Health-Promoting Phytochemicals: Comprehensive Review of Processing and Analytical Strategies

Shashank Tidke[1], Vidya G[2], Samanvitha Suresh[1], Poojitha M[1], N. Rajeswari[1]
[1]*Department of Biotechnology, Dayananda Sagar college of Engineering, Kumaraswamy layout, Bangalore India, 560111*
[2] *Department of chemistry, Dayananda Sagar College of Engineering, Kumaraswamy layout, Bangalore India, 5600111*

Abstract **Plant phytochemicals are naturally occurring compounds found in plants that contribute to their color, flavor, and resistance to diseases and pests. These compounds are not essential nutrients like vitamins and minerals but have been found to offer various health benefits to humans. This review article investigates the extensive applications of plant phytochemicals in therapeutics. It includes various categories of phytochemicals, such as flavonoids, alkaloids, glucosinolates, phenolic acids, stilbenes, tannins, saponins, terpenoids, lignans and organosulfur compounds, highlighting their biological activities and positive effects. We explore various extraction and identification techniques, including traditional methods like solvent extraction and advanced methods such as supercritical fluid extraction and chromatography. Furthermore, we discuss recent advancements in the production of phytochemicals, including biotechnological approaches and sustainable practices aimed at enhancing yield and potency. By providing a comprehensive overview of the current state of phytochemical research and its industrial applications, this review underscores the potential of these bioactive compounds in developing innovative products that meet the growing consumer demand for natural and effective solutions.**

Keywords: Phytochemicals, Anticancer, Antimicrobial, Antimalarial, Phytochemical Screening.

I. INTRODUCTION

Medicinal plants are generally used to treat common as well as chronic diseases, because they are rich in bioactive compounds, or simply called, phytochemicals. Phytochemicals are plant-based bioactive compounds produced by plants for their protection. These compounds can be derived from a variety of sources, including whole grains, fruits, vegetables, nuts, and herbs. A diet low in nutritious foods like fruits, vegetables, and whole grains, and high in ultra-processed grains and sugary drinks, increases the risk of chronic diseases. Improving dietary quality by adding essential nutrients could prevent up to 20% of global deaths. The Mediterranean diet (MedDiet), rich in olive oil, berries, and honey, is linked to increased longevity and reduced cancer and cardiovascular disease risk by inactivating carcinogens and inhibiting tumor growth. To date, over a thousand phytochemicals have been discovered. Some of the significant phytochemicals are alkaloids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides. These phytochemicals demonstrate potent antioxidant properties and exhibit a range of activities, including antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral effects. Additionally, they play roles in regulating gene transcription, enhancing gap junction communication, boosting immunity, and offering protection against various cancers (Kumar et al., 2023, 1). Plant secondary metabolites (PSMs) are vital for human health and form many pharmaceutical drugs' backbone. Indeed, more than 25% of the existing drugs belong to PSMs. The most widely recognized PSM-derived drugs include morphine (from *Papaver somniferum*), digitoxin (from *Digitalis purpurea*), taxol (from *Taxus baccata*), artemisinin (from *Artemisia annua*), and quinine (from *Cinchona officinalis*). Additionally, vinblastine and vincristine (from Catharanthus roseus), and aspirin (initially isolated as salicylic acid from *Filipendula ulmaria*) are also notable. Plants exposed to various abiotic stress conditions produce these secondary metabolites in higher concentrations as a coping mechanism.

The extraction of compounds from plants is central to natural product research, with ongoing advancements in extraction methods. Modern green techniques such as supercritical fluid extraction, ultrasound-assisted extraction, accelerated solvent extraction, microwave-assisted extraction, and enzyme-assisted methods are gaining popularity (Bitwell et al., 2023, 2) . Following extraction, compounds need characterization and analysis to determine their chemical composition and biological properties. Chromatographic methods like affinity chromatography, thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gel filtration, and adsorption chromatography are used for separation and quantification. Spectroscopic methods, including nuclear magnetic resonance (NMR) spectroscopy, UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), mass spectrometry (MS), and advanced techniques like UPLC-Q-Orbitrap HRMS, provide structural insights. This review summarizes major phytochemicals and their medicinal uses, highlighting recent advancements in their production. It also covers key techniques for extraction, purification, and identification, aiming to enhance understanding and support further research in phytochemistry for developing effective natural products.

Overview of Key Phytochemicals and Associated Health Benefits

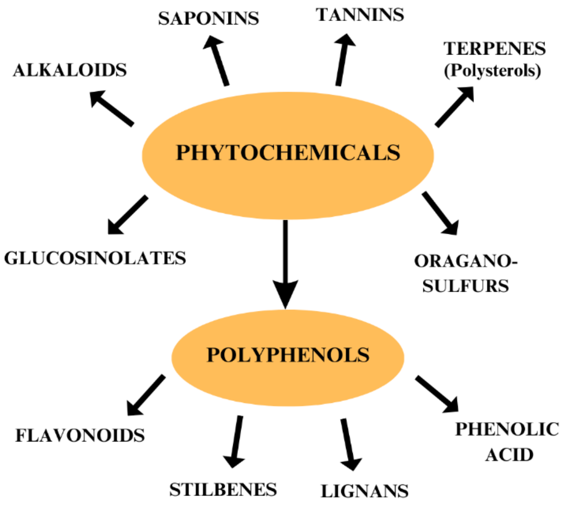

Fig.1*:* Major Classification of Phytochemicals

Phytochemicals are naturally occurring compounds found in plants. These chemicals are responsible for various biological activities in the plants themselves, including growth, reproduction, and protection against pests and diseases. In human nutrition and health, phytochemicals are recognized for their potential health benefits and roles in disease prevention. Fig.1 illustrates the major classes of phytochemicals, while Table.1 provides a detailed discussion of these categories.

