Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2026, Volume 17, Issue 4, Page 655-692

http://www.jmaterenvironsci.com

"Advancing Phytochemical Research: Techniques and Applications of Plant Secondary Metabolites"

Lawan I. [1-2] *, Mustapha R. K. [1],

[1]*Department of Chemistry, Faculty of Science, Northwest University, Kano, Nigeria*
[2]*Department of Chemistry/Biochemistry, Federal Polytechnic Idah, Kogi State, Nigeria*

**Corresponding author, Email address: imranlawanadam@gmail.com*

Received *15 Mar 2026,*
Revised *29 Apr 2026,*
Accepted *30 Apr 2026*

Keywords:
- ✓ *Plant secondary metabolites;*
- ✓ *phytochemicals;*
- ✓ *extraction;*
- ✓ *bioactive compounds*
- ✓ *analytical techniques;*

Citation*: Lawan. I., Mustapha R.K. (2026) Advancing Phytochemical Research: Techniques and Applications of Plant Secondary Metabolites, J. Mater. Environ. Sci., 17(4), 655-692.*

Abstract: This study employs a systematic literature review to explore the extraction, analysis, and applications of plant secondary metabolites. Relevant literature was carefully selected from reputable academic sources, with a focus on recent advancements in extraction methods, analytical techniques, and practical applications. The review addresses key aspects of phytochemical research, including the preparation of plant materials, selection of appropriate solvents, and optimization of extraction methods. Both conventional and modern extraction approaches are discussed, alongside the spectroscopic and chromatographic techniques commonly used for the analysis of secondary metabolites. The reviewed studies were critically examined and compared to provide a clear, accurate, and comprehensive overview of current trends and challenges in the field.

1. Introduction

Secondary metabolites are a heterogeneous group of organic compounds that are produced by plants and are not directly involved in vital physiological processes such as development, reproduction, or growth (Bocso & Butnariu 2022). Unlike primary metabolites, which are vital for the metabolic processes, secondary metabolites are generally produced as specialized products that are important for adaptation and survival of the plants under natural conditions (Elshafie *et al*., 2023; Naji *et al*., 2024). These compounds are derived from complex biosynthetic routes that are initiated with primary metabolic intermediates and are sometimes taxon-, tissue-, or developmental stage-specific (Xie *et al*., 2025). The presence of many metabolites in different plant materials and their many documented bioactivities show that they could be a natural source of medicine for treating a wide range of illnesses (Mustapha *et al*., 2022). The compounds are well known for their roles in plant defence against herbivores, pathogens, and abiotic stress, and in regulating interactions with other organisms, including pollinators and symbiotic microorganisms (Zhan *et al*., 2022; Devi *et al*., 2023; Kabir *et al*., 2025). Plant secondary metabolites encompass several major classes, including alkaloids, phenolic compounds (such as flavonoids and tannins), and terpenoids, each characterized by distinct chemical structures and biological activities (Naji *et al*., 2024). These compounds

contribute significantly to the chemical diversity observed in plants and are estimated to number in the hundreds of thousands to over a million distinct structures across plant species (Zhan *et al*., 2022) Apart from the ecological importance, secondary metabolites of plants are also significant to humans because of the broad range of biological activities that these compounds exhibit (Elshafie *et al*., 2023). They are widely used in various sectors, including the pharmaceutical, nutraceutical, agricultural, and industrial sectors, and show a range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Devi *et al*. 2023; Ouahabi *et al*. 2023; Pramanik *et al*., 2024). Primary metabolites are those compounds which are directly involved in the normal growth, development, and reproduction of an organism (Bocso and Butnariu 2022; Kabir *et al*., 2025). They are ubiquitous in all living organisms, participating in the most important metabolic processes, including glycolysis, the TCA cycle, and photosynthesis (Brizzolara *et al*., 2020; Salam *et al*., 2023). Primary metabolites include carbohydrates, amino acids, nucleotides, lipids, etc., which are the building blocks of life, providing the cell with the necessary energy, structural components, and biosynthetic raw materials. As they are the most essential components, they are always being synthesized in the active growth phase of the cell (Salam *et al*., 2023; Hammad 2025). On the other hand, secondary metabolites are those that are not vital for fundamental survival, although they are important for survival and adaptation to the environment. The secondary metabolites are usually species-specific and are produced as a response to particular developmental stages or environmental stimuli. Some examples of secondary metabolites are alkaloids, terpenoids, flavonoids, and phenolic compounds (Salam *et al*., 2023; Simsek & Whitney, 2024). A major difference between these two groups is related to their importance, as primary metabolites are vital for the cell, while secondary metabolites play an important role in the fitness and survival of the organism under certain ecological conditions (Salam *et al*., 2023; Bocso and Butnariu 2022). In addition, primary metabolites often serve as the starting material for the biosynthesis of secondary metabolites, and this is an inherent relationship between the two metabolic pathways (Salam *et al*., 2023). Current studies suggest that there is no sharp boundary between primary and secondary metabolism, and they are interconnected through complex networks (Simsek & Whitney, 2024).

2. Methodology

This particular study will make use of a systematic literature review to assess the extraction, analysis, and applications of secondary metabolites found in plants. The relevant literature will be chosen from credible sources, focusing on recent developments in the extraction and analysis methods and their applications. The information used for this particular literature review has been chosen based on its relevance to important domains such as plant preparation, solvents used for extraction, extraction methods, and analysis. The traditional as well as contemporary methods used for extraction have been taken into consideration, along with spectroscopic and chromatographic methods used for analysis. The information collected for the literature review has been thoroughly reviewed and presented in a logical fashion to maintain a logical flow of ideas. The information gathered for this particular literature review has provided a comprehensive understanding of the subject based on current trends, challenges faced, and developments taking place in the field (Aourabi *et al*., 2021; Diass *et al*., 2021; El Amri *et al*., 2025; Kadda *et al*., 2026).

3. Major Classes of Secondary Metabolites

Secondary metabolites are chemically varied organic compounds in plants that are not metabolically essential but carry out crucial roles in interactions with the environment, stress, and

defense (Elshafie *et al*., 2023; Ashraf *et al*., 2018). Secondary metabolites can be grouped based on biosynthesis and chemical characteristics into terpenoids, phenolic compounds, and nitrogen-containing compounds, including alkaloids, with subgroups like glycosides and sulfur-containing compounds (Elshafie *et al*., 2023; Tang *et al*., 2024).

3.1 Terpenoids (Isoprenoids)

The largest and most diverse group of secondary metabolites is terpenoids, consisting of tens of thousands of individual compounds. The biosynthetic route of terpenoids is initiated through the MVA and MEP pathways, using isoprene units consisting of five carbon atoms. The structural classification of terpenoids is as follows:
Monoterpenes(C_{10}), sesquiterpenes(C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) (Sana *et al*., 2025). Functionally, terpenoids play a crucial role in the development, signalling, and adaptation of plants to the environment. They are involved in the defence of plants through repellent properties against herbivores and pathogens, and also mediate attractants for pollinators through volatiles. Additionally, terpenoids have pharmacological properties, including anti-inflammatory, antimicrobial, and anticancer properties (Tang *et al*., 2024; Hilal *et al*., 2024).

3.2 Phenolic Compounds

Phenolic compounds are an abundant and widespread group of plant secondary metabolites. These compounds have been described as having one or more hydroxyl groups attached to an aromatic ring and are primarily biosynthesized through the shikimate and phenylpropanoid pathways. The phenolic compound group includes flavonoids, tannins, lignin, coumarin, and phenolic acids (Elshafie *et al*., 2023; Ozyigit *et al*., 2023). Phenolics play a vital role in plant defence mechanisms, such as scavenging reactive oxygen species, thereby protecting plant cells from oxidative stress. Phenolics also play a vital role in plant cell structure, pigmentation, and protection from ultraviolet light. Phenolic compounds possess remarkable biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, thus portraying their tremendous importance in pharmaceutical and nutraceutical industries (Ozyigit *et al*., 2023; Tang *et al*., 2024).

