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A Study on Sustainable and Innovative Extraction Techniques used for Bioactive Compounds from Agro-Industrial Wastes and Plants

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Abstract-

Agro-industrial waste is increasingly recognized as a promising source of bioactive phytochemicals such as phenolic compounds, carotenoids, flavonoids, and anthocyanins. Recovering and utilizing these compounds present a strategic pathway for reducing environmental impact while simultaneously offering a source of natural antioxidants. This study reviews advanced extraction methodologies employed in the isolation of phenolic compounds and other phytochemicals from raw fruits, vegetables, and their industrial residues. Various conventional and advanced extraction techniques such as maceration, digestion, decoction, soxhlet extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) are briefly discussed along with studies that utilized them. Researchers placed particular emphasis on identifying the optimal extraction parameters including extraction time, temperature, pressure, solvent type, and its concentration to assess the effectiveness of various extraction techniques. UAE, MAE and SFE techniques are reported with less consumption of solvents and result with better extraction yields in lesser extraction time as compared to traditional extraction methods. Employing green solvents could not only enhance recovery efficiency but also contributes to environmental sustainability by reducing the overall load of agro waste.

Keywords: Agro industrial waste, Phytochemicals, Extraction, Antioxidant

Introduction-

Unprecedented population growth in developing countries and food scarcity has become the immediate challenge of this hour. Consumption of mainly animal-based products, processed food, unhealthy and unsustainable food consumption in developed countries could lead to a burden on food resources.[1,2] While in developing countries, lack of production, processing and distribution may be one of the reasons for food scarcity, various innovative food processing and preservation techniques, and modern methods to increase crop production are adopted by the governments in order to meet the food requirements of the growing population. Sustainable food systems can ensure global food security with minimal environmental impact.[3] A sustainable diet prioritizes environmental sustainability, food

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security, and health, while respecting cultural diversity, biodiversity, and affordability.[4] Food waste has a significant global impact on health, society, economy, and the environment, posing challenges for sustainable development.[5] Food scarcity is exacerbated by substantial food waste, generated through both consumer behaviour and production processes, highlighting the need for improved efficiency and sustainability in the food system. The food processing industries, which use raw fruits and vegetables, generate substantial amounts of plant waste, and increase the burden on environmental health. Many industries are engaged in the production of packed fruit juices and beverages and consume a lot of fruits and vegetables just to add flavour, nutrients, and antioxidants to the products. The byproducts and wastes of these industries also contain many organic compounds, some of which are valuable—like phenolic compounds, carotenoids, flavonoids, anthocyanins, dietary fibers, polysaccharides, essential oils, and pigments. The contribution of phenolic compounds is significant.[6,7] Fruits and vegetables are a rich source of phenolic compounds, and concentrations of phenolic compounds depend on the ripeness, colour, and type of plant material. For example, grapes are among the richest sources of phenolic compounds. They contain carbohydrates in the form of sugar, organic acids, and polyphenolic compounds as their constituents along with water. During the preparation of juice, a significant amount of valuable phenolic compounds remains in the byproducts. Most of the phenolic content of grapes is present in their seeds, followed by the skin and pulp.[8,9] Phenolic compounds are likely the key contributors to the antioxidant properties found in foods and are also known for their anti-inflammatory effects. The ability of these compounds to neutralize free radicals depends on the number of -OH groups present in the compound, the nature of substitution in the aromatic ring, the position of hydroxyl groups, and their proximity to the -COOH group within the compound.[6,10]

The flavonoid family of polyphenols comprises flavanols, isoflavones, flavanones, flavones, and chalcones. They have been reported to possess antiviral, antioxidant, antimicrobial, anticancer, and anti-inflammatory properties and that's why these natural products are valued for their potential therapeutic and biological properties.[11,12,13,14]

Carrot is a root vegetable, rich in carotenoids particularly beta-carotene which serves as a precursor to vitamin A. This nutrient is responsible for maintaining good eyesight, healthy skin, and a strong immune system, and makes the carrot a valuable component of a balanced diet. Carotenoids are the natural compounds responsible for colour and possess many biological activities. Wasted pulp during carrot juice preparation contains a certain quantity of carotenoids and can be extracted with the help of extraction methods.[15,16] Red, purple, and blue colours of fruits and vegetables are due to the presence of water-soluble coloured anthocyanins, which belong to the phenolic group. The colour of anthocyanins is affected by pH, structure, and temperature. As phenols are acidic in nature, these compounds appear blue at high pH and red at low pH. Many tropical fruits, blackberries, currants, coloured leafy vegetables, tubers, and grapes are rich in anthocyanin content. These coloured pigments were used as food colorants and as natural dyes conventionally. Anthocyanins have profound use in pharmaceuticals due to their potential health benefits.[17] Valuable compounds can be extracted from