Table 1: Types of Phytochemicals with their health benefits

Type of phytochemicals	Description	Health Benefits
Phenolic acids	Plant phenolics, such as anthocyanins, flavonoids, and phenolic acids, are natural compounds with diverse structures. Phenolic acids, in particular, are notable for their high solubility in both lipids and water, which enhances their effectiveness as antioxidants in preventing oxidative deterioration in emulsions.	Anti-inflammatory and anti-cancer effects, helps with liver damage and allergies, reduce LDL oxidation and rheumatoid arthritis symptoms, neuro- and cardio-protective properties. (Kiokias et al., 2020, 4)
Lignans	Lignans are natural compounds derived from the shikimic acid pathway, primarily consisting of phenylpropanoid units. They are categorized into classical lignans, linked at β-β′ positions, and neolignans, with different linkages. These compounds are widely distributed across over 70 plant families, including Lauraceae, Annonaceae, Orchidaceae, and Schisandraceae. Lignans typically appear as dimers but can also be trimers or tetramers, and are often found in free form or as glycosides.	Anti-Inflammatory, anti-viral, anti-cancer help with cardiovascular and bone health, neuroprotection, and menopause symptom relief. (Cui et al., 2020,3) (Jang et al., 2022, 5)
Stilbenes	Stilbenes are natural polyphenols with two aromatic rings linked by an ethylene group, existing mainly in the E (trans) form. They protect plants from stressors like UV radiation and pests.	anticancer, anti-inflammatory, antimicrobial, and neuroprotective effects. (Reinisalo et al., 2015, 6),

Flavonoids	Flavonoids are a significant group of natural plant compounds known as polyphenols, which play a key role in plant growth and defense. These low-molecular-weight phenolic compounds are widespread in the plant kingdom. Depending on the attachment of the B ring and the saturation and oxidation level of the C ring, flavonoids are classified into several subgroups: flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanins, and chalcones.	antiviral properties, antibacterial activity, support heart health by protecting LDL from oxidative damage, anti-inflammatory, anti-cancer. (Roy et al., 2022, 7)
Alkaloids	Alkaloids are a group of naturally occurring chemical compounds that typically contain basic nitrogen atoms, though some neutral or weakly acidic compounds are also classified as alkaloids. Certain synthetic compounds are also considered alkaloids. In addition to carbon, nitrogen, and hydrogen, alkaloids may contain sulfur and, less commonly, elements like bromine, phosphorus, or chlorine.	anti-cancer, anti-inflammatory, anti-malarial, and anti-microbial properties, potential benefits for Parkinson's and Alzheimer's diseases. (Rajput et al., 2022, 8)
Terpenoids	They are a large group of natural compounds derived from mevalonic acid (MVA) and consisting of multiple isoprene units. With over 50,000 identified, mainly from plants, they play crucial roles in plant growth and defense	antitumor, anti-inflammatory, and antimicrobial activities, Anti-diabetic, antioxidant, anti-inflammatory, anti-rheumatic, anti-arthritic. (Yang et al., 2020, 9)
Glucosinolates	Glucosinolates are naturally occurring compounds predominantly found in the order Capparales, which includes families like Brassicaceae, Caricaceae, and Capparaceae. All glucosinolates share a core structure consisting of a β-thioglucose molecule linked via a sulfur atom to a (Z)-N-hydroximinosulfate ester, with a side chain that varies depending on its amino acid origin.	Antimicrobial, anticancer, antioxidant. (Ishida et al., 2014, 10)
Saponins	They are versatile phytochemicals found in plants, marine life, and microorganisms. They feature a hydrophobic aglycone linked to a hydrophilic sugar moiety, which gives them amphiphilic properties. Saponins are classified into triterpenoid types, such as oleananes and ursanes, and steroidal types, including dioscin and diosgenin.	Anti-inflammatory, antiviral, cardioprotective, and anticancer activities; enhance immune responses and offer hypocholesterolemic and antioxidant benefits. (Timilsena et al., 2023, 11)
Tannins	They are complex polyphenolic compounds found in gymnosperms and angiosperms, known for forming strong complexes with macromolecules. They are categorized into hydrolyzable tannins, which are esterified with gallic or ellagic acids and can be broken down into these acids, and condensed tannins, which are flavonoid polymers that can form insoluble products.	Anti-microbial with bacteriostatic and bactericidal activity, Antiviral, Antiparasitic, Anti-inflammatory, and Antioxidant activities. (Das et al., 2020, 12)

II.PLANT PHYTOCHEMICALS USED AS THERAPEUTICS

Plant phytochemicals are bioactive compounds with therapeutic properties, including anti-inflammatory, antioxidant, antimicrobial, and anticancer effects. Found in various plant parts, these compounds are gaining attention in both traditional and modern medicine. As research into alternative therapies and new drugs expands, understanding the mechanisms and benefits of phytochemicals could offer promising opportunities for drug development and improved health.