3.3 Alkaloids (Nitrogen-Containing Compounds)

Alkaloids represent a heterogeneous group of nitrogenous secondary metabolites, primarily of amino acid origin, such as tryptophan, tyrosine, and ornithine (Bhambhani *et al*., 2021). Alkaloids have been widely distributed throughout the plant kingdom and have been well known for their pronounced physiological and pharmacological activity. Some of the well-known alkaloids include morphine, quinine, and nicotine (Bribi 2018). From a pharmacological perspective, alkaloids have been widely utilized for analgesic, antimalarial, anticancer, and antimicrobial activity (Adamski *et al*., 2020). in modern science, alkaloids have been recognized for their importance in drug discovery and development, primarily for their pronounced activity and structural diversity (Hilal *et al*., 2024; Tang *et al*., 2024).

3.4 Glycosides and Other Specialized Metabolites

Glycosides are a class of secondary metabolites, where a carbohydrate part called glycone is linked to another part called an aglycone through a covalent bond. These compounds can be

classified based on the nature of their aglycones, and they include cardiac glycosides, cyanogenic glycosides, and saponins (Kowsalya *et al*., 2025). These compounds act as storage compounds for bioactive molecules, and they can be hydrolyzed to release bioactive molecules when they are required. Besides glycosides, plants have been reported to produce other sulfur-containing compounds like glucosinolates, among other secondary metabolites. These compounds have been reported to be involved in various roles, including herbivore deterrence, plant-microbe interactions, and stress protection (Sana *et al*., 2025; Elshafie *et al*., 2023).

3.5 Importance of Secondary Metabolites

3.5.1. Medicine

A significant function of secondary metabolites in medicine pertains to their capacity as sources of essential pharmaceuticals. Some drugs that people use now come from secondary metabolites, either directly or indirectly. Alkaloids, terpenoids, and phenolic derivatives, like morphine, quinine, and artemisinin, and flavonoids, are often found to have direct drug potential or are used as lead compounds to make semi-synthetic derivatives with better pharmacokinetics and pharmacodynamics (Atanasov *et al*., 2021; Elshafie *et al*., 2023). Secondary metabolites have many pharmacological effects, such as being antioxidants, antimicrobials, anti-inflammatories, anticancer agents, and antivirals (Kaushik *et al*., 2021; Bhatti *et al*., 2022). Phenolic compounds and flavonoids are also well-known for their strong antioxidant properties, which protect the body from oxidative stress caused by free radicals. This keeps the body from getting hurt. This has been a crucial element in the prevention and management of chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Bhatti *et al*., 2022). Another important thing about secondary metabolites is that they are important for fighting antimicrobial resistance (AMR), which is a big health problem that affects the whole world. Some natural secondary metabolites have unique ways of working, like affecting microbial cell membranes, stopping enzymes from working, and stopping nucleic acid synthesis (Thirumurugan *et al*., 2018). The multiple targeted action lowers the chance of developing resistance compared to regular antibiotics. Additionally, certain secondary metabolites exhibit a synergistic effect with existing antibiotics, enhancing their efficacy and circumventing resistance (El-Readi *et al*., 2021). Consequently, natural products persist in motivating the creation of novel pharmaceuticals, particularly in areas where synthetic substances have achieved minimal success (Newman & Cragg, 2020; Atanasov *et al*., 2021).

3.5.2 Agriculture

The plant's secondary metabolites help it fight off both biotic and abiotic stresses. One of their most important jobs is to protect plants from pests and diseases. Alkaloids, flavonoids, and phytoalexins are secondary metabolites that work as natural pesticides and antimicrobials. They stop fungi, bacteria, and insects that eat plants from growing and developing. This natural defence system reduces the need for chemical pesticides and promotes environmentally friendly farming (Elshafie *et al*., 2023). Secondary metabolites also play an important role in helping plants tolerate stress and adapt to their environment. Under conditions such as drought, high salinity, and extreme temperatures, plants increase the production of antioxidants, including flavonoids and phenolic compounds. These substances help reduce oxidative stress within the plant, thereby enhancing stress tolerance and supporting better crop yields under harsh environmental conditions (Kumari *et al*., 2024). In addition, secondary metabolites are becoming increasingly valuable in sustainable and

bio-based agriculture. Compounds derived from plants and microorganisms are widely used as biostimulants, biofertilizers, and biopesticides. These applications contribute to improved soil fertility, enhanced plant growth, and increased resistance to diseases (Marks *et al*., 2025).

3.5.3 Industrial and Biotechnological Applications

Secondary metabolites have wide-ranging industrial applications, including their use in natural dyes, pigments, biofuels, renewable energy sources, biopolymers, and biodegradable products. Advances in biotechnology, particularly in metabolic engineering and synthetic biology, have made it possible to produce these compounds on a large scale for industrial purposes (Liko *et al*., 2016; Jan *et al*., 2021; Xu et al., 2023).

3.5.4 Environmental Applications

Secondary metabolites also contribute significantly to environmental sustainability. They are involved in the bioremediation of pollutants, eco-friendly pest control, and the reduction of chemical pollution. Their use aligns closely with the principles of green chemistry and sustainable development (Yang *et al*., 2020).

3.5.5 Cosmetics and Personal Care Applications

In the cosmetics industry, secondary metabolites are increasingly valued for their antioxidant, anti-aging, anti-inflammatory, and photoprotective properties. These bioactive compounds are widely incorporated into skincare and personal care products to enhance skin health and protection (Yang *et al*., 2020; Li *et al*., 2024).

3.5.6 Food Preservation

Secondary metabolites play a crucial role in food preservation due to their natural antimicrobial and antioxidant properties. Compounds such as phenolics, terpenoids, and essential oils have been shown to inhibit the growth of foodborne pathogens and spoilage organisms (Elshafie *et al*., 2023). Beyond their antimicrobial effects, these compounds also exhibit strong antioxidant activity, which is essential in preventing oxidative spoilage. Oxidation can deteriorate food quality, leading to rancidity, colour changes, and nutrient loss. Polyphenols and flavonoids help neutralize free radicals, thereby maintaining the quality and shelf life of food products (Zhang *et al*., 2024). Furthermore, secondary metabolites are becoming key components in functional foods, nutraceuticals, and advanced food preservation technologies. Their presence in fruits, vegetables, and plant extracts not only enhances food quality but also provides additional health benefits (Abu-Reidah, 2023).

4. Plant Material and Pre- treatment

4.1. Plants Species Selection

Significantly, the selection of plant species influences the nature, type, and biosynthetic potential of secondary metabolites. Ethnobotanical knowledge remains a key criterion for species selection due to its strong association with therapeutic efficacy and biological activity (Elshafie *et al*., 2023; Zhao *et al*., 2023). In addition, chemotaxonomic relationships play an important role, as certain plant families such as Fabaceae, Rutaceae, and Lamiaceae are known to be associated with specific classes of metabolites, including flavonoids, terpenoids, and alkaloids (Jan *et al*., 2021;

Barthwal & Mahar, 2024). Environmental factors, including soil nutrients, climate, and biotic stress, also significantly influence the biosynthesis of secondary metabolites. Both qualitative and quantitative variations may occur due to differences in geographic origin, even within the same species (Salam *et al*., 2023; Kumari *et al*., 2024).

4.1.1 Plant Part Selection

The tissue-specific distribution of secondary metabolites makes the selection of appropriate plant parts such as leaves, roots, bark, and seeds essential for obtaining optimal yields and bioactivity (Xu *et al*., 2023; Barthwal & Mahar, 2024). Leaves are metabolically active and typically contain high levels of phenolics and flavonoids, which are closely associated with antioxidant activity (Yang *et al*., 2023; Jan *et al*., 2021). Roots often contain allelochemicals, terpenoids, and alkaloids that contribute to plant defense mechanisms (Salam *et al*., 2023). Similarly, bark is rich in tannins, lignans, and other defense-related secondary metabolites, which have long-standing importance in pharmacognosy (Akbar *et al*., 2024). Seeds, on the other hand, contain high concentrations of protective compounds such as saponins and glycosides, which are vital for plant survival and have various industrial applications (Yang *et al*., 2023).

