CHAPTER 2

Review of Literature

2.1. "Green Remedies: The Role of Medicinal Plants in Modern Healthcare"

According to the World Health Organization (WHO), more than 17.5 million people die each year from cardiovascular disease (WHO, 2018). Alternative and complementary medicines, such as medicinal plants, have become increasingly popular as treatment choices, particularly in underdeveloped nations. A variety of variables influence their use, including cultural concerns, accessibility of availability, and false safety assumptions. The usage of medicinal herbs may be considered a remote practice. Each society has its own set of techniques for utilization, which are generally based on common knowledge. Thus, the public uses natural goods without fully understanding the hazards and efficacy of each species (Chrysant and Chrysant, 2017; Tribess et al., 2015). Herbal practice continues to play an important role in the management and treatment of many health conditions, particularly in India's remote and rural areas (Malik et al., 2015). The understanding of medicinal plant conservation and use has created a link between environmental conservation and indigenous knowledge (Cameron, 2008). In the present day, however, the practice of herbal therapy is disappearing, even in areas where it was historically developed and fostered by oral tradition down centuries. The fall in herbal medicine use is mostly due to a shift in people's views about allopathic medication, as well as its widespread availability even in small towns, despite people's awareness of its potential negative effects. This circumstance may result in the loss of traditional and useful knowledge about plants used in healthcare management in the future (Harsha et al., 2002).

The use of plants to treat a variety of human ailments has a long history. Various plant parts, including as leaves, stems, bark, and roots, are utilized to prevent, alleviate, or restore anomalies to normal. Because the practice of "herbal remedies" does not strictly adhere to facts obtained via scientific methods, conventional medicine considers "herbal medicines" to be an alternative medicine. However, most pharmaceutical medicines currently prescribed by physicians have a lengthy history of use as herbal treatments, such as opium, aspirin, digitalis, and quinine. Modern medicine uses active chemicals extracted from higher plants, and around 80% of these active substances show a good link between their modern therapeutic application and traditional uses (Sarkar et al., 2015). Experts from various fields have prepared detailed information on the use of various medicinal herbs and their active components as antioxidants, antidiabetic, antihypertensive, antiatherosclerosis, gastroprotective, analgesic, anticancer, antidepressant, antiasthma, antiobesity, antiatherosclerosis, antimicrobial, anti-inflammatory agents, and immunomodulators, as well as their safety concerns and toxic effects (Eddouks et al., 2012).

2.2. Unveiling the Power of Plant Secondary Metabolites

Secondary plant metabolites are a variety of chemical substances produced by plant cells via metabolic routes deriving from core metabolic processes. Albrecht Kossel, who won the Nobel Prize in physiology or medicine in 1910, was the first to define the term secondary metabolite (Jones and Kossel, 1953). Czapek defined them as end-products three decades later (Bourgaud et al., 2001). According to him, these compounds are produced from nitrogen metabolism through ‘secondary alterations like deamination. In the middle of the twentieth century, breakthroughs in analytical techniques such as chromatography enabled the recovery of an increasing number of these compounds, laying the groundwork for the development of the subject of phytochemistry.

Secondary metabolites have been demonstrated to have a variety of biological effects, providing scientific justification for the use of herbs in traditional medicine in many ancient civilizations. They have been described as antibacterial, antifungal, and antiviral, and hence can protect plants from infections. Furthermore, they are significant UV absorption chemicals, preventing serious leaf damage from light. Some plants used as feed grasses, such as clover or alfalfa, have been shown to display estrogenic characteristics and interact with animal fertility (Bennets et al., 1946).