III.PHYTOCHEMICALS AND ANTICANCER ACTIVITY

Current cancer treatments—surgery, radiotherapy, and chemotherapy—are effective but come with limitations such as damage to healthy cells, lack of specificity, drug resistance, and side effects. Surgery is most effective in early stages, while radiation and chemotherapy are used in later stages but can harm healthy tissues. Phytochemicals, naturally occurring bioactive compounds, offer promising alternatives to improve treatment efficacy and reduce side effects (Choudhari et al., 2020, 13). Annona muricata (Soursop or Graviola) is a tropical plant from the Annonaceae family, traditionally used for its therapeutic properties. The plant contains Annonaceae Acetogenins (AGEs), a class of phytochemicals that inhibit NADH oxidase and disrupt mitochondrial complex-1. Studies show A. muricata leaf extract reduces invasion and motility in MDA-MB-231 breast cancer cells and induces apoptosis in MCF-7 cells. Acetogenins like annonacin, bulletin, desacetyluvaricin, and bullatalicin also exhibit strong anticancer activity, positioning A. muricata as a potential candidate for cancer chemoprevention (Perinbarajan et al., 2024, 14).

Phytochemicals and Antimicrobial activity

Antibiotic resistance has become a global health crisis, making the search for effective alternatives crucial. Plant-based remedies show promise, with extracts from *Calligonum polygonoides, Asphodelus tenuifolius, Pulicaria crispa,* and *Fagonia cretica* demonstrating activity against bacteria like *Staphylococcus carnosus* and *Klebsiella pneumoniae.* Compounds such as alkaloids, flavonoids, carvacrol in essential oils, and organosulfur compounds like allicin are potent antibacterial. Exploring these bioactive compounds and metabolomics is essential for discovering new antimicrobial treatments. Medicinal plants also offer potential for treating parasitic diseases. Malaria, caused by Plasmodium species, remains a major health issue, with resistance to current drugs and challenges in distribution highlighting the need for alternatives. Plants like *Azadirachta indica* (neem), *Anacardium occidentale* (cashew), and *Triumfetta cordifolia* have shown anti-plasmodial effects (Ezenyi et al., 2020, 15). For giardiasis, garlic (*Allium sativum*) and other natural remedies show promise. Amoebiasis and toxoplasmosis can be managed with plants like *Cephaelis ipecacuanha* and *Brucea antidysenterica*. Plants such as blueberries and *Olea europaea* are effective against cryptosporidiosis (F. Ullah et al., 2020, 16). Flavonoids, found in various fruits and vegetables, are noted for their antiviral properties. Compounds like EGCG from green tea and baicalein are effective against rotavirus and human cytomegalovirus. Flavonoids such as fisetin and quercetagetin target dengue, hepatitis C, and chikungunya viruses. Additionally, phenolic acids and flavonoids like luteolin and genistein show activity against Japanese encephalitis, Zika, and herpes B. Alkaloids, terpenes, lignans, and coumarins from plants also exhibit antiviral effects, with compounds such as homonojirimycin and betulin targeting various viruses (Ghildiyal et al., 2020, 17).

IV.ADVANCEMENT IN PRODUCTION OF PHYTOCHEMICALS

With increasing consumer preference for natural products, medicinal plants are becoming more popular than synthetic drugs. Traditional methods and environmental conditions, however, impede the production of plant metabolites. Plant tissue culture provides a solution by allowing controlled, year-round production, and when paired with metabolic engineering and machine learning, it further boosts the efficiency of phytochemical production.

1. Micropropagation

Callus and suspension cultures are used to produce a range of secondary metabolites by manipulating biosynthesis pathways. Callus cultures have been utilized for synthesizing tropane alkaloids, α-tocopherol, ajmaline, serpentine, reserpine, flavonoids, scopolamine, paclitaxel, stilbene, resveratrol, and anthocyanins. Differentiated organ cultures, such as shoots or roots, can also produce metabolites similar to those in native plants (Chandran et al., 2020,18). In biotechnology, in vitro plant production is preferred for its uniform cultivation of plants through controlled conditions and growth regulators, enhancing phytochemical yields. Micropropagation enables the clonal production of genetically identical, pathogen-free plants efficiently (Jareonsin & Pumas, 2021, 19).

2. Hairy root culture in the production of Bioactive compound

Plant hairy root cultures represent a promising alternative for producing compounds typically synthesized in plant roots. *Agrobacterium rhizogenes*-mediated transformation has enabled the induction of hairy roots in plants, facilitating in vitro production of secondary metabolites found in roots, including nicotine, tropane alkaloids, ginsenosides, anthraquinones, and artemisinin. Hairy roots are favored for their high productivity, stability, and efficiency in compound development. Hairy root can be used as a biological farm to yield recombinant proteins, pharmaceuticals, flavors and pigments, and many more products. These hairy roots are generally grown in the bioreactor using *Agrobacterium rhizogenes* for large scale production (Mishra & Ranjan, 2008, 20).