4.1.2 Importance of Standardized Plant Selection

Standardization of plant species, selected parts, harvesting time, and environmental conditions is essential for improving reproducibility and enabling meaningful comparisons across phytochemical studies (Salam *et al*., 2023; Rahman *et al*., 2023).

4.2 Collection of Plant Materials

The collection of plant materials is a critical step in phytochemical research, as it directly affects the quality, reproducibility, and reliability of secondary metabolite extraction and analysis. Adhering to proper collection protocols helps preserve bioactive compounds and minimizes variability arising from environmental and physiological factors (Barthwal & Mahar, 2024; Jan *et al*., 2021).

4.2.1 Selection Criteria and Harvesting Conditions

Plant materials should be selected based on ethnobotanical relevance, pharmacological importance, and chemotaxonomic evidence, as these factors increase the likelihood of obtaining biologically active compounds (Elshafie *et al*., 2023; Zhao *et al*., 2023). The stage of plant growth, seasonal variation, and environmental conditions significantly influence metabolite biosynthesis, as production is often regulated by stress responses and developmental stages (Salam *et al*., 2023; Kumari *et al*., 2024). Harvesting is therefore typically carried out at specific phenological stages when metabolite concentration is at its peak. For example, leaves are often collected during the flowering stage, whereas roots and bark are usually harvested during dormancy to maximize bioactive compound accumulation (Xu *et al*., 2023; Jan *et al*., 2021). Furthermore, environmental parameters such as soil composition, altitude, and climate should be carefully documented, as they contribute to metabolite variability (Rahman *et al*., 2023; Yang *et al*., 2023).

4.2.2 Handling, Processing, and Storage

Collected plant materials should be thoroughly cleaned to remove contaminants such as soil, dust, and microbial residues. This should be followed by appropriate drying methods such as shade

drying, controlled oven drying, or freeze-drying to prevent enzymatic degradation and the loss of volatile compounds (Barthwal & Mahar, 2024; Kumari *et al*., 2024). Drying conditions must be carefully optimized to avoid degradation of thermolabile compounds, particularly phenolics and flavonoids. After drying, plant materials are typically ground into fine powders to increase surface area and enhance extraction efficiency (Jan *et al*., 2021). The processed samples should then be stored in airtight containers and protected from light, moisture, and oxygen to minimize oxidation and degradation (Salam *et al*., 2023).

4.3 Identification of Plant Materials

Accurate identification of plant materials is essential to ensure the validity, reproducibility, and scientific reliability of phytochemical studies. Misidentification can result in inconsistent findings, reduced bioactivity, and incorrect conclusions about therapeutic potential (Zhao *et al*., 2023; Elshafie *et al*., 2023).

4.3.1 Taxonomic Identification

Plant identification is primarily carried out using classical taxonomic methods, including morphological characterization and comparison with herbarium specimens. Key features such as leaf morphology, floral structures, and reproductive organs are examined using standard botanical keys (Barthwal & Mahar, 2024). Voucher specimens should be prepared and deposited in recognized herbaria for future reference and verification. This practice ensures traceability and enhances the reproducibility of research outcomes (Xu *et al*., 2023; Jan *et al*., 2021).

4.3.2 Molecular Identification (DNA Barcoding)

Modern plant identification increasingly incorporates molecular techniques, particularly DNA barcoding, which provides precise and reliable species identification. Common genetic markers such as rbcL, matK, and ITS regions are widely used to distinguish closely related species (Zhao *et al*., 2023; Xu *et al*., 2023). DNA barcoding is especially useful when morphological identification is difficult due to phenotypic variation, incomplete specimens, or processed plant materials. Combining molecular approaches with traditional taxonomy significantly improves identification accuracy and reduces the risk of errors (Salam *et al*., 2023).

4.3.3 Chemotaxonomic and Phytochemical Identification

Chemotaxonomy involves the use of characteristic secondary metabolites as markers for plant classification and identification. Specific metabolite profiles such as alkaloids in Apocynaceae or flavonoids in Fabaceae serve as biochemical signatures that support taxonomic classification (Akbar *et al*., 2024; Jan *et al*., 2021). Advanced analytical techniques, including high performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS), are widely used to profile phytochemical constituents and confirm species identity (Barthwal & Mahar, 2024; Kumari *et al*., 2024).

4.4 Drying Methods

Drying is a critical post-harvest processing step in phytochemical research, as it directly affects the stability, concentration, and integrity of secondary metabolites. Fresh plant materials contain high moisture levels, which can promote enzymatic degradation, microbial growth, and chemical

instability if not properly controlled (Barthwal & Mahar, 2024; Jan *et al*., 2021). Selecting an appropriate drying method is therefore essential to preserve bioactive compounds and ensure reproducibility in extraction and analysis (Kumari *et al*., 2024; Yang *et al*., 2023).

4.4.1 Air Drying (Shade Drying)

Air drying, particularly shade drying, is one of the most common and traditional methods. It involves drying plant materials at ambient temperature in a well-ventilated environment, protected from direct sunlight to prevent photodegradation of sensitive compounds (Elshafie *et al*., 2023; Salam *et al*., 2023). This method is especially suitable for preserving thermolabile compounds such as flavonoids, phenolics, and essential oils (Yang *et al*., 2023; Kumari *et al*., 2024). However, it is relatively slow and may expose samples to oxygen and contaminants, potentially causing oxidation or microbial growth if not properly managed (Jan *et al*., 2021). Despite these limitations, it remains widely used due to its simplicity and low cost (Barthwal & Mahar, 2024).

4.4.2 Oven Drying

Oven drying involves the use of controlled temperatures, typically between 40–60°C, to accelerate moisture removal (Kumari *et al*., 2024; Xu *et al*., 2023). This method reduces drying time and minimizes microbial growth and enzymatic activity (Salam *et al*., 2023). However, high temperatures may degrade heat-sensitive compounds such as essential oils and certain phenolics. Therefore, optimizing temperature and drying duration is essential. Moderate temperatures (40–50°C) are generally effective in preserving antioxidant compounds while ensuring efficient drying (Kumari *et al*., 2024).

4.4.3 Freeze Drying (Lyophilization)

Freeze drying, or lyophilization, is considered one of the most effective methods for preserving the chemical integrity and bioactivity of secondary metabolites. It involves freezing the plant material followed by sublimation of ice under vacuum conditions, thereby removing moisture without exposing samples to high temperatures (Barthwal & Mahar, 2024; Jan *et al*., 2021). This technique is particularly suitable for preserving thermolabile and volatile compounds, as it minimizes thermal degradation and oxidation (Yang *et al*., 2023; Xu *et al*., 2023). Although highly effective, freeze drying is expensive and requires specialized equipment, which may limit its use in some settings (Salam *et al*., 2023).

4.5 Grinding and Particle Size Reduction

Grinding and particle size reduction are essential preprocessing steps that improve extraction efficiency by increasing surface area and enhancing solvent penetration (Barthwal & Mahar, 2024; Jan *et al*., 2021).

4.5.1 Grinding Techniques

Various grinding methods are used depending on the nature of the plant material and the target compounds. These include manual grinding, mechanical milling (e.g., hammer and ball mills), and cryogenic grinding (Kumari *et al*., 2024). Manual grinding is suitable for small-scale studies but lacks uniformity, while mechanical milling provides efficient and consistent particle size reduction for large-scale applications (Jan *et al*., 2021). Cryogenic grinding, which uses liquid nitrogen, is particularly useful for preserving thermolabile and volatile compounds by minimizing heat generation (Yang *et al*., 2023; Xu *et al*., 2023).