2.3. The Antioxidant Power of Medicinal Plants in Disease Prevention

Plant materials have been used in phytomedicines since ancient times. These can be produced from any part of the plant, such as bark, leaves, flowers, seeds, and so on (Cragg and Newman, 2001), implying that any portion of the plant could have active components. Knowing the chemical elements of plants is desirable since it will help with the production of complicated chemical substances. Many researchers have reported on the phytochemical screening of numerous plants (Siddiqui et al., 2009; Ashokkumar et al., 2010; Chitravadivu et al., 2009). The recent development of functional foods and pharmaceutical products based on medicinal and food plants (specifically fruits and vegetables) has improved all aspects of life, including the alleviation of physical disorders, the reduction in the use of synthetic antibiotics, and an increase in life expectancy (Li et al., 2017; Nollet and Gutierrez-Uribe, 2018). Indeed, these plants have long been employed as safe, effective, and sustainable sources of natural antioxidants or free radical scavengers, notably phenolic components like phenolic acids, flavonoids, tannins, stilbenes, and anthocyanins (Nollet and Gutierrez-Uribe, 2018). These phenolics are mostly thought to confer antioxidant activity on medicinal and food plants, making a significant contribution in the fight against many pathological conditions such as cancer, diabetes, aging, cardiovascular, and other degenerative diseases (Uritu et al., 2018; Etkin, 1986; Watson and Preedy, 2016). The negative effects of oxidative stress on human health have become a major concern. The World Health Organization (WHO) estimates that 80% of the world's population relies on traditional medicine for primary health care, with plant extracts and active components accounting for the majority of this therapy. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide) than enzymatic antioxidants (e.g., SOD, GPx, and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance causes cell damage (Aruoma, 1998; Lefer and Granger, 2000; Smith et al., 2000; Bhatia et al., 2003; Peuchant et al., 2004) and health issues (Steer et al., 2002; Uchida, 2000). A lack of antioxidants, which can neutralize reactive free radicals, promotes the development of degenerative diseases (Shahidi et al., 1992), such as cardiovascular disease, cancer, neurodegenerative diseases, Alzheimer's disease (Matteo and Esposito, 2003), and inflammatory diseases. One alternative is to supplement the diet with antioxidant chemicals found naturally in plants (Knekt et al., 1996). These natural plant antioxidants can so act as a form of preventive medicine.

The most practical strategy to combat degenerative diseases is to boost antioxidant activity in our bodies, which can be accomplished by consuming vegetables, fruits, or edible plants (Adefegha and Oboh, 2005). There is a growing interest in natural antioxidants, such as polyphenols found in medicinal and food plants, which may help reduce oxidative damage.

Natural antioxidants boost plasma antioxidant capacity and lower illness risk (Shekhar et al., 2011). Different components of the plant, such as seeds, leaves, and bark of the stem and root, are known to contain significant amounts of phytoconstituents such as phenolics, flavonoids, and tannins, which have the capacity to block the excessive production of free radicals and so can act as antioxidants.

2.4. Cardiovascular diseases

2.4.1. Unravelling the Impact of Hypertension on Cardiovascular Health

Cardiovascular diseases (CVDs) are a primary cause of weakness and premature mortality, and hence constitute a major public health issue (Al Disi et al., 2015). High blood pressure (BP), known as a silent killer, is caused by a variety of reasons, including the combination of hereditary and environmental components that disrupt BP homeostasis (Wang and Xiong, 2012). Hypertension (HTN) is the most frequent risk factor for acute myocardial infarction, accounting for around 16.5% of deaths globally each year. It is also the leading cause of morbidity and mortality associated with cardiovascular diseases (Anwar et al., 2016). It is anticipated that by 2025, 29% of the world's adults, or about 1.56 billion individuals, will suffer from HTN (Roger et al., 2011). Although some current medications are used to treat clinical hypertension, they are accompanied with a variety of negative effects.