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solid plant wastes through solid-state fermentation, a process leveraging microorganisms like bacteria, yeast, and fungi to break down plant wastes via hydrolysis. Various conventional methods to extract antioxidant compounds from fruit pulp like orange pomace are known, such as solvent extraction, Soxhlet extraction, and maceration, Accelerated Solvent Extraction (ASE), Microwave-assisted extraction, UV-assisted extraction, and Supercritical Fluid Extraction (SFE).[18]

Extraction Methods- Conventional solvent extraction technique has been widely used for the extraction of secondary metabolites from plants. These secondary metabolites enable the plant to survive in its environment and defend against threats to their survival.[19] Maceration – The fruit or vegetable residue is soaked in the chosen solvent in powdered or coarse form at room temperature for three to four days. Extraction is followed by cleaning of the extract via decantation or filtration after standing for some time. To avoid solvent loss, maceration should be carried out in a covered container. A challenging task in this extraction is the selection of a suitable solvent, as it is the solvent which outlines the class of phytochemicals in the plant residue. This technique was used conventionally for the extraction of phenolic compounds and anthocyanins from fruit and vegetable residues, but it is time-consuming and could give significant efficiency under optimum conditions. [20,10] Digestion technique uses warming of plant residue with the solvent and avoids the decomposition of phytochemicals due to heating. Maximum warming could reach up to 50 °C in case of hard plant parts. The vessel used for extraction should be preheated and later the plant material should be warmed at an appropriate temperature, followed by shaking the mixture at regular intervals. [20]

Decoction extraction technique involves the boiling of plant residue or plant material in water up to 100 °C. Duration of boiling depends upon the part of the plant and the bioactive compounds to be extracted. Delicate parts of plants can be boiled for 15–20 minutes, while hard parts can be boiled for 60 minutes. Cooling, straining, and filtration of the mixture are the next steps after boiling. Extraction using decoction is appropriate for phytochemicals which do not break down with increasing temperature and remain intact. However, the extract could contain undesirable products in addition to the desired ones. [10,21,22]

Alara O.R. et al. have studied the extraction of phenolic compounds from the leaves of Vernonia cinera using **Soxhlet extraction**. This method is widely used as compared to other conventional methods like percolation, diffusion and maceration. In this technique, plant material is kept in a porous bag made of cellulose and this bag is further kept in the Soxhlet paraphernalia. The solvent is heated to vaporize and penetrate the plant material bag and condensed till the extraction is completed, and the extract has shown significant antioxidant activity.[23] The extraction of bioactive compounds from fruit and vegetable residues generally involves extraction with organic solvents by heating or stirring. These conventional techniques are limited by their time-consuming nature, high organic solvent consumption, and potential for degradation of the desired compound during extraction—compromising extraction efficiency and product quality. The choice of organic solvent and its quantity could cause health issues. [24,25] Advanced extraction techniques coupled with optimal solvent selection could enhance efficiency and extraction yield. Some advanced techniques are listed below.

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Accelerated Solvent Extraction (ASE)- A stainless steel extraction cell with plant material and solvent is subjected to high pressure and temperature and kept between inert silica layers. High temperature accelerates solubility and diffusion rate, whereas high pressure increases solvent interaction and penetration with the plant material. Later, the collection of extract is done in precleaned vials and further analysed. ASE is reported as a better extraction technique than Soxhlet, digestion, percolation, decoction and maceration because it consumes less time and solvent compared to traditional methods of extraction. [26, 27, 10]

Person et al. worked on the extraction of natural rubber from the sunflower plant using the ASE technique. Acetone was used as a solvent for the extraction of resin, methanol for the extraction of chlorophyll, and hexane for the extraction of natural rubber from sunflower tissue resins. Experiments were performed with different extraction times, number of cycles, and extraction temperatures for these three solvents. Results were reported better with ASE, and better recommendations are: 16 min acetone extraction at 40 °C (2 cycles), 5 min methanol extraction at 60 °C (5 cycles), followed by two cycles of 16 min hexane extraction at 40 °C. [27]

Gomes, S.V. et al. investigated the leaves of seventeen species of Passiflora for extraction of TPC and TFC using Box-Behnken design to increase the extraction yield using the ASE technique. They reported five cycles with 64% ethanol at 80 °C as the favourable conditions for better extraction of phenolic compounds. [28]

Brachet A. et al. investigated the proper conditions and parameters for extraction of cocaine and benzoylecgonine from coca leaves using the ASE method. They experimented with various temperatures, pressures, and extraction time ranges. Better results were obtained with a temperature of 80 °C, 10 min extraction time and 20 megapascals pressure using the ASE technique compared to traditional methods.[29] Extraction conditions could vary with the type of phytochemicals to be extracted, parent plant material, other byproducts, and interaction of the solvent with them.

