like HMF. The efficiency of extraction may have increased at elevated temperature but the extracted compound started to degrade under these intense conditions **(35).** PLE worked on principle in which the solution resided in liquid state when the pressure was increased by keeping the temperature constant. Temperature ranged between 50C and 200C. liquefied solvents like water and alcohol were mostly used in this technique that was inexpensive, eco-friendly and nonpoisonous **(47).**

Table 6: Applications of PLE for extraction of bioactive compounds from plant

Under observation plant	Extracted bioactive compound	Treatment	Solvent used	Result & conclusion	Ref
Red cabbage	Anthocyanins	Temperature (80–120 ◦C), sample amount (1–3 g), extraction time (6–11 min), at a fixed pressure of 50 bar	Water + 5 vol% of ethanol + formic acid (0–5 vol%)	662 µg/g (±3) of anthocyanin content obtained with the optimized PLE method	**(48)**
Sweet potatoes	Antioxidant potential Total anthocyanins Total flavonoids	15 min, 2 cycles at optimum Temperature 90 ◦C	80 % ethanol	Desired results obtained from PLE technique with higher extraction yield of Antioxidant potential, Total anthocyanins, total flavonoids	**(48)**
Rosemary leaves	Rosmarinic acid Carnosic acid Carnosol	5-9 heat up + 3 min, 60 % at optimum T/P 183 ◦C/130 bar	Ethanol	Maximum yield of Rosmarinic acid, Carnosic acid and Carnosol obtained though PLE	**(48)**
Berries	Total phenolic compounds Phenolic compounds composition Antioxidant potential	5 min, 2 cycles, 50 % at optimum T/P 60 and 100 ◦C/103.4 bar	Water (pH 11.5)	Desirable yield of Total phenolic compounds, Phenolic compounds composition and Antioxidant potential of berries achieved	**(49)**

Chicory roots	Chlorogenic acid Dicaffeoylquinic acid	5–7 min heat + 30 min at optimum T 107 ◦C, 95 ◦C	46 % ethanol 57 % ethanol	PLE is suitable for extraction of Chlorogenic acid and Dicaffeoylquinic acid from chicory roots	**(50)**
Carrots	Total phenolic compounds	5 min at Optimum T/P 80 ◦C/103.4 bar	Water/ acetone/ ethanol	Results obtained through PLE have higher extraction yield of Total phenolic content	**(51)**

Studies on the eco-friendly technique of pressurized liquid extraction have demonstrated increased yields of bioactive compounds from varied plant sources like red cabbage **(48)**, sweet potatoes **(48)**, rosemary leaves **(48)**, berries **(49)**, chicory roots **(50)** and carrots **(51)** as mentioned in **Table 6**. The properties of the recovered soluble extracts were mostly determined by the extraction temperature; at 150°C, the greatest protein levels were found. PLE was one of the leading novel extraction techniques because of its benefits automated method, pressurized extraction solvent remained in liquid state at temperatures above its boiling point, enabled the use of a wide range of solvents and thus allows the extraction of a wide range of solutes of different polarities, low solvent consumption and reduced extraction duration, no filtration necessary but has its drawbacks as well like possible degradation of thermolabile analytes due to elevated temperatures especially when combined to long extraction durations **(46)**.

Enzyme Assisted Extraction (EAE)

Polyphenols were being release from cell by breaking the cell wall with the help of enzymes, particularly efficient for the compounds bounded within the cell medium, in enzyme assisted extraction process. This technique was operated at moderate condition and protected the purity and stability of sensitive compounds **(35)**. Right solvent selection and substrate specific enzyme was important for the extraction procedure to be effectively carried out. The enzymes like cellulase, pectinesterase, hemicellulase, fucosyltransferase, pectinase, α-amylase, and protease was used to break the cell wall to release polyphenols. Water, ethanol, acetone and their mixtures were used as solvents. Water has been a common solvent for the polar compounds extraction (47).

Table 7: Applications of EAE for extraction of bioactive compounds from plant

Under observation plant	Extracted compound	Treatment	Results & conclusions	Ref
Saba banana peel (Musa acuminata x balbisiana) or Kepok Kuning banana	Pectin	55 °C, pH 5.0 for 120 min using 0.103 g/mL pectinase Mixture of enzymatic hydrolysate and 95% ethanol (1:3 (v/v))	10.80 % extraction rate of pectin with EAE	**(51)**
Beetroot	Betalains betacyanin, and betaxanthin	Enzymolysis: Enzymatic mix 25 U/g containing cellulase (37%),	Yield (mg/mL U) betaxanthin 11.37 ± 0.45, betacyanin	**(52)**