Blood pressure (BP) is controlled by multiple systems, including nitric oxide (NO), neurological mechanisms, and renal-endocrine mechanisms. Various antihypertensive agents, including diuretics, β-blockers, calcium-channel blockers, and rennin angiotensin system blockers (e.g., angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers), are used to treat hypertension (Vardanyan et al., 2016; Charlton and Thompson, 2016). However, antihypertensive drugs can cause side effects such as reduced renal function, dry cough, and angioedema (Roger et al., 2011). Therefore, herbal medicine can be used to manage hypertension (Leung et al., 2017). Natural herbal medications with potential antihypertensive efficacy and fewer side effects can be an effective alternative to synthetic drugs when combined with a change in lifestyle and modest exercise. Antihypertensive drug side effects have prompted researchers to seek novel therapies in the form of metabolites or extracts from medicinal plants that control hypertension while causing fewer negative effects (Popovi et al., 2016). Recently, multiple ethnobotanical investigations conducted in various parts of the world revealed that hundreds of plants are utilized globally for empirical hypertension treatment (Nunes et al., 2015; Lee and Hur, 2017). On the other hand, the findings of various ethnobotanical surveys revealed that questioned patients used medicinal plants to treat hypertension because phytotherapy is less expensive, more effective, and superior to contemporary treatment (Roger et al., 2011; Ahmad et al., 2015). Nature is the best source of solutions for many health issues. Today, herbal medicines are utilized alone or in conjunction with chemical ones to treat a variety of disorders (Lee and Hur, 2017; Eddouks et al., 2017). There are numerous medicinal plants suggested by native cultures for the treatment of hypertension, which opens new avenues of research into the antihypertensive effects of medicinal plants (Leung et al., 2015; Nunes et al., 2015).

2.4.2. Exploring the Anti-inflammatory Efficacy of Medicinal Plants

Inflammation is the body's primary defense response against foreign stimuli such as injury or pathogen-induced infection. Inflammation is a necessary immunological response that allows the organism to survive an injury (Adegbola et al., 2017). Because inflammation plays an important role in restorative, healing, and aggressive processes, it is thought to be a beneficial pathological process in countering pathogen-induced stress and noxious situations (Fullerton and Gilroy, 2016; Medzhitov, 2010). Inflammation is a complex process that involves various cellular interactions and can be characterized as acute or chronic (Vanucci-Bacqué and Bedos-Belval, 2021).

Acute inflammation protects the body by healing injuries and resisting microbial invasion, whereas chronic inflammation targets critical cells, moieties, and organs of the body, resulting in the development of various chronic pathologies such as cardiovascular disease, skeletal muscle disorders, inflammatory bowel disease, diabetes, cancer, and neurological diseases, which further accelerates the aging process. There are several therapies available to control and suppress an inflammatory crisis; steroids, nonsteroid anti-inflammatory drugs, and immunosuppressants are practical examples of these medications that have been linked to side effects. Thus, to get a higher pharmaceutical response while minimizing undesired side effects, we must incorporate natural anti-inflammatory components into prescription therapy (Bagad et al., 2013; Ghasemian, 2015). Herbal remedies are promoting subjects in medicine; thus, we must expand our knowledge of them. Complementary, alternative, and traditional treatments are important sources of herbal medication suggestions, but contemporary medicine must prove these guidelines scientifically before putting them into practice. Plants have long been used to improve human health. Plants create a variety of biologically active chemicals in response to invading pathogens and environmental stressors. These tiny organic compounds originate from secondary metabolism and have a variety of biological functions. Among the several functions, anti-inflammatory effects are highlighted (Nardi et al., 2016; Virshette et al., 2019).

Inflammation is recognized as an evolutionarily conserved process of protection and a vital survival factor (Liu et al., 2017). It consists of complex sequential changes in the tissue that eliminate the initial cause of the cell injury, which could have been caused by infectious agents or substances from their metabolism (microorganisms and toxins), as well as physical agents (radiation, burn, and trauma), or chemicals (caustic substances) (Fialho et al., 2018; Jang et al., 2016). Inflammation symptoms include local redness, swelling, discomfort, heat, and loss of function (Virshette et al., 2019).