3. Shoot culture in the production of Bioactive compound

In vitro shoot cultures are a promising source of high-value chemicals, including medicines, antioxidants, and flavorings. They retain tissue differentiation from the parent plant, enabling the synthesis of secondary metabolites not found in unorganized cell suspensions (Krol et al., 2021, 21). Shoot cultures have been effectively used to extract compounds like Phenylpropanoid and Naphtodianthrone from Hypericum perforatum, known for their anti-inflammatory, anti-tumoral, anti-viral, anti-microbial, and antioxidant properties (Gadzovska Simic et al., 2014, 22). Utilizing stress responses in plants to enhance secondary metabolite production is a promising strategy. Shoot cultures of A. integrifolia, exposed to biotic elicitors such as yeast and pectin extracts, have shown increased accumulation of compounds like rosmarinic acid, chlorogenic acid, apigenin, and quercetin, which possess antioxidant properties. This suggests that shoot cultures could be valuable for producing natural antioxidants (M. A. Ullah et al., 2021, 23).

4. Metabolic Engineering

Producing therapeutic phytochemicals is challenging due to their complex structures and high costs associated with chemical synthesis and plant extraction. Plant extraction can be inefficient, leading to shortage of drugs, requiring large amounts of plant material. Metabolic engineering aims to establish a green manufacturing industry by using cell factories for the bioproduction of chemicals, especially plant natural products (PNPs), which are useful as flavors, fragrances, and medicines. Microbial production offers scalable, controlled, and on-demand synthesis of PNPs, overcoming supply chain issues and enabling production of intermediates and novel derivatives. The microbial production of phytochemicals allows for higher purity and yield than traditional plant extraction, with host selection being the key. Microbes like *Streptomyces*, *Corynebacterium glutamicum*, and *Yarrowia lipolytica* are used for specific applications, but *E. coli* and *S. cerevisiae* are common due to their ease of genetic manipulation and scale-up potential. Engineering strategies, including the elimination of competing pathways, have notably increased PNP production, as demonstrated by a six-fold rise in strictosidine production through pathway optimization in yeast (Cravens et al., 2019, 24). 5Strategies to increase natural product yields include upregulating or silencing specific genes or introducing new genes. Genomic technologies, such as RNA-seq, are vital for identifying relevant genes, while metabolomics and proteomics enhance pathway understanding and enzyme discovery. Collaborative projects on medicinal plants have provided data to elucidate biosynthetic pathways (Wilson & Roberts, 2014, 25). For instance, improvements in ginsenoside Rh2 production in yeast were achieved through enzyme engineering and cofactor adjustments. Tools like AlphaFold2 are advancing enzyme optimization for therapeutic phytochemicals (Holtz et al., 2024, 26).

5. Combining In-Vitro Culture with Machine Learning Technologies

Advances in genomics, bioinformatics, and synthetic biology could transform phytochemical discovery and production by improving our understanding of biosynthetic pathways. Effective use of plant genomics and functional data requires integrating computational and experimental methods. Future developments may involve using genomic insights and transcriptome data to reconstruct biosynthetic pathways in microbial systems, facilitating scalable production of complex phytochemicals and enhancing our understanding of plant natural product synthesis. Research is also exploring strategies to optimize the extraction and production of phenolic compounds from medicinal plants. Traditional aqueous-alcoholic solvent extraction methods face challenges in fully recovering these compounds. Innovative approaches combine advanced machine learning with controlled cultivation of Bryophyllum species under abiotic stress to identify factors affecting phenolic production. Using neurofuzzy logic, the study improves extraction processes and uncovers the potential of underexplored medicinal plants, benefiting medicine, agriculture, and nutrition (García-Pérez et al., 2020, 27).

V.TECHNIQUES USED IN THE EXTRACTION OF PHYTOCHEMICAL

Phytochemical Extraction is a critical stage in separating health-promoting phytochemicals from various plant sources. The selection of method mostly related on the nature of the natural compound and plant matrix. Various extraction methods are shown in Fig. 2 and Table 2.

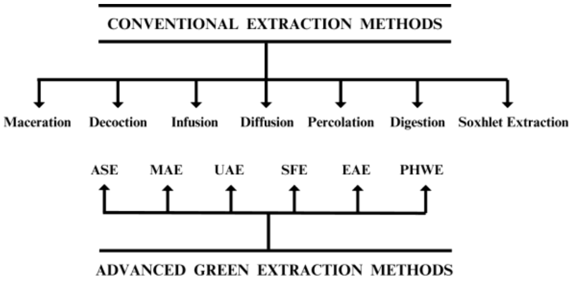

Fig.2: Methods used for extraction of bioactive phytochemicals; ASE- Accelerated solvent extraction; MAE- Microwave-Assisted Extraction; UAE- Ultrasound assisted extraction; SFE- Supercritical fluid extraction; EAE- Enzyme assisted extraction; PHWE- Pressurized hot water extraction;

Table 2: Methods used for extraction of bioactive phytochemicals.