Another study reported by Jentzer et al. used the ASE technique for extraction of steviol glycosides from dried stevia leaves in order to analyse the influence of temperature, static time and number of cycles on extraction yield. Steviol glycosides are natural sweeteners with low calories and could be a better alternative to sugar. The team reported 100 °C extraction temperature, 4 min static time and 1 cycle as the ideal extraction parameters. Yield was reported higher with the reduction of particle size of parent material to less than 0.5 mm.[30]

Microwave-Assisted Extraction (MAE) -This technique involves heating plant material along with a solvent using microwaves and has been found suitable for the extraction of natural products such as saponins, polyphenols and flavonoids. As these compounds are polar in nature, microwaves directly interact with polar compounds, making the extraction efficient. [31] Low viscosity of solvent favours the solvation process, and a high dielectric constant of the solvent enhances heating. [32,33]

Polar compounds are capable of producing heat by rotation under the influence of microwaves. This generated heat enhances solvent penetration into the plant species and ultimately enhances the efficiency of extraction. Some advanced microwave-assisted extractions used in recent days for

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improved extraction include **pressurized microwave-assisted extraction (PMAE)** and **solvent-free microwave-assisted extraction (SFMAE)**. [34] Use of oil solvents for recovery of carotenoids could be a better alternative to the use of environmentally unfriendly organic solvents in MAE. Carotenoids are oil-soluble pigments and oil solvents could reduce oxidation time and degradation rate of the extract. On the other hand, flaxseed oil has potential health benefits, and enriched flaxseed oil with soluble carotenoids will prove more beneficial from a health perspective. [16,35]

Elik, A. et al. worked on the extraction of carotenoids from carrot juice waste pulp using the MAE technique and flaxseed oil as a solvent, to estimate the effect of MAE on extraction yield using optimal conditions. The favourable extraction conditions reported are: 9.39 min extraction time, 165 W microwave powers, and an oil-to-waste ratio of 8.60:1. [35,36]

Ultrasonic-assisted extraction (UAE) is also a technique used for the extraction of bioactive compounds from fruit and vegetable residues by using high-frequency sound waves. This technique is reported to show better extraction results as compared to conventional extraction methods, like better yield, low cost of equipment, and reduced consumption of time and organic solvents. The PLE technique for extraction of phytochemicals is environmentally safe and a faster technique. It provides selective and fast extraction using high pressure and temperatures. Ethanol and water are found to show better results in the PLE technique, and both these solvents are safe to use. SFE (supercritical fluid extraction) is also environmentally safe, as it extracts bioactive compounds without toxic contaminants, with greater selectivity and low solvent use. [37,10]

Zhang, L et al. have investigated the antifatigue activity of flavonoids extracted from lotus leaves using the ultrasound-assisted extraction method (UAE). They used 100 g of dried powdered lotus leaves, soaked them in ethanol for 4 hours, followed by ultrasonication for 15 to 40 min at 40 °C. Extracted flavonoids were examined, and favourable extraction conditions for maximum extraction yield were obtained. Twenty-five minutes of ultrasonic time with 70% ethanol solution was reported as the optimal condition for extraction. [38]

Petigny, L et al. worked on the extraction of phytochemicals from boldo leaves using UAE. This plant is a good source of alkaloids and flavonoids and is known for its antioxidant activity. UAE was used to reduce the extraction time and amount of solvent and enhance the efficiency and extraction yield. Various studies have reported enhanced extraction yield of bioactive compounds from vegetables using high-power UAE compared to traditional methods. About one-fourth the time was required with UAE for equal extraction yield compared to conventional maceration. They concluded that 40 min of ultrasonication of plant residue with 23 W/cm² power at 36 °C was the optimal condition for extraction. [39] UAE is reported to accelerate the extraction speed and efficiency and reduce the required amount of solvent.

Extraction of saponins from red lentil, fenugreek, soybean and lupin with the assistance of UAE using different solvents—ethanol, ethanol—water and water—reported the highest saponin-rich extract from edible seeds with the use of ethanol. [40]

Extraction of some thermolabile biochemicals from plant material could become difficult at higher

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temperatures, as these substances get degraded or decompose and change their properties. Hence, selection of extraction temperature in this case is important to preserve their originality.