Overall, this complicated biological response results in the restoration of homeostasis. However, if inflammatory mediators are released in excess and detrimental signal-transduction pathways are activated, the inflammatory process might persist, resulting in a mild but chronic proinflammatory state. A low-grade inflammatory state is linked to a variety of illnesses and chronic health issues, including obesity, diabetes, cancer, and heart disease. As a result, the identification of a new generation of therapeutic drugs for the treatment of inflammation is desirable (Liu et al., 2017). The treatment of inflammation entails certain systems that can be employed as therapeutic targets. Medicinal plants serve a significant role in the discovery of new and strong medications, as they produce secondary metabolites with clinically curative effects (Li et al., 2020; Zaynab et al., 2018).

2.4.3. The Role of Angiotensin-converting enzyme (ACE) Inhibitors in Hypertension Management

Despite the high incidence of morbidity and mortality caused by hypertension, management remains poor. In addition to various successful nonpharmacologic therapies, many people require antihypertensive medicine to decrease their blood pressure, and they frequently require multiple medications at once (Coates, 2003). Among the many options in antihypertensive medication are those that target the renin-angiotensin-aldosterone (renin) pathway. Currently, renin system inhibitors include angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB). Angiotensin converting enzyme 1, which is present throughout the animal world, is an integral membrane-bound protein with active sites directed to extracellular areas. Mammals express two isoforms: a single domain germinal isoform essential for male fertility, and a double domain somatic isoform that plays an important role in the renin-angiotensin system. Both somatic domains are active, with varying substrate affinities. ACE (angiotensin I converting enzyme 1 [EC 3.4.15.1], peptidyl-dipeptidase A, peptidyl-dipeptidase I, dipeptidyl carboxypeptidase I (DCP1), peptidyldipeptidase I, kininase II, peptidase P, carboxycathepsin) was first identified as a key component of the renin-angiotensin system, with the primary function of converting angiotensin I to angiotensin II and degrading bradykinin. In combination with renin, it appeared that the primary purpose of ACE in mammals was as part of the homeostatic mechanism responsible for maintaining appropriate blood pressure and electrolyte balance. Mammalian ACE activity is commonly detected by evaluating the decrease in substrate cleavage in the presence of ACE inhibitors such captopril and lisinopril. ACE hydrolyzes peptides by removing a dipeptide from the C-terminus, such as converting angiotensin I to angiotensin II or degrading bradykinin. It can also function as an endopeptidase, as demonstrated by the cleavage of peptides having amidated C-termini. Although ACE plays an important role in mammals as a component of RAS, its relevance to animals must be more complex for two reasons (Corvol et al., 1995). One is the gene's inherent complexity. The second is the presence of the gene product in animals that lack an identifiable RAS but exhibit similar biochemical activity of the enzymes. This may be most obvious in the case of the D. melanogaster Ance product, which is very efficient at cleaving basic pairs of residues from the C-terminus of a peptide, as would be found after cleavage by a prohormone convertase (Isaac at el., 1998), and thus relevant in the processing of a variety of bioactive peptides, including tachykinins in both mammals and insects (Vanden Broeck et al., 1999).

2.5. Extraction of bioactive compound

2.5.1. Essential Steps in the Extraction of Medicinal Plant Compounds

Extraction is the critical initial stage in the analysis of medicinal plants since it is required to extract the desired chemical components from the plant materials before further separation and characterisation. The basic operation involved processes such as prewashing, drying of plant materials or freeze drying, grinding to generate a homogeneous sample, and often optimizing the kinetics of analytic extraction as well as increasing the surface contact of the sample with the solvent. Proper procedures must be followed to ensure that possible active ingredients are not lost, altered, or destroyed during the extraction of plant materials. If the plant was chosen for its traditional applications (Fabricant and Farnsworth, 2001), the extract must be prepared in accordance with the traditional healer's instructions to accurately imitate the traditional 'herbal' medication. The solvent system chosen is largely determined by the type of the bioactive chemical under consideration. Various solvent solutions are available for extracting bioactive compounds from natural sources. Hydrophilic chemicals are extracted using polar solvents such as methanol, ethanol, or ethyl-acetate. More lipophilic chemicals are extracted using dichloromethane or a 1:1 combination of dichloromethane and methanol. Chlorophyll is sometimes removed via hexane extraction (Cos et al., 2006).