Technique	Description
Maceration	Maceration is an extraction method involving soaking plant materials in solvents like water, ethanol, or methanol for 3–7 days with occasional shaking. After soaking, the liquid is strained, and the solid residue is pressed to extract additional liquid, followed by filtration or decantation. Simplicity, No Need for Specialized Equipment, Preservation of Thermolabile Compounds,Cost-Effective, Flexibility Time-consuming, Labor-intensive, Solvent type and polarity affect efficiency. (Kumar et al., 2023, 1)
Digestion	Digestion is an extraction method using warmed solvent (35 to 50 °C) for 0.5 to 24 hours with shaking to improve extraction efficiency while minimizing degradation of bioactive compounds. Increased Efficiency, Faster Extraction, Better Extraction of Tough Materials. Risk of Degradation, Requires Temperature Control, Limited Temperature Range. (Bitwell et al., 2023, 2)
Infusion	Infusions involve extracting soluble compounds from plant materials by soaking them in water. Traditionally, the process involves treating powdered plant material with cold water for 15 minutes, then boiling for 30 minutes, followed by straining. Simple and effective extraction method, Quick preparation, Adjustable potency with dilution. Alcohol inclusion preserves aroma and strength Prone to microbial contamination, Limited shelf life, less suitable for industrial use, requires prompt use or proper preservation to prevent spoilage. (Kumar et al., 2023, 1).
Percolation	Percolation is a more efficient extraction method than maceration, as it continuously replaces saturated solvent with fresh solvent, leading to higher yields of desired compounds. This continuous flow often results in better extraction of specific phytochemicals compared to methods like refluxing. Extracts higher concentrations of active components, achieves better efficiency, Continuous renewal of solvent enhances extraction. Complexity and cost of the process, which requires specialized equipment and careful optimization of solvent concentrations and extraction conditions. (Zhang et al., 2018, 28).
Soxhlet Extraction	Soxhlet extraction involves placing a sample in a thimble within an extractor. Solvent in a connected flask is heated, evaporates, and condenses onto the sample. This solvent then dissolves target compounds, which are siphoned back into the flask. The cycle repeats until extraction is complete. Continuous fresh solvent supply improves extraction efficiency, Effective heat application aids in compound extraction; No filtration needed for the final extract, Allows simultaneous processing of multiple samples; Relatively low cost and straightforward operation. Time-consuming. Large solvent use; Sensitive compound degradation; No agitation acceleration; Economic and environmental risks. (Spinei & Oroian, 2023, 29).
Accelerated solvent extraction (ASE)	accelerated solvent extraction also called Pressurized Liquid Extraction (PLE) is an automated method for extracting compounds from solid samples using solvents at high temperatures and pressures. It improves extraction efficiency by keeping the solvent

	in a liquid state above its boiling point. Key factors include sample size, solvent type, temperature, pressure, pH, and flow rate, which influence the process. Fast extraction, Low solvent volume, no filtration needed, Enhanced solubility, Higher yield, Eco-friendly. High equipment costs, Precise optimization needed, Maintenance issues, Complex controlling, Limited accessibility. (Quitério et al., 2022,)
Ultrasound assisted extraction (UAE)	Ultrasound-assisted extraction uses sound waves to create cavitation bubbles in a solvent, which disrupts cellular structures and enhances solvent penetration. This method, influenced by solvent properties, generates high-velocity jets from collapsing bubbles, improving compound extraction. It is environmentally friendly due to its low energy and solvent use and achieves high yields quickly. Low energy and solvent consumption, improved extraction efficiency, and reduced extraction time. Need to compare green solvents with traditional ones to fully understand their efficacy, and potential limitations in solvent choice based on the physical properties of the medium. (Picot-Allain et al., 2021,31)
Supercritical fluid extraction (SFE)	Supercritical Fluid Extraction (SFE) uses fluids above their critical points for high solvation power. Carbon dioxide (CO_2) is commonly used due to its safety and environmental benefits. The process involves cooling and pressurizing CO_2, then heating it to extract compounds from the sample. A co-solvent like ethanol can be added to enhance solubility. The solvent and extract are then separated by reducing pressure and temperature. Selective extraction, Mild temperatures, environmentally friendly, Cost-effective, Easy solvent recycling. High equipment cost, Challenges with extraction of polar compounds which needs co-solvents, Complex process, High setup and operational expenses. (Osorio-Tobón, 2020, 32).
Enzyme assisted extraction (EAE)	Enzyme-assisted extraction (EAE) uses enzymes to break down cell walls in plant or food materials, making bioactive compounds more accessible for extraction. Enzymes like cellulase, protease, or pectinase are applied under controlled conditions to enhance extraction efficiency, typically using water as the solvent and low temperatures to prevent degradation of sensitive compounds. Enhanced recovery. Environmentally friendly Low temperature, Energy-efficient, Higher yields. Limited enzyme range. Specific to vegetables and beverages Complex optimization, Variable conditions. (Sridhar et al., 2021, 33).