4.5.2 Particle Size Reduction

Particle size reduction facilitates the breakdown of plant tissues, allowing for the release of secondary metabolites during extraction (Xu *et al*., 2023; Salam *et al*., 2023). Smaller particle sizes increase surface area and improve solvent–solid interactions, thereby enhancing extraction efficiency (Jan *et al*., 2021). However, excessive size reduction may lead to particle agglomeration, reduced permeability, and degradation of sensitive compounds (Barthwal & Mahar, 2024).

4.5.3 Influence of Particle Size on Extraction Efficiency

Particle size significantly affects extraction yield and phytochemical composition. Fine particles generally improve extraction efficiency due to increased surface area and better solvent accessibility (Jan *et al*., 2021; Kumari *et al*., 2024). However, excessively fine particles may cause filtration challenges, reduced solvent flow (especially in Soxhlet or column extraction), and increased co-extraction of impurities such as chlorophyll and waxes (Barthwal & Mahar, 2024; Xu *et al*., 2023). In contrast, coarse particles may result in incomplete extraction due to limited solvent penetration (Salam *et al*., 2023).

4.5.4 Factors Affecting Grinding Efficiency

Several factors influence the efficiency of particle size reduction, including:

- Moisture content: High moisture levels can lead to clogging and reduced grinding efficiency, whereas excessively dry materials may become too brittle and prone to degradation (Salam *et al*., 2023).
- Temperature: Heat generated during the grinding process can degrade thermolabile compounds, making the use of cooling systems or cryogenic techniques necessary (Yang *et al*., 2023).
- Equipment type and operating conditions: Factors such as grinding speed, duration, and the type of grinding mechanism significantly affect particle size distribution and uniformity (Jan *et al*., 2021).
- Plant matrix composition: The physical nature of the plant material also plays a role, as fibrous materials require different grinding techniques compared to softer plant tissues (Xu *et al*., 2023).

4.6. Storage Conditions

Proper storage of plant materials is a crucial step in phytochemical investigations, as it directly influences the stability, integrity, and bioactivity of secondary metabolites. Poor storage conditions can lead to oxidation, enzymatic degradation, volatilization, and microbial contamination, ultimately compromising the quality and reproducibility of experimental results (Barthwal & Mahar, 2024; Jan *et al*., 2021). Therefore, optimizing storage conditions is essential to preserve phytochemical composition prior to extraction and analysis (Kumari *et al*., 2024; Yang *et al*., 2023).

4.6.1 Temperature Control

Temperature is one of the most critical factors affecting the stability of secondary metabolites. Elevated temperatures accelerate chemical degradation, oxidation, and the loss of volatile compounds, particularly in thermolabile metabolites such as flavonoids, phenolics, and essential oils (Yang *et al*., 2023; Xu *et al*., 2023). Dried plant materials are typically stored at room temperature (20–25°C) under controlled conditions. However, long-term storage often requires refrigeration

(4°C) or freezing (−20°C) to minimize degradation (Salam *et al*., 2023; Kumari *et al*., 2024). Freezing is especially recommended for samples rich in sensitive bioactive compounds, as it helps preserve their structural integrity and biological activity (Jan *et al*., 2021).

4.6.2 Protection from Light

Exposure to light, particularly ultraviolet (UV) radiation, can cause photodegradation of secondary metabolites, resulting in structural changes and reduced bioactivity (Barthwal & Mahar, 2024; Yang *et al*., 2023). To prevent this, plant materials and extracts should be stored in amber-colored or opaque containers and kept in dark environments. This minimizes photochemical reactions and helps maintain compound stability during storage (Kumari *et al*., 2024; Xu *et al*., 2023).

4.6.3 Moisture and Humidity Control

Moisture and humidity play a significant role in promoting microbial growth and enzymatic activity in stored plant materials. High humidity can lead to fungal contamination, hydrolysis of compounds, and degradation of bioactive constituents (Salam *et al*., 2023; Rahman *et al*., 2023). To reduce these risks, plant materials should be thoroughly dried before storage and kept in low-humidity conditions using desiccators or silica gel packs. Airtight containers are recommended to prevent moisture absorption and maintain sample integrity (Jan *et al*., 2021; Barthwal & Mahar, 2024).

4.6.4 Oxygen Exposure and Oxidation

Exposure to oxygen can cause oxidative degradation of secondary metabolites, particularly phenolics and other unsaturated compounds. This process may reduce antioxidant activity and alter chemical composition (Yang *et al*., 2023; Kumari *et al*., 2024). To minimize oxidation, plant materials should be stored in airtight or vacuum-sealed containers. In some cases, inert gas flushing (e.g., nitrogen) may be used for highly sensitive samples to further reduce oxidative damage (Jan *et al*., 2021; Xu *et al*., 2023).

4.6.5 Storage Duration and Stability

The duration of storage significantly affects the stability of secondary metabolites. Prolonged storage may lead to gradual degradation, even under optimal conditions, resulting in reduced extraction yield and changes in phytochemical profiles (Salam *et al*., 2023; Rahman *et al*., 2023). Studies suggest that short-term storage under controlled conditions is preferable, and samples should be processed as soon as possible after collection and drying. For long-term storage, periodic quality assessment is recommended to monitor changes in metabolite composition (Kumari *et al*., 2024; Jan *et al*., 2021).

4.6.6 Storage of Extracts

In addition to raw plant materials, proper storage of extracts is equally important. Extracts should be kept in airtight, light-resistant containers at low temperatures, typically 4°C or −20°C, depending on their stability (Barthwal & Mahar, 2024). The type of solvent used also influences stability, with ethanol and methanol extracts generally showing better preservation compared to aqueous extracts (Yang *et al*., 2023).

5. Principles of Extraction

5.1 Solubility and Polarity Concepts

Solubility and polarity are fundamental principles that govern the extraction, separation, and characterization of plant secondary metabolites. The efficiency of phytochemical extraction largely depends on the chemical compatibility between the solvent and the target compounds, which is determined by their polarity and intermolecular interactions (Barthwal & Mahar, 2024; Jan *et al*., 2021).

5.1.1 Concept of Solubility

Solubility refers to the ability of a solute (e.g., a secondary metabolite) to dissolve in a solvent to form a homogeneous solution. In phytochemical extraction, solubility is influenced by factors such as molecular structure, functional groups, temperature, and the properties of the solvent (Kumari *et al*., 2024; Xu *et al*., 2023). Secondary metabolites exhibit diverse solubility behaviors due to differences in their chemical structures. For example, polar compounds such as phenolics, flavonoids, and glycosides are generally soluble in polar solvents like water, methanol, and ethanol. In contrast, non-polar compounds such as terpenoids and lipids dissolve more readily in non-polar solvents such as hexane and chloroform (Yang *et al*., 2023; Jan *et al*., 2021). Solubility also plays a crucial role in determining the rate and extent of extraction. Compounds that are more soluble in a given solvent are extracted more efficiently. Therefore, careful selection of the solvent is essential to ensure compatibility with the physicochemical properties of the target metabolites and to achieve optimal extraction efficiency (Barthwal & Mahar, 2024).

5.1.2 Concept of Polarity

Polarity refers to the distribution of electrical charge within a molecule, which determines how it interacts with other substances. It plays a vital role in solvent selection and extraction efficiency, following the principle of "like dissolves like," where polar solvents dissolve polar compounds and non-polar solvents dissolve non-polar compounds (Jan *et al*., 2021; Xu *et al*., 2023).
Solvents can be broadly classified based on their polarity:

- Polar solvents: water, methanol, ethanol
- Moderately polar solvents: acetone, ethyl acetate
- Non-polar solvents: hexane, petroleum ether

The polarity of secondary metabolites is largely influenced by the presence of functional groups such as hydroxyl (–OH), carbonyl (C=O), and glycosidic linkages. Compounds containing multiple polar functional groups generally exhibit higher polarity and are more soluble in polar solvents (Yang *et al*., 2023; Kumari *et al*., 2024).