Because the target chemicals might range from non-polar to polar and thermally labile, the extraction procedures used must be appropriate. Plant samples are extracted using a variety of processes, including sonification, heating under reflux, soxhlet extraction, and others. In addition, plant extracts are made by macerating or percolating fresh green plants or dried powdered plant material in water and/or organic solvent systems.

The use of green extraction techniques to extract phytoconstituents from natural sources reduces the number of solvents used and waste generated during the extraction process. Traditional extraction procedures produce a large amount of solvent waste, which poses numerous environmental and health hazards. Furthermore, adopting automated modern techniques reduces exposure to solvents and vapor. Green extraction is based on analytical processes that consume less energy, allow for the use of various solvents and sustainable natural resources, and result in a safer and superior extract/product. According to a life cycle analysis of waste generated in Active Pharmaceutical Ingredient (API) manufacturing plants, solvent-related waste accounts for 80% of total waste. If other pharmaceutical businesses produce similar levels of solvent waste, addressing solvent selection, use, recovery, and disposal will go a long way toward resolving the problem.

2.5.2. The Role of Non-Thermal Advanced Extraction in Unveiling Therapeutic Potential

Regardless of their unrivalled chemical variety, natural products such as plant extracts, whether pure compounds or standardized extracts, give limitless prospects for new therapeutic discoveries. The usage of herbal remedies in Asia reflects a long history of human interactions with nature. Plants used in traditional medicine include a diverse spectrum of chemicals that can treat both chronic and infectious disorders (Duraipandiyan et al., 2006). Men turned to ethnopharmacognosy after experiencing unpleasant effects and microbial resistance to chemically manufactured medications. They discovered literally thousands of phytochemicals from plants as safe and broadly useful alternatives with less side effects. Numerous positive biological activities, including anticancer, antibacterial, antioxidant, antidiarrheal, analgesic, and wound healing, have been reported. People frequently claim that various natural or herbal products provide significant benefits. However, clinical trials are required to demonstrate the efficacy of a bioactive chemical to validate this traditional claim. Clinical trials designed to better understand the pharmacokinetics, bioavailability, efficacy, safety, and drug interactions of newly created bioactive chemicals and their formulations (extracts) must be carefully evaluated. Clinical trials are meticulously structured to protect participants' health while also answering specific research questions by testing for both immediate and long-term adverse effects and measuring their outcomes before the treatment is widely used on patients (Dincheva et al., 2023; Shilpha et al., 2017).

2.5.3. Green Extraction Techniques: A Path to Cleaner and More Efficient Processes

Security concerns, such as solvent toxicity and the existence of solvent residues in extracts, along with low yields, have prompted the development of alternative extraction technologies, such as clean or green technologies, that can reduce or eliminate the use of organic solvents. These procedures, also known as cold extraction techniques, do not alter the stability of the extracted molecules and use less energy to extract (Tiwari et al., 2015). According to Jacotet-Navarro et al., (2016), the goal of these green extraction procedures is to obtain a higher extraction rate, more efficient energy consumption, improved mass and heat transfer, smaller equipment size, and fewer processing stages.

The use of these technologies also aims to protect the natural environment and its resources (Mustafa and Turner, 2011). Many methods have been developed to extract bioactive phenols from different plant matrices. These methods range from the most common extraction techniques, such as conventional solvent extraction, to sophisticated techniques like microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction, supercritical fluid extraction, and enzyme-assisted extraction (EAE). These extraction procedures rely on input parameters to maximize phenolic extraction from plant samples. Temperature, incubation period, particle size, pressure, concentration of specific input factor, solvent type, and solute-to-solvent ratio are some of the input factors.