VI.PURIFICATION TECHNIQUES

Purification Techniques involve methods used to isolate and purify compounds from mixtures, often for further analysis or use in pharmaceuticals, cosmetics, or research. These techniques exploit various physical and chemical properties of compounds, such as size, charge, solubility, and affinity, to achieve separation and purification. This section discusses various purification techniques used to isolate and purify bioactive compounds. Fig.3 describes various purification techniques of phytochemicals.

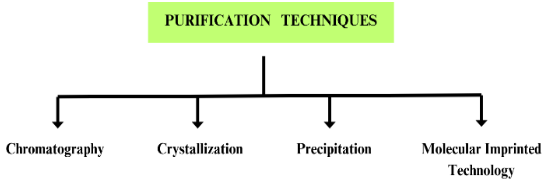

Fig.3: Major Techniques for Purification of Phytochemicals

1. Gel Chromatography and Adsorption Chromatography:

Gel filtration chromatography separates molecules based on size using a porous gel medium, with larger molecules eluting first as they cannot enter the beads. Adsorption chromatography separates substances based on their affinities for a solid support, leading to different retention times. A recent study on willow bark (Salicaceae) used hot water extraction followed by sequential chromatography to isolate antioxidant phytochemicals. Hydrophilic weak cation exchange resins, gel permeation, and adsorption chromatography were used to separate compounds like (+)-catechin and salicin, which were then quantified and characterized using high-performance liquid chromatography and NMR spectroscopy (Dou et al., 2021, 34).

2. Thin Layer Chromatography:

Thin Layer Chromatography (TLC) separates compounds based on their distribution between a stationary solid phase and a liquid mobile phase. Compounds more soluble in the mobile phase travel further, while less soluble ones stay closer to the origin. In a study on isolating Gossypol acetic acid from cottonseed, TLC was used for preliminary purification to ensure accurate analysis. TLC isolated different enantiomers of gossypol acetic acid, facilitating visual monitoring and purification. It was complemented by silica gel-based methods like column chromatography and HPLC for enhanced isolation and study.

3. HPLC:

High-Performance Liquid Chromatography (HPLC) separates compounds based on their interactions with a stationary phase in a packed column and a mobile phase solvent, determined by the polarity and affinity of the compounds. HPLC is crucial for purifying and analyzing phytochemicals in pomegranate peel extracts identified nine key polyphenolic compounds, including protocatechuic acid and ellagic acid, in the ethanol extract with the highest total phenolics and flavonoids. The presence of active compounds like cinnamic acid and p-coumaric acid underscores HPLC's role in providing a detailed phytochemical profile, supporting their applications in pharmaceuticals and cosmetics (El-Hamamsy & El-Khamissi, 2020, 36).

4. Crystallization and Precipitation:

Crystallization is a cost-effective method to obtain solid bioactive crystals from saturated solutions but may face challenges like low yield, slow rates, and potential impurities. Precipitation, on the other hand, quickly produces solid particles with a precipitating agent but can result in lower purity and the formation of aggregates. The purification of rosmarinic acid from hydroethanolic extracts was achieved without preparative chromatography by partitioning with a low-toxicity solvent, cold evaporation under vacuum, and recrystallization from a hot aqueous solution. This method, yielding about half the amount reported analytically, offers a practical approach for purifying phenolic acids that typically resist precipitation.

5. Molecularly Imprinted Technology:

Molecularly imprinted polymers (MIPs) are synthetic polymers used to isolate and purify target molecules with high selectivity, reusability, and low cost. MIPs are created by copolymerizing monomers with template molecules, which are removed to leave selective binding sites. Synthesis methods include bulk, precipitation, and surface molecular imprinting (SMIT). SMIT, using solid surfaces like magnetic Fe3O4, offers faster binding and higher separation efficiency. MIPs are applied in solid-phase extractions (MISPE, MIDSPE) and chromatographic separation. Bulk polymerization has been used to isolate sinapic acid from broccoli, while MMIPs have been effective for hesperetin separation from Citrus reticulata (Susanti et al., 2024, 37).

VII.IDENTIFICATION TECHNIQUES

Compound identification and screening are vital in analytical chemistry and pharmaceutical research. Fig. 4 shows various analytical techniques such as FTIR, Mass Spectroscopy, NMR, UPLC-Q-Orbitrap-HRMS, and UV-Vis spectroscopy.