5.1.3 Relationship Between Solubility, Polarity, and Extraction Efficiency

The relationship between solubility and polarity is crucial for maximizing extraction yield and selectivity. Appropriate solvent selection based on polarity enhances mass transfer, diffusion, and dissolution of target compounds, thereby improving extraction efficiency (Barthwal & Mahar, 2024; Jan *et al*., 2021). Sequential extraction using solvents of increasing polarity (e.g., hexane → ethyl acetate → methanol → water) is commonly employed to separate complex plant matrices into distinct groups of metabolites (Salam *et al*., 2023; Xu *et al*., 2023). This approach enables selective isolation of compounds based on their polarity, facilitating further analysis and purification. However, the use of inappropriate solvents may result in low extraction yields, co-extraction of

impurities, or degradation of sensitive compounds. This underscores the importance of a thorough understanding of solubility polarity relationships in phytochemical extraction (Kumari *et al*., 2024; Yang *et al*., 2023).

5.1.4 Factors Influencing Solubility and Polarity in Extraction

Several factors influence the interaction between solubility and polarity during extraction, including:

- Temperature: Increasing temperature generally enhances solubility and extraction efficiency; however, it may also lead to the degradation of thermolabile compounds (Xu *et al*., 2023).
- Solvent composition: The use of solvent mixtures (e.g., ethanol–water) can optimize polarity and improve extraction efficiency by enhancing solute–solvent interactions (Jan *et al*., 2021).
- pH: The solubility of ionizable compounds, such as alkaloids and phenolic acids, is highly dependent on pH, which affects their ionization state (Kumari *et al*., 2024).
- Molecular size and structure: Larger or more structurally complex molecules may exhibit limited solubility, even when polarity conditions are favorable (Yang *et al*., 2023).

Optimizing these parameters is essential for achieving efficient, reproducible, and high-yield extraction outcomes.

5.1.5 Importance in Phytochemical Analysis and Standardization

A comprehensive understanding of solubility and polarity is crucial for method development, compound isolation, and analytical characterization in phytochemical research. These principles guide the selection of appropriate solvents for extraction, chromatography, and spectroscopic techniques, ensuring accurate identification and quantification of secondary metabolites (Barthwal & Mahar, 2024; Jan *et al*., 2021). In pharmaceutical and nutraceutical applications, polarity-based extraction strategies are vital for quality control, standardization, and formulation development, as they directly influence the composition, consistency, and bioactivity of plant extracts (Elshafie *et al*., 2023).

5.2 Diffusion and Mass Transfer

Diffusion and mass transfer are fundamental physicochemical processes that govern the efficiency of extracting secondary metabolites from plant matrices. These processes control the movement of bioactive compounds from plant tissues into the extraction solvent, thereby influencing the rate, yield, and selectivity of extraction (Barthwal & Mahar, 2024; Jan *et al*., 2021).

5.2.1 Concept of Diffusion

In phytochemical extraction, diffusion refers to the movement of secondary metabolites from the intracellular regions of plant tissues into the surrounding solvent (Xu *et al*., 2023; Salam *et al*., 2023). The rate of diffusion is influenced by several factors, including concentration gradient, temperature, molecular size, and solvent viscosity. A higher concentration gradient between the plant matrix and the solvent enhances the rate of compound transfer, thereby improving extraction efficiency (Jan *et al*., 2021). Additionally, smaller molecules diffuse more rapidly than larger and more complex molecules, which may experience steric hindrance within plant tissues (Yang *et al*., 2023). Pre-treatment processes such as grinding and drying play a crucial role in facilitating diffusion by disrupting plant cell walls, reducing physical barriers, and increasing the accessibility of intracellular metabolites (Kumari *et al*., 2024; Barthwal & Mahar, 2024).

5.2.2 Concept of Mass Transfer

Mass transfer refers to the overall movement of solutes from one phase to another and includes both diffusion and convective transport mechanisms. In the context of plant extraction, it describes the transfer of secondary metabolites from the solid plant matrix into the liquid solvent phase (Jan *et al*., 2021; Xu *et al*., 2023).

5.2.3 Stages of Mass Transfer

Mass transfer during extraction typically occurs in three main stages:

Penetration of the solvent into plant tissues

Dissolution of secondary metabolites in the solvent

Diffusion of the dissolved compounds into the bulk solution

These stages collectively determine the efficiency of extraction methods such as maceration, Soxhlet extraction, and advanced techniques like ultrasound-assisted extraction (Kumari *et al*., 2024; Salam *et al*., 2023).

5.2.4 Factors Affecting Diffusion and Mass Transfer

Several factors influence the rate of diffusion and mass transfer during phytochemical extraction:

- Particle size: Reduction in particle size increases surface area, thereby enhancing diffusion and mass transfer. However, excessively fine particles may hinder solvent flow and reduce overall extraction efficiency (Barthwal & Mahar, 2024; Jan *et al*., 2021).
- Temperature: Higher temperatures increase molecular motion and solvent diffusivity, which accelerates mass transfer. Nevertheless, excessive heat may degrade thermolabile secondary metabolites (Yang *et al*., 2023; Xu *et al*., 2023).
- Solvent properties: Solvent characteristics such as polarity, viscosity, and diffusivity significantly influence mass transfer. Low-viscosity solvents generally promote faster diffusion and better penetration into plant tissues (Jan *et al*., 2021; Kumari *et al*., 2024).
- Agitation and mixing: Agitation improves mass transfer by reducing the boundary layer thickness surrounding plant particles, thereby enhancing solvent solute interactions (Salam *et al*., 2023).
- Extraction time: Longer extraction times allow equilibrium to be reached between the solute in the plant matrix and the solvent. However, prolonged extraction may lead to degradation or oxidation of sensitive compounds (Barthwal & Mahar, 2024).

5.2.5 Role in Extraction Techniques

The principles of diffusion and mass transfer are fundamental to both conventional and modern extraction techniques. In maceration, diffusion is the primary mechanism driving extraction, whereas in Soxhlet extraction, continuous solvent cycling enhances mass transfer efficiency (Jan *et al*., 2021). Advanced techniques such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) significantly improve mass transfer by disrupting plant cell structures and enhancing solvent penetration (Kumari *et al*., 2024; Xu *et al*., 2023).

5.2.6 Importance in Extraction Efficiency and Optimization

Efficient diffusion and mass transfer are essential for maximizing extraction yield, minimizing solvent consumption, and improving overall process efficiency. Poor mass transfer can lead to

incomplete extraction, low yield, and inconsistent phytochemical profiles (Barthwal & Mahar, 2024; Jan *et al*., 2021). Optimizing extraction parameters based on mass transfer principles enables scalable and reproducible processes, which are critical for pharmaceutical, nutraceutical, and industrial applications (Elshafie *et al*., 2023).

5.3 Influence of Temperature, Time, Solvent Type, and pH

The efficiency of extracting plant secondary metabolites is strongly influenced by key operational parameters, including temperature, extraction time, solvent type, and pH. These factors directly affect mass transfer, solubility, compound stability, and selectivity, ultimately determining both the yield and composition of phytochemical extracts (Barthwal & Mahar, 2024; Jan *et al*., 2021).

5.3.1 Temperature

Temperature plays a crucial role in extraction efficiency. Increasing temperature reduces solvent viscosity and surface tension, which enhances solvent penetration into plant tissues and facilitates the release of secondary metabolites (Xu *et al*., 2023; Kumari *et al*., 2024). However, excessively high temperatures may cause thermal degradation, oxidation, or volatilization of thermolabile compounds such as flavonoids, phenolics, and essential oils (Yang *et al*., 2023; Jan *et al*., 2021). Therefore, an optimal temperature range must be maintained to balance extraction efficiency with compound stability. Moderate temperatures (40–60°C) are commonly recommended to preserve bioactivity while ensuring efficient extraction (Kumari *et al*., 2024).