Phenolics have been extracted employing Solvents (Alberti et al. 2014; Bachir at al. 2014) MAE (Bouras et al., 2015; Dahmoune et al., 2014, 2015) UAE and various other methods such as supercritical fluid extraction, cold extraction, supercritical CO_2 extraction, and semi continuous hot-cold extraction (Monrad et al., 2012; Pinela et al., 2018). UAE generates acoustic waves in the solvent, resulting in the formation of cavitation bubbles and internal agitation inside the matrix. The formed cavitation bubbles burst at the surface of the plant sample matrix, disrupting the plant cell wall and aiding in the release of phenolic bioactives into the solvent (Chemat et al., 2017). UAE use ultrasonic baths with various solvent concentrations, solvent-to-sample ratios, duration, and temperature, which are typically sonicated at 40 KHz. All these characteristics can vary and must be tuned for the specific plant sample utilized. Supercritical fluid extraction (SFE) is a method for recovering extracts from solid matrices that uses solvents at or near their critical temperatures and pressures. SFE extracts phenols from plant matrices using supercritical fluids (CO_2 and water). Carbon dioxide (critical point 7.38 MPa, 304.2 K) is the most utilized supercritical fluid (Prado et al., 2015).

SFE consists of two steps: (i) extracting phenolic chemicals and (ii) separating the phenolic extract from the solvent. SFE is a popular extraction technique because of its advantages over other methods. SFE has several advantages, including faster, more selective, and enhanced phenolic recovery without the use of hazardous organic solvents. Increased time owing to solutes reduces the diffusion rate from the solid matrix into the supercritical fluid, which is the most prevalent disadvantage of SFE.

2.5.4. Eco-Friendly Extraction: The Advantages of Green Solvents in the Food Industry

Ethanol, methanol, ethyl acetate, acetone, heptane, dimethyl sulfoxide (DMSO), and water are among the most often used extraction solvents. The food sector likes solvents that are non-toxic and easy to use. Because of their poisonous nature, DMSO and methanol solvents for extraction raise food safety concerns; as a result, they are commonly used as solvents for a variety of non-food applications. DMSO can dissolve both polar and non-polar molecules and is entirely soluble in organic solutions, including water. Methanol was also shown to be suitable for extracting polyphenols with lower molecular weights, whilst water was advised for extracting polyphenols with higher flavanols (Dai et al., 2010). Thus, a mixture of the two has been suggested. Methanol inhibits polyphenol oxidase and is thus recommended for phenolic component extraction from the raw plant matrix (Yao et al., 2004). Water is highly efficient in extracting non-polar chemicals, whereas organic solvents are effective in extracting organic compounds from plant biomass. The hydrophilic chemicals found in plants are the most active and can be extracted easily using water as a solvent. Water was also found to be an acceptable extraction medium for tea catechins, when compared to methanol (80%) and ethanol (70%) (Khokhar et al., 2002). Do et al., (2014) found significantly increased antioxidant activity in Limnopholia aromatic extracts prepared using absolute ethanol, with the highest yield achieved by combining 50% acetone and 50% water. They advocated employing appropriate amounts of aqueous and organic solvents for the efficient extraction of active principles that are soluble in both water and organic solvent. Organic solvents are often employed to efficiently extract phenolic chemicals; however, their residuals may be detrimental to consumers' health even in tiny amounts. Thus, when utilizing organic solvents as an extraction medium, it is critical to take every precaution to eliminate all extracting solvents from the filtrate. The use of aqueous medium in extraction offers various advantages. Water is regarded the cheapest and safest solvent, although it is not as efficient as organic solvents, particularly in extracting active chemicals with antioxidant potential (Shah et al., 2014). Turkmen et al., (2006) used water, acetone, N, N dimethylformamide, and ethanol/methanol as extraction solvents to extract green tea and mate tea components and reported that 50% aqueous ethanol and 50% aqueous acetone yielded the highest yield and thus antioxidant potential.