		xylanase (35%), pectinase (28%) Temp. 25 °C Time 240 min pH 5.5 ± 0.1	14.67 ± 0.67	
Tomato waste (peel and seeds)	Lycopene	Enzymolysis: Celluclast:Pectinex 1:1 Enzyme: substrate ratio 0.2 mL/g Time 5 h Temp. 40 °C pH = 4.5 Extraction: Ethyl acetate extraction Solvent–substrate ratio 5 mL/g Time 1 h	Higher lycopene recovery	**(52)**
Soy (Glycine max L.) grit	Protein	50°C, 3 hours, pH 5.5, carbohydrases (cellulase, pectinase and xylanase)	1 h alkaline extraction increased protein yield for 21%, 2 h alkaline extraction enhanced protein yield for 13%	**(53)**
Tomatoes	Carotenoid-containing chromoplasts	45–55°C, pH 5–5.5, 180 min, polygalacturonase, pectin lyase, cellulose, xylanase	Recovery yield [about 4.30 ± 0.08 (mg_{Lyc}/Kg_{tomato})/U] obtained	**(54)**

Different bioactive compounds like pectin from banana peel **(51)**, betalains betacyanin, and betaxanthin from beet root **(52)**, lycopene from tomatoes **(52)**, protein from soy **(53)** and carotenoid-containing chromoplasts from tomatoes **(54)** with the help of Enzyme Assisted Extraction (EAE) under the optimized conditions as mentioned in **Table 7.**

Combination techniques

The main aim of food processing industries was to introduce eco-friendly along with cost effective technique that was done in two ways (1). Increase the output yield (2). Harness the production process. Until now there was no such idyllic technique to achieve all in one but by acquiring stability between expenditure, choice of solvent without compromising on quality. MAE, UAE and EAE were the dominant methodology in achieving higher yield and valuable product among all other mentioned above. It was a good idea to combine two of any techniques for better results **(55)**. Combination techniques, MAE and EAE, have been applied to enzyme pretreatment. This novel proficient extraction technique has solvent usage and cost effective extraction method. SFE and MAE was another desirable combination method for eco-friendly and effective extraction process. PEF and MAE combination technique was preferable for the extraction of bioactive compounds from plant based because of their low energy consumption and take less processing time **(47)**.

Table 8 Applications of combination techniques for extraction of bioactive compounds from plant

Combination technique	Under observation plant	Extracted compound	Treatment	Results & conclusions	Ref
Enzyme assisted supercritical carbon dioxide (SC-CO_2) extraction	Barbary fig	Isorhamnetin conjugates	Rapidase: pectin lyase, polygalacturonase, rhamnogalacturonase and arabinanase, arabinogalactanase, galactanase, endoglucanase and cellobiohydrolase SFE: CO_2, 20% ethanol, 60°C 100 bar	The highest extraction yields for Rapidase, Viscozyme were obtained at 100 bar, 60 °C and 20 % of ethanol as co-solvent. The use of Rapidase 2.4-fold and Viscozyme increased 2.8-fold the total extraction yield	**(56)**
Supercritical Fluid extraction And pressurized Liquid extraction	Biquinho pepper (capsicum)	Capsiate-rich oleoresin and Phenolics	PLE & SFE conditioned at 75% ethanol, 10 MPa, 65°C	Lowest cost of manyfacturing for capsiate and phenolic compounds at the solvent to feed mass ratios of 4.9 and 10	**(57)**
Ultrasound assisted, enzymatic extraction	Three leaf akebia seed	Protein isolates	Ultrasonication: 500 W, 40°C, 1 hours, bath model Cellulose assisted hydrolysis	Higher yields (19.38%), higher protein content (52.78%) were obtained	**(58)**

Challenges and future prospects

Novel extraction techniques have been hailing throughout the world but it has some limitations also:

1. Non practicality of these techniques on industrial scale, only suitable for laboratory scale.
2. There was need of SOPs for extraction parameters, choice of solvent, quality and safety control.

These extraction techniques has been proved to be promising to the brighter future and needed more research and collaboration among academia, researchers, industry and scientists for the implementation and innovation in these extraction techniques for sustainable economy **(59).**

Conclusion

The improvisation of extraction technologies from plant sources has been advanced to enhance efficiency, productivity, and minimize environmental concerns. Several advanced techniques, such as MAE, UAE, PLE, SFE, Enzyme assisted extraction, DES extraction, reverse micelles extraction, and subcritical water extraction, have been developed to extract bioactive chemicals and proteins from plants. These methods have been known for their improved efficiency, time-saving properties, and environmentally friendly approach. These innovative extraction procedures offer advantages compared to traditional extraction methods by maintaining the quality of essential oils, proteins, and other therapeutic bioactive compounds, reducing chemical risks, and improving the efficiency of the extraction processes. The continual enhancements and application of these innovative extraction techniques contribute to the effective exploitation and preservation of plant materials in various industries, such as pharmaceuticals, cosmetics, food and beverages, and medicine, among others.

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