5.3.2 Time

Extraction time is another critical parameter that influences the extent of solute diffusion and the attainment of equilibrium between the plant matrix and the solvent. Generally, longer extraction times improve yield by allowing sufficient time for mass transfer processes to occur (Salam *et al*., 2023; Jan *et al*., 2021). However, prolonged extraction can lead to degradation of sensitive compounds, oxidation, and the co-extraction of unwanted substances such as pigments and waxes (Barthwal & Mahar, 2024; Yang *et al*., 2023). The optimal extraction time varies depending on the method used and the nature of the plant material. Notably, modern techniques such as ultrasound-assisted and microwave-assisted extraction significantly reduce extraction time while maintaining high efficiency (Xu *et al*., 2023; Kumari *et al*., 2024).

5.3.3 Solvent Type

Solvent selection is one of the most important factors in the extraction of secondary metabolites. The effectiveness of a solvent depends on its polarity, viscosity, boiling point, and compatibility with the target compounds (Jan *et al*., 2021; Barthwal & Mahar, 2024). Polar solvents such as water, methanol, and ethanol are widely used for extracting polar compounds, including phenolics, flavonoids, and glycosides. Moderately polar solvents like acetone and ethyl acetate are suitable for semi-polar compounds, while non-polar solvents such as hexane and petroleum ether are used for lipophilic compounds like terpenoids and fatty acids (Yang *et al*., 2023; Xu *et al*., 2023). Additionally, the use of solvent mixtures (e.g., ethanol–water) has been shown to improve extraction efficiency by optimizing polarity and enhancing solute–solvent interactions (Kumari *et al*., 2024).

5.3.4 pH

pH significantly influences the solubility, stability, and ionization state of secondary metabolites, particularly alkaloids, phenolic acids, and other ionizable compounds (Kumari *et al*., 2024; Xu *et al*., 2023). Under acidic conditions, alkaloids are typically protonated and become more soluble in aqueous solvents, which enhances their extraction efficiency. In contrast, phenolic compounds often exhibit greater solubility under slightly alkaline conditions due to deprotonation of hydroxyl groups (Jan *et al*., 2021; Yang *et al*., 2023). However, extreme pH conditions can lead to hydrolysis, degradation, or structural changes in sensitive compounds, thereby reducing their biological activity (Salam *et al*., 2023).

6.Conventional Extraction Methods

6.1. Maceration

Maceration is a simple and widely used extraction technique that begins with proper preparation of plant material. The sample is first collected, cleaned, dried, and ground to an appropriate particle size to increase surface area and enhance solvent penetration (Salam *et al*., 2023; Xu *et al*., 2023). A suitable solvent is then selected based on the polarity and solubility of the target compounds, commonly including water, ethanol, methanol, or hydroalcoholic mixtures (Jan *et al*., 2021; Yang *et al*., 2023). The powdered plant material is subsequently immersed in the solvent in a closed container and left to stand at room temperature for a defined period, typically 24–72 hours or longer. Occasional stirring or agitation is applied to improve mass transfer (Barthwal & Mahar, 2024; Kumari *et al*., 2024). After extraction, the mixture is filtered to separate the liquid extract from plant residues (Xu *et al*., 2023). The filtrate is then concentrated using evaporation techniques, such as rotary evaporation, to obtain the crude extract containing the desired secondary metabolites (Jan *et al*., 2021).

Maceration offers several advantages that contribute to its widespread use in phytochemical research. It is simple to perform, requires minimal equipment, and is suitable for both laboratory and field applications (Barthwal & Mahar, 2024). The method is also cost-effective due to its low energy and infrastructure requirements, making it particularly useful in resource-limited settings (Kumari *et al*., 2024). Because the process is carried out at room temperature, it helps preserve thermolabile compounds such as flavonoids and essential oils by minimizing thermal degradation (Yang *et al*., 2023; Salam *et al*., 2023). Additionally, maceration is versatile and can be applied to a wide range of plant materials and solvents, allowing the extraction of diverse classes of secondary metabolites (Jan *et al*., 2021). It is also easily scalable for larger extraction processes without requiring major modifications (Xu *et al*., 2023). Despite these advantages, maceration has several limitations. The process is relatively time-consuming, often requiring several hours to days to achieve adequate extraction (Barthwal & Mahar, 2024; Jan *et al*., 2021). It may also yield lower extraction efficiency compared to advanced techniques due to limited mass transfer (Kumari *et al*., 2024). Furthermore, large volumes of solvent are typically required, which can increase both cost and environmental impact (Xu *et al*., 2023). Prolonged soaking, particularly in aqueous media, may encourage microbial growth if not properly controlled (Salam *et al*., 2023). In addition, the non-selective nature of maceration can result in the co-extraction of unwanted compounds such as chlorophyll, waxes, and pigments, which may interfere with downstream analysis (Yang *et al*., 2023).

6.2 Soxhlet Extraction

Soxhlet extraction is a widely used conventional method for the continuous extraction of plant secondary metabolites. It operates on the principle of repeated solvent reflux and siphoning, allowing efficient transfer of soluble compounds from solid plant matrices into a solvent without the need for constant solvent replacement (Barthwal & Mahar, 2024; Jan *et al*., 2021). This method is particularly suitable for the exhaustive extraction of compounds with limited solubility in a given solvent (Kumari *et al*., 2024).

6.2.1 Principles of Soxhlet Extraction

The Soxhlet extraction process is based on continuous solvent recycling combined with diffusion and mass transfer mechanisms. In this method, the solvent is heated to its boiling point, causing it to vaporize. The vapor passes through a distillation arm into a condenser, where it cools and condenses back into liquid form. The condensed solvent then flows into the extraction chamber containing the plant material, where it dissolves the target secondary metabolites (Xu *et al*., 2023; Salam *et al*., 2023). As the solvent accumulates, it reaches a certain level and is automatically siphoned back into the boiling flask, carrying the dissolved compounds with it. This cycle of evaporation, condensation, extraction, and siphoning is repeated multiple times, ensuring continuous contact between fresh solvent and plant material (Jan *et al*., 2021; Yang *et al*., 2023). The repeated cycles maintain a strong concentration gradient, thereby enhancing diffusion and improving extraction efficiency (Barthwal & Mahar, 2024).

6.2.2 Extraction Efficiency

The high efficiency of Soxhlet extraction is mainly due to its continuous solvent recycling mechanism, which ensures repeated interaction between fresh solvent and plant material. This enhances both mass transfer and diffusion, allowing for near-complete recovery of secondary metabolites (Xu *et al*., 2023; Salam *et al*., 2023). The method maintains a strong concentration gradient throughout the process, facilitating efficient solute transfer into the solvent phase. It is therefore particularly effective for compounds with limited solubility, as repeated cycles enable gradual and thorough extraction (Jan *et al*., 2021; Kumari *et al*., 2024). Additionally, the elevated temperatures used during solvent reflux improve solubility, solvent penetration, and diffusion rates, further enhancing extraction efficiency (Yang *et al*., 2023). However, efficiency depends on factors such as extraction time, number of cycles, solvent type, and particle size (Barthwal & Mahar, 2024). Excessive heating or prolonged extraction may lead to degradation of sensitive compounds (Jan *et al*., 2021).