Water, ethanol, deep eutectic solvents, synthetic ionic liquids, and carbon dioxide, as well as water as a supercritical fluid in extraction, are increasingly preferred by the food industry due to their non-toxicity, food safety, and recycling (Pateiro et al., 2021; Chemat et al., 2019). Ionic liquids are salt mixtures in the liquid phase at room temperature, comprising ionic components that bind ionically (Choi and Verpoorte, 2019). These solvents have very low vapor pressure, high conductivity, are very stable at high temperatures, and have a wide range of polarity, but their food-safety risk, high cost, and poor environmental degradability hinder their proper utilization in the extraction sector (Vekariya, 2017).

2.6. The Efficiency of Response Surface Methodology (RSM)

RSM as a process optimization tool. RSM is a statistical method for experimental modeling, minimizing experimental runs, optimizing processes, and assessing process interaction. This aids in determining the optimal response for the specific extraction approach (Nishad et al., 2021). Traditionally, optimizing a process was a time-consuming operation because it included changing one aspect at a time without considering the interaction of many factors and ensuring the persistence of real optimum conditions. Furthermore, traditional designs cannot modify the number of experiments or the quantities of inputs, which significantly increases the time required to run the experiments. The use of RSM to carry out an experiment can reduce the number of runs, time, and observations. It also lowers the cost of experimentation and allows for the extraction of relevant data from analyses while maintaining their quality. Nowadays, RSM is a ubiquitous instrument used in a variety of scientific disciplines, including industrial science, biological science, chemical science, physical science, clinical science, food science, and engineering sciences, among others. All these sciences include various processes that are reliant on process parameters such as incubation time, temperature, and pH.

RSM has been successfully utilized to standardize electrochemical methods defined by pH, reaction duration, and applied current (Su'arez-Escobar et al., 2016). The textile sector uses RSM to optimize the bioremediation process for wastewater treatment (Asgher et al., 2014). Food science employs the RSM optimization tool for the extraction of bioactive chemicals from plant matrices, the synthesis or degradation of specific nutraceuticals, and food analysis (Chakraborty et al., 2020). Furthermore, in chemical engineering to optimize catalyst activity (Helmi et al., 2020), and in clinical science to create liposomes (Bo et al., 2015). These are only a few examples of how RSM is used to optimize various processes; there are several other applications for RSM. One of the areas mentioned above is food science, which finds the numerous uses of RSM in standardizing or optimizing the process for extraction of phytochemicals (phenolics, flavonoids, alkaloids) or macromolecules (polysaccharides, proteins, oils), synthesis of specific ingredient for the food industry (Aissa et al., 2012), studying the degradation of specific metabolite (Sivasubramanian and Namasivayam, 2015), identification of bioactive molecules (Francescato et al., 2013), fractionation of compounds, quantification of specific metabolite (Mabood et al., 2017) and also for getting clarified juice with stability (Tomadoni et al., 2016). Extraction variables such as solvent type and volume, time, and temperature all have a substantial impact on the extraction process. The combination of variables determines the best circumstances for producing an appropriate amount of bioactives. RSM is used to extract natural bioactives from plant matrices (Granato et al., 2014; Kumar et al., 2019). Serious analytical limitations associated with arbitrary extraction times and temperatures can be overcome by using statistical models that precisely describe the individual and combinatorial effects of variables. This appears to be the most capable methodology for recovering an extract with maximum functionalities from plant materials.

2.7. Novel plant extracts in food product development

Advances in the study of plant-derived phytocompounds have created new opportunities for use in the food sector and medicine. With their broad biological effects against many diseases, these chemicals hold great promise for enhancing human health and disease prevention. Furthermore, continuous research into medicinal plants and ways for enhancing their characteristics will likely result in exciting breakthroughs, bringing long-term solutions to global health concerns (Kowalczewski and Zembrzuska, 2023). Plant extracts are frequently used in the food business for a variety of purposes. The effectiveness of plant extracts is determined by the physicochemical qualities, biological activities, and bioavailability of their active components. However, the limited stability and disagreeable taste of plant extracts limit their use in the food business. Encapsulation is an emerging technology for preserving the biological activity and bioavailability of these bioactive substances while permitting their use in a variety of food products. Encapsulation is the most effective method for not only preserving but also enhancing their physicochemical properties, overcoming the limitations of low water solubility, and improving the sustained release, bioavailability, and application of these functional ingredients in food systems (Reddy et al., 2022).