Extraction of bioactive compounds from spices like ginger, garlic and turmeric using traditional methods and UAE suggested enhanced content of vitamin C, phenolic compounds, and carotenoids obtained with the use of the UAE method at 70 °C. [41]

Some studies have reported better efficiency of ionic solvents in the UAE method as compared to conventionally used organic solvents. [42] While some have concluded that deep eutectic liquids are more efficient than traditionally used organic solvents, such as better extraction yields of flavonoids from common buckwheat sprouts and effective extraction of anthocyanins from wine lees using eutectic solvents. [43,44]

Supercritical Fluid Extraction (SFE) - Gases can be liquefied by applying pressure, but above a certain temperature, they can't be liquefied regardless of the pressure applied. This temperature is termed the critical temperature. Above the critical temperature, gas cannot be liquefied by applying any pressure, whereas critical pressure is the minimum pressure required to liquefy a gas at its critical temperature to overcome the kinetic energy of gas molecules. When a substance is heated above its critical temperature, it can exist in two indistinguishable phases: liquid and gas. At the critical point, the substance becomes a supercritical fluid. These days, supercritical fluids are used in extraction of phytochemicals and in chromatography at commercial scale. [10,45] The supercritical fluid extraction method selectively and efficiently extracts soluble compounds from biological material by using properties of fluids above the critical point. Supercritical fluids possess properties of both liquids and gases (high diffusivity, low viscosity), which favour effective extraction. [46]

Several studies have reported the use of supercritical fluids—mainly supercritical CO₂—as a solvent in extraction of bioactive compounds from plants and residues of fruits and vegetables, which is better than organic solvents in various ways. An investigation by Espinosa-Pardo, F.A. et al. for the extraction of phenolic compounds from dry and fermented orange pomace using supercritical CO₂ and cosolvents involved the use of supercritical fluid extraction. The solvent used was supercritical carbon dioxide. They conducted the SFE at different temperatures, pressure ranges, and with various cosolvents like pure ethanol and ethanol—water mixtures. Extraction yield was found to be enhanced with SFE at high pressure and with 90% ethanol as cosolvent. However, the antioxidant properties of extracts decreased at high pressure. [46]

SFE is proven helpful in extraction of thermolabile bioactive compounds due to the low extraction temperature employed. High solvation power of SFE CO₂ for nonpolar phytochemicals enhances extraction yield, and its low solubility for polar compounds can be improved with the use of polar cosolvents. Low toxicity and cost make SFE CO₂ more popular in the field of natural product extraction, but adjustment of temperature and pressure should be done carefully in case of thermolabile compounds. [10] Better yield of lycopene was extracted from dried tomato skin at 40–70 °C temperature and 25–45 MPa pressure using SFE CO₂. [47]

Enhanced extraction yield with the use of SFE CO2 from cherry leaves was reported compared to

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methanol solvent extraction. The optimal extraction conditions employed were 30 MPa pressure, 43 °C temperature, and 120 min extraction time. [48] Extraction of phenolic compounds from potato peel using supercritical CO₂ as a cosolvent with 20% methanol, at 80 °C and 350 bar, with 18.0 g/min flow rate, reported better yield of phenolic compounds. [49] A study by Trentini, C.P. et al. regarding the effect of temperature and pressure on Macauba oil yield reported better yield using supercritical CO₂ and compressed propane as the solvent. [50] Various researchers have used these extraction methods to recover the valuable compounds from plant wastes and agro industrial wastes and some of them are listed in the above content and few are listed below.

Ana Paula da Fonseca Machado et al. worked on the extraction of antioxidants from residues of blackberry fruit. They used the pressurized liquid extraction (PLE) technique with different solvents and temperature ranges to evaluate their effect on yield and antioxidant activity. Experiments were performed with temperature ranges of 60, 80, and 100 °C. A positive effect of temperature was observed on overall yield. Ethanol and 50% water were found to show better results with PLE at 100 °C for total phenolic compound yield. Anthocyanin yields were reported better with acidified water as solvent at 60 and 80 °C.[51]

The extraction of phenolic compounds from orange peel waste was investigated using conventional solvent extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) methods. Three solvents were employed for the study: deionized water, ethyl acetate, and aqueous ethanol (80% v/v). Among the techniques evaluated, UAE demonstrated superior energy efficiency, making it a favorable option for sustainable extraction processes. Conversely, MAE yielded the highest concentration of phenolic compounds, particularly when 80% ethanol in water was used as the solvent, indicating its effectiveness in maximizing compound recovery.[52]

Conclusion:

The agro-food industries present a substantial opportunity for the recovery of bioactive compounds from fruit and vegetable by-products, which are rich in antioxidant, anti-inflammatory and many other therapeutic activities. Advanced extraction techniques such as Supercritical Fluid Extraction (SFE), Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE) have demonstrated high efficiency in isolating these compounds. Specifically, the use of CO₂ as a cosolvent in SFE, combined with organic solvents, enhances the extraction of thermolabile phytochemicals. The utilization of fruit and vegetable processing waste through the recovery of bioactive compounds using green solvents and advanced extraction technologies offers a promising strategy for mitigating environmental pollution.

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