6.2.3 Solvent Consumption

Despite its effectiveness, Soxhlet extraction is often associated with high solvent consumption. A substantial volume of solvent is required in the boiling flask to sustain continuous reflux cycles, which can increase operational costs and environmental impact (Xu *et al*., 2023; Kumari *et al*., 2024). Although the solvent is recycled within the system, losses may occur due to evaporation, handling, and transfer, especially during prolonged extraction (Salam *et al*., 2023). Furthermore, the use of organic solvents such as hexane, chloroform, and methanol raises concerns related to toxicity, safety, and environmental sustainability (Yang *et al*., 2023). To address these issues, strategies such as optimizing solvent selection, reducing extraction time, and implementing solvent recovery systems have been developed (Jan *et al*., 2021). The use of greener solvents, such as ethanol and

water, is also encouraged to minimize environmental impact while maintaining efficiency (Barthwal & Mahar, 2024).

6.3 Decoction and Infusion in Secondary Metabolite Extraction

Decoction and infusion are traditional extraction techniques widely used for isolating plant secondary metabolites, particularly in herbal medicine and pharmacognosy. Both methods primarily use water as the extraction solvent, making them environmentally friendly, cost-effective, and safe for human use (Elshafie *et al*., 2023; Jan *et al*., 2021). These techniques are especially suitable for extracting polar and water-soluble compounds, including phenolics, flavonoids, tannins, and glycosides (Kumari *et al*., 2024; Yang *et al*., 2023).

6.3.1. Decoction

6.3.1.1 Principle

Decoction is an extraction method that relies on the application of heat to enhance the solubility, diffusion, and mass transfer of bioactive compounds from plant materials into water. Boiling disrupts plant cell walls, thereby facilitating the release of intracellular secondary metabolites (Xu *et al*., 2023; Salam *et al*., 2023). In this method, dried and powdered plant material is immersed in water and heated to boiling for a specific duration, typically between 15 and 60 minutes, depending on the nature of the plant material and the target compounds. After heating, the mixture is allowed to cool and is subsequently filtered to obtain the extract (Jan *et al*., 2021; Husaini *et al*., 2023). Decoction is particularly effective for extracting compounds from hard plant parts such as roots, bark, and seeds, where heat assists in breaking down rigid structural components and improving the release of bioactive constituents (Kumari *et al*., 2024).

6.3.2 Infusion

6.3.2.1 Principle

Infusion is based on the principles of diffusion and solubility, where bioactive compounds dissolve into water without prolonged exposure to high temperatures. This approach helps preserve sensitive compounds by minimizing thermal degradation (Jan *et al*., 2021; Yang *et al*., 2023). In infusion, plant materials typically leaves or flowers are soaked in hot or cold water and allowed to stand for a short period, usually 5–30 minutes. The mixture is then filtered to obtain the extract. Cold infusion may be employed for highly thermolabile compounds that are prone to degradation at elevated temperatures (Kumari *et al*., 2024; Xu *et al*., 2023; Husaini *et al*., 2023). Infusion is a gentle extraction method that effectively preserves thermolabile and volatile compounds due to the absence of prolonged heating (Elshafie *et al*., 2023). It is simple, rapid, and requires minimal equipment, making it suitable for both laboratory applications and traditional practices (Jan *et al*., 2021). However, infusion generally yields lower extraction efficiency compared to decoction and other conventional methods due to its mild extraction conditions (Yang *et al*., 2023). It is also less effective for extracting compounds from hard plant tissues and is mainly limited to readily soluble constituents (Salam *et al*., 2023).

6.3.3 *Comparative Perspective*

Decoction and infusion differ primarily in terms of temperature, extraction time, and suitability for different plant materials. Decoction involves higher temperatures and longer extraction times, making it more suitable for hard and woody plant parts such as roots and bark. In

contrast, infusion uses milder conditions and is ideal for soft tissues such as leaves and flowers (Kumari *et al*., 2024). Although both methods are valued for their simplicity, safety, and alignment with traditional practices, their limitations in selectivity and efficiency have led to the increasing adoption of advanced extraction techniques in modern phytochemical research (Jan *et al*., 2021; Xu *et al*., 2023).

6.4 Application of Traditional Extraction Methods in Herbal Medicine

Traditional extraction methods, including maceration, decoction, and infusion, play a crucial role in herbal medicine by enabling the recovery of bioactive secondary metabolites responsible for therapeutic effects. These methods are deeply rooted in ethnomedicinal practices and continue to serve as primary approaches for preparing herbal remedies worldwide (Elshafie *et al*., 2023; Jan *et al*., 2021). Their continued relevance is largely due to their simplicity, safety, and compatibility with natural products, making them indispensable in both traditional and modern phytotherapy (Kumari *et al*., 2024).

6.5 Role in Extraction of Bioactive Compounds

Traditional extraction methods are essential for isolating pharmacologically active secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, and glycosides. These compounds exhibit a wide range of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects, which underpin the therapeutic efficacy of herbal medicines (Yang *et al*., 2023; Salam *et al*., 2023). Decoction and infusion are particularly effective for extracting water-soluble compounds, whereas maceration allows for the extraction of both polar and moderately polar constituents, depending on the solvent used (Jan *et al*., 2021; Xu *et al*., 2023).

6.6. Use in Preparation of Herbal Formulations

Traditional extraction techniques are widely used in the preparation of various herbal formulations, including teas, decoctions, tinctures, and syrups. Decoction is commonly applied for preparing remedies from hard plant parts such as roots and bark, whereas infusion is preferred for softer tissues like leaves and flowers (Kumari *et al*., 2024). Maceration is frequently employed in the preparation of tinctures and liquid extracts, where plant materials are soaked in alcohol or hydroalcoholic solutions to facilitate the extraction of a broader range of phytochemicals (Elshafie *et al*., 2023).

6.7 Therapeutic Significance

The use of traditional extraction methods in herbal medicine is closely linked to their ability to preserve the bioactivity and synergistic interactions of phytochemicals. Unlike highly purified compounds, crude extracts obtained through these methods often contain multiple constituents that work together synergistically to enhance therapeutic efficacy (Jan *et al*., 2021; Yang *et al*., 2023). Furthermore, the relatively mild extraction conditions especially in infusion and maceration help preserve thermolabile and volatile compounds, which are essential for maintaining the pharmacological properties of herbal preparations (Salam *et al*., 2023).

6.8 Safety and Accessibility

Traditional extraction methods are generally regarded as safe due to the use of non-toxic solvents such as water and ethanol, both of which are suitable for human consumption (Elshafie *et*

al., 2023). Their low cost and minimal equipment requirements make them highly accessible, particularly in resource-limited and rural settings, where they often serve as a primary source of healthcare (Kumari *et al*., 2024). Additionally, these methods align with the principles of sustainability and green chemistry, as they rely on renewable plant resources and environmentally friendly solvents (Jan *et al*., 2021).

6.9 Challenges and Limitations

Despite their widespread application, traditional extraction methods present several challenges in herbal medicine. These include a lack of standardization, variability in extraction efficiency, and inconsistency in phytochemical composition, all of which can affect the quality and reproducibility of herbal products (Xu *et al*., 2023; Yang *et al*., 2023). Moreover, prolonged heating during decoction may lead to degradation of sensitive compounds, while the relatively mild conditions in infusion may result in incomplete extraction and reduced therapeutic efficacy (Salam *et al*., 2023).

6.10 Relevance in Modern Phytotherapy

In contemporary research, traditional extraction methods continue to serve as baseline techniques for evaluating and comparing advanced extraction technologies. They also play a key role in validating ethnomedicinal knowledge and supporting the development of novel phytopharmaceuticals (Jan *et al*., 2021; Kumari *et al*., 2024). The integration of traditional methods with modern scientific approaches enhances standardization, quality control, and therapeutic reliability, ensuring their continued relevance in global healthcare systems (Elshafie *et al*., 2023).

7. Modern/Advanced Extraction Techniques

7.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is an advanced technique widely used for the efficient recovery of plant secondary metabolites. It utilizes ultrasonic waves, typically in the range of 20–100 kHz, to enhance mass transfer and disrupt plant cell structures, thereby facilitating the release of bioactive compounds into the extraction solvent (Jan *et al*., 2021; Kumari *et al*., 2024). UAE has gained considerable attention due to its high efficiency, shorter extraction time, and reduced solvent consumption compared to conventional extraction methods (Xu *et al*., 2023).