2.8. Advancing Oral Bioavailability of Natural Antioxidants through Encapsulation

The consumption of natural bioactive compounds such as polyphenols and anthocyanins are highly desirable, but the difficulties associated with these compounds' susceptibility to adverse external effects, or damaging food processing conditions, as well as their chemical instability, have resulted in significant effort to improve oral bioavailability. Therefore, microencapsulation is a potential notion. The use of microencapsulated bioactive compounds as functional ingredients in various food and beverage applications has great promise since it allows for the addition of natural antioxidants to a variety of food products (Diplock et al., 1999). Such components are typically encased in a wall material, which imparts valuable and/or eliminates worthless qualities to the original element (Gharsallaoui et al., 2007).

In addition to their health benefits as antioxidants and anticancer agents, anthocyanins can be employed as colorants. It is a water-soluble pigment that can be used instead of red to blue food colors (Reynertson et al., 2006). Concerns about the harmful and carcinogenic consequences of artificial colorants, as well as the increased demand for healthful meals, have prompted consumers to replace them with natural colorants. The inclusion of functional substances into food is frequently limited by their flavor and instability during processing and storage. Encapsulation techniques are frequently utilized to overcome these restrictions and generate greater value-added goods (Celli et al., 2015).

Ionic gelation is a microencapsulation technique that can be carried out utilizing atomization, dripping (coextrusion, extrusion), or electrostatic spray methods. This approach has the benefit of employing mild conditions because it does not use high temperatures, strong stirring, or organic solvents, allowing the encapsulation of compounds that would deteriorate under other conditions (Colak et al., 2016; Mukai-Corrêa et al., 2005). Hydrogels are defined as polymeric networks having hydrophilic qualities, and physical hydrogels known as "ionotropic hydrogel" are created by mixing a polyelectrolyte with a multivalent ion with the opposite charge (Ahmed, 2015).

Hydrogels are often utilized for encapsulation because of their excellent capacity to adsorb water and biological fluids. According to Chan et al., (2010), liquid extracts, such as concentrated herbal aqueous extracts, can be encapsulated by adsorbing them onto prefabricated calcium-alginate hydrogel beads. Adsorption to encapsulate hydrophilic molecules may be an alternate strategy for preventing core material losses into the ionic solution. Hydrogel beads are immersed in a concentrated solution, allowing active chemicals from the extract to permeate into the blank beads. In a study conducted by Otálora et al., (2018) in encapsulated cactus (*Opuntia megacantha*) betaxanthins by ionic gelation and spray drying, it was discovered that encapsulation for spray drying, and ionic gelation allowed greater pigment stability at low% RH than lyophilised pulp, which was used as a control. The encapsulates had a high total dietary fiber content and anti-radical activity, making this yellow orange natural colorant a valuable biofunctional addition. Co'rdoba et al., (2013) encapsulated yerba mate polyphenols in calcium alginate beads containing starch and discovered an increase in encapsulation efficiency from 55% (control sample, without starch) to 65%.

The authors discovered that starch granules filled holes in the calcium alginate matrix, preventing polyphenols from diffusing into the cross-linking solution during ionic gelation. Adding inulin to an alginate matrix increased carqueja extract encapsulation effectiveness from 49 to 73.8% (Balanč et al., 2016). The authors postulated that polar polyphenol molecules interact with inulin's hydroxyl groups. Stojanovic et al., (2012) similarly showed a positive effect of inulin on encapsulation efficiency when dealing with thyme extract, with an increase of around 55% when compared to alginate microbeads without inulin (51% efficiency).