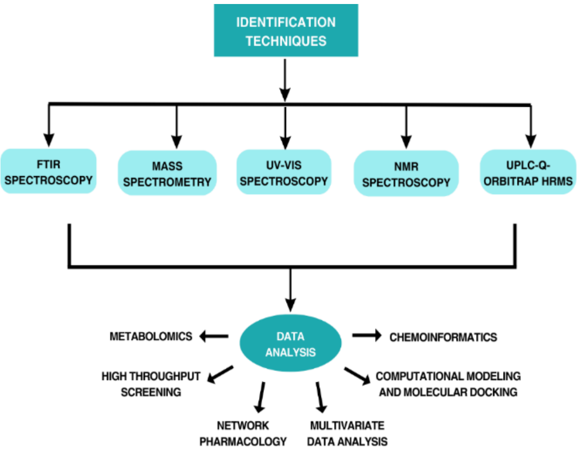

Fig .4: Major Techniques for Identification of Phytochemicals

These analytical techniques play key roles such as, FTIR identifies functional groups, MS provides molecular weight and structure, NMR reveals molecular frameworks, UPLC-Q-Orbitrap-HRMS combines high-resolution mass analysis with chromatography, and UV-Vis detects chromophore compounds. Together, these methods enable thorough analysis, supporting the discovery and validation of bioactive compounds and advancing drug development. Following phytochemical screening, techniques like metabolomics, cheminformatics, high-throughput screening, network pharmacology, and molecular docking aid in data interpretation. These methods provide insights into metabolite profiles, chemical interactions, and potential therapeutic uses, enhancing drug formulation and optimization from phytochemicals.

VIII.CONCLUSION

Current research underscores the promising health benefits of phytochemicals, particularly their antimicrobial and anticancer properties, as well as their versatility in various industrial applications. Natural compounds are promising for disease prevention, with phytochemicals vital in food and pharmaceuticals. Advancements in phytochemical production, including micropropagation, hairy root, and shoot cultures, have improved yield and efficiency. Metabolic engineering enhances compound production, while combining in-vitro techniques with machine learning optimizes processes and accelerates research. These innovations enable more effective and scalable production for various applications. Extraction methods like MAE, PLE, SFE, and UAE efficiently produce phytochemical-rich extracts using minimal solvents and shorter times. Combining these methods could enhance efficiency, and future work should scale them for industrial use while preserving bioactivity. The primary techniques for purifying phytochemicals—such as chromatography (including Gel-Adsorption, Affinity, HPLC, and TLC), crystallization and precipitation, and molecularly imprinted technology—each offer unique benefits. Chromatography delivers precise separation, while crystallization and precipitation are effective for isolating compounds. Molecularly Imprinted Technology provides selective purification. These methods collectively improve the purity and yield of bioactive compounds, facilitating their use in research and various industries. Each screening technique offers distinct and complementary insights into chemical samples. NMR provides detailed structural data, and UPLC-Q-Orbitrap-HRMS combines high-resolution mass spectrometry with advanced chromatography for precise detection and quantification. Ongoing improvements in these processes are likely to enhance the effectiveness and reliability of phytochemical-based treatments, contributing to more precise and sustainable health interventions.

REFERENCES

[1] Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., & K, S. (2023). Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*, *28*(2), 887.

[2] Bitwell, C., Indra, S. Sen, Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, *19*, e01585.

[3] Cui, Q., Du, R., Liu, M., & Rong, L. (2020). Lignans and their derivatives from plants as antivirals. *Molecules*, *25*(1), 183.

[4] Kiokias, S., Proestos, C., & Oreopoulou, V. (2020). Phenolic acids of plant origin—A review on their antioxidant activity in vitro (o/w emulsion systems) along with their in vivo health biochemical properties. *Foods*, *9*(4), 534.

[5] Jang, W. Y., Kim, M.-Y., & Cho, J. Y. (2022). Antioxidant, anti-inflammatory, anti-menopausal, and anti-cancer effects of lignans and their metabolites. *International Journal of Molecular Sciences*, *23*(24), 15482.

[6] Reinisalo, M., Kårlund, A., Koskela, A., Kaarniranta, K., & Karjalainen, R. O. (2015). Polyphenol stilbenes: molecular mechanisms of defence against oxidative stress and aging-related diseases. *Oxidative Medicine and Cellular Longevity*, *2015*(1), 340520.

[7] Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., Islam, S., & Islam, M. R. (2022). Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed Research International*, *2022*(1), 5445291.

[8] Rajput, A., Sharma, R., & Bharti, R. (2022). Pharmacological activities and toxicities of alkaloids on human health. *Materials Today: Proceedings*, *48*, 1407–1415.

[9] Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in pharmacological activities of terpenoids. *Natural Product Communications*, *15*(3), 1934578X20903555.

[10] Ishida, M., Hara, M., Fukino, N., Kakizaki, T., & Morimitsu, Y. (2014). Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. *Breeding Science*, *64*(1), 48–59.

[11] Timilsena, Y. P., Phosanam, A., & Stockmann, R. (2023). Perspectives on saponins: food functionality and applications. *International Journal of Molecular Sciences*, *24*(17), 13538.

[12] Das, A. K., Islam, M. N., Faruk, M. O., Ashaduzzaman, M., & Dungani, R. (2020). Review on tannins: Extraction processes, applications and possibilities. *South African*

Journal of Botany, *135*, 58–70.

[13] Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P., & Prakash, O. (2020). Phytochemicals in cancer treatment: From preclinical studies to clinical practice. *Frontiers in Pharmacology*, *10*, 1614.

[14] Perinbarajan, G. K., Sinclair, B. J., Mossa, A.-T., Ohja, N., & Jeelani, P. G. (2024). Silica/Annona muricata nano-hybrid: Synthesis and anticancer activity against breast cancer. *Heliyon*, *10*(3).