7.1.1 Principle of UAE

The principle of UAE is based on acoustic cavitation, which involves the formation, growth, and collapse of microscopic bubbles in a liquid medium under ultrasonic irradiation. The collapse of these bubbles generates localized high temperatures and pressures, along with microjets and shockwaves that disrupt plant cell walls (Yang *et al*., 2023; Salam *et al*., 2023). This mechanical disruption enhances solvent penetration, increases surface area, and accelerates diffusion and mass transfer, ultimately leading to improved extraction efficiency (Jan *et al*., 2021; Xu *et al*., 2023).

7.1.2 Advantages of Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) offers several advantages over conventional extraction techniques, making it a preferred method in modern phytochemical research. One of its primary benefits is the significant reduction in extraction time, which is achieved through enhanced mass transfer and improved solvent penetration into plant tissues. Additionally, UAE requires lower volumes of solvent, thereby improving cost-effectiveness and supporting environmentally

sustainable practices. The technique is also associated with higher extraction efficiency and improved yields of target secondary metabolites. Due to the shorter exposure to heat, UAE is particularly suitable for the extraction of thermolabile compounds, as it minimizes thermal degradation. Furthermore, UAE is considered energy-efficient and environmentally friendly, aligning with the principles of green extraction technologies (Jan *et al*., 2021; Kumari *et al*., 2024).

7.1.3 Limitations of Ultrasound-Assisted Extraction (UAE)

Despite its numerous advantages, UAE has certain limitations that may affect its applicability. The localized high temperatures and pressures generated during acoustic cavitation can lead to the degradation of sensitive bioactive compounds. In addition, the technique may face challenges in large-scale industrial applications due to limitations in scalability. The cost of ultrasonic equipment and its maintenance requirements can also be a constraint, particularly in resource-limited settings. Moreover, the formation of free radicals during cavitation may negatively impact the stability of certain phytochemicals, potentially altering their chemical structure and bioactivity (Xu *et al*., 2023; Yang *et al*., 2023).

7.1.4 Applications in Phytochemical Research

Ultrasound-assisted extraction (UAE) has been extensively applied in the extraction of various classes of plant secondary metabolites, including phenolics, flavonoids, alkaloids, and essential oils. It is particularly effective for recovering antioxidant and bioactive compounds used in pharmaceutical, nutraceutical, and food industries (Salam *et al*., 2023; Jan *et al*., 2021). In addition, UAE is widely employed in analytical sample preparation, where rapid and efficient extraction is essential for subsequent chromatographic and spectroscopic analyses (Kumari *et al*., 2024).

7.1.5 Comparison with Conventional Methods

Compared to traditional extraction techniques such as maceration and Soxhlet extraction, UAE offers significantly shorter extraction times, higher yields, and reduced solvent consumption. While conventional methods rely primarily on passive diffusion, UAE enhances extraction efficiency through mechanical disruption of plant cell structures and intensified mass transfer. As a result, UAE is considered a more efficient and modern alternative for phytochemical extraction (Xu *et al*., 2023; Jan *et al*., 2021).

7.2 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) is an advanced technique that utilizes microwave energy (300 MHz–300 GHz) to enhance the recovery of plant secondary metabolites. This method has gained considerable attention due to its rapid extraction process, reduced solvent consumption, and high efficiency compared to conventional extraction techniques (Jan *et al*., 2021; Kumari *et al*., 2024). MAE is widely applied in phytochemical research for extracting compounds such as phenolics, flavonoids, alkaloids, and essential oils (Xu *et al*., 2023).

7.2.1 Principle of MAE

The principle of MAE is based on the interaction between microwave radiation and polar molecules or ionic species present in the solvent and plant matrix. This interaction leads to rapid heating through mechanisms such as dipole rotation and ionic conduction, resulting in efficient energy transfer (Yang *et al*., 2023; Xu *et al*., 2023). Microwave energy causes polar molecules, such as water and ethanol, to align with the oscillating electromagnetic field, generating heat through

molecular friction. This rapid internal heating increases pressure within plant cells, leading to cell wall rupture and facilitating the release of intracellular secondary metabolites. Consequently, mass transfer is enhanced, resulting in improved extraction efficiency (Jan *et al*., 2021; Kumari *et al*., 2024).

7.2.2 Advantages of MAE

Microwave-assisted extraction offers several advantages over conventional methods. It significantly reduces extraction time while requiring lower solvent volumes, thereby improving cost-effectiveness and environmental sustainability. The technique also provides high extraction efficiency and improved yields of target compounds. Moreover, the shorter exposure time helps preserve thermolabile compounds by minimizing thermal degradation. MAE is also energy-efficient, making it a suitable approach for green and sustainable extraction processes (Jan *et al*., 2021; Kumari *et al*., 2024).

7.2.3 Limitations of MAE

Despite its advantages, MAE has certain limitations that must be considered. There is a risk of thermal degradation if temperature and operating conditions are not properly controlled. In addition, MAE is less suitable for non-polar solvents, as they exhibit poor absorption of microwave energy. The high initial cost of equipment and challenges associated with scaling up the process for industrial applications may also limit its widespread use. These factors highlight the need for careful optimization of operating parameters when applying MAE in phytochemical extraction (Xu *et al*., 2023; Yang *et al*., 2023).

7.2.4. Applications in Phytochemical Research

Microwave-assisted extraction (MAE) is widely applied in the extraction of various classes of secondary metabolites, including phenolics, flavonoids, alkaloids, and essential oils, due to its high efficiency and rapid processing time (Salam *et al*., 2023). It is particularly valuable in analytical sample preparation, where fast and efficient extraction is required for chromatographic and spectroscopic analyses (Jan *et al*., 2021). In addition, MAE is increasingly utilized in pharmaceutical, nutraceutical, and food industries for the development of high-quality plant-based products (Kumari *et al*., 2024).

7.2.5 Comparison with Other Extraction Methods

Compared to conventional techniques such as maceration and Soxhlet extraction, MAE provides significantly faster extraction, higher yields, and reduced solvent consumption. Unlike ultrasound-assisted extraction (UAE), which relies on acoustic cavitation, MAE operates through microwave-induced heating and internal cell rupture. This makes it particularly effective for extracting polar compounds, as microwave energy is efficiently absorbed by polar solvents and molecules (Xu *et al*., 2023; Jan *et al*., 2021).

7.3 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is an advanced and environmentally friendly technique that utilizes fluids at temperatures and pressures above their critical points to extract bioactive compounds from plant materials. Carbon dioxide (CO_2) is the most commonly used supercritical fluid due to its non-toxicity, non-flammability, and tunable solvating properties (Jan *et al*., 2021; Kumari *et al*., 2024). SFE has gained widespread attention in pharmaceutical, nutraceutical, and

food industries because of its high selectivity, rapid extraction rate, and ability to preserve thermolabile compounds due to relatively moderate operating temperatures (Xu *et al*., 2023; Salam *et al*., 2023).

7.3.1 Principle of SFE

SFE operates on the principle that a supercritical fluid exhibits properties intermediate between those of gases and liquids, including high diffusivity, low viscosity, and enhanced solvation capacity (Yang *et al*., 2023). When CO_2 is subjected to conditions above its critical temperature and pressure, it enters a supercritical state. In this state, it can penetrate plant matrices like a gas while dissolving solutes like a liquid. The solvating power of the supercritical fluid can be precisely controlled by adjusting parameters such as pressure, temperature, and the addition of co-solvents. This tunability allows for selective extraction of specific secondary metabolites (Jan *et al*., 2021; Kumari *et al*., 2024).

7.3.2 Advantages of SFE

Supercritical fluid extraction offers several advantages over both conventional and other advanced extraction methods. It provides high selectivity for target