[15] Ezenyi, I. C., Verma, V., Singh, S., Okhale, S. E., & Adzu, B. (2020). Ethnopharmacology-aided antiplasmodial evaluation of six selected plants used for malaria treatment in Nigeria. *Journal of Ethnopharmacology*, *254*, 112694.

[16] Ullah, F., Ayaz, M., Sadiq, A., Ullah, F., Hussain, I., Shahid, M., Yessimbekov, Z., Adhikari-Devkota, A., & Devkota, H. P. (2020). Potential role of plant extracts and phytochemicals against foodborne pathogens. *Applied Sciences*, *10*(13), 4597.

[17] Ghildiyal, R., Prakash, V., Chaudhary, V. K., Gupta, V., & Gabrani, R. (2020). Phytochemicals as antiviral agents: recent updates. *Plant-Derived Bioactives: Production, Properties and Therapeutic Applications*, 279–295.

[18] Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology Reports*, *26*, e00450.

[19] Jareonsin, S., & Pumas, C. (2021). Advantages of heterotrophic microalgae as a host for phytochemicals production. *Frontiers in Bioengineering and Biotechnology*, *9*, 628597.

[20] Mishra, B. N., & Ranjan, R. (2008). Growth of hairy-root cultures in various bioreactors for the production of secondary metabolites. *Biotechnology and Applied Biochemistry*, *49*(1), 1–10.

[21] Krol, A., Kokotkiewicz, A., Szopa, A., Ekiert, H. M., & Luczkiewicz, M. (2021). Bioreactor-grown shoot cultures for the secondary metabolite production. *Plant Cell and Tissue Differentiation and Secondary Metabolites: Fundamentals and Applications*, 187–247.

[22] Gadzovska Simic, S., Tusevski, O., Maury, S., Delaunay, A., Joseph, C., & Hagège, D. (2014). Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response in Hypericum perforatum L. shoot cultures. *The Scientific World Journal*, *2014*(1), 609649.

[23] Ullah, M. A., Gul, F. Z., Khan, T., Bajwa, M. N., Drouet, S., Tungmunnithum, D., Giglioli-Guivarc'h, N., Liu, C., Hano, C., & Abbasi, B. H. (2021). Differential induction of antioxidant and anti-inflammatory phytochemicals in agitated micro-shoot cultures of Ajuga integrifolia Buch. Ham. ex D. Don with biotic elicitors. *AMB Express*, *11*, 1–13.

[24] Cravens, A., Payne, J., & Smolke, C. D. (2019). Synthetic biology strategies for microbial biosynthesis of plant natural products. *Nature Communications*, *10*(1), 2142.

[25] Wilson, S. A., & Roberts, S. C. (2014). Metabolic engineering approaches for production of biochemicals in food and medicinal plants. *Current Opinion in Biotechnology*, *26*, 174–182.

[26] Holtz, M., Acevedo-Rocha, C. G., & Jensen, M. K. (2024). Combining enzyme and metabolic engineering for microbial supply of therapeutic phytochemicals. *Current Opinion in Biotechnology*, *87*, 103110.

[27] García-Pérez, P., Lozano-Milo, E., Landín, M., & Gallego, P. P. (2020). Combining medicinal plant in vitro culture with machine learning technologies for maximizing the production of phenolic compounds. *Antioxidants*, *9*(3), 210.

[28] Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine*, *13*, 1–26.

[29] Spinei, M., & Oroian, M. (2023). Structural, functional and physicochemical properties of pectin from grape pomace as affected by different extraction techniques. *International Journal of Biological Macromolecules*, *224*, 739–753.

[30] Chaves, J. O., De Souza, M. C., Da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. da F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A. V., & Barbero, G. F. (2020). Extraction of flavonoids from natural sources using modern techniques. *Frontiers in Chemistry*, *8*, 507887.

[31] Picot-Allain, C., Mahomoodally, M. F., Ak, G., & Zengin, G. (2021). Conventional versus green extraction techniques—A comparative perspective. *Current Opinion in Food Science*,

40, 144–156.

[32] Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, *57*, 4299–4315.

[33] Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D.-V. N., & Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: A review. *Environmental Chemistry Letters*, *19*, 3409–3443.

[34] Dou, J., Heinonen, J., Vuorinen, T., Xu, C., & Sainio, T. (2021). Chromatographic recovery and purification of natural phytochemicals from underappreciated willow bark water extracts. *Separation and Purification Technology*, *261*, 118247.

[35] Öztürk, C., Aksoy, M., & Küfrevioğlu, Ö. İ. (2020). Purification of tea leaf (Camellia sinensis) polyphenol oxidase by using affinity chromatography and investigation of its kinetic properties. *Journal of Food Measurement and Characterization*, *14*, 31–38.

[36] El-Hamamsy, S. M. A., & El-Khamissi, H. A. Z. (2020). Phytochemicals, antioxidant activity and identification of phenolic compounds by HPLC of pomegranate (Punica granatum L.) Peel extracts. *Journal of Agricultural Chemistry and Biotechnology*, *11*(4), 79–84.

[37] Susanti, I., Pratiwi, R., Rosandi, Y., & Hasanah, A. N. (2024). Separation methods of phenolic compounds from plant extract as antioxidant agents candidate. *Plants*, *13*(7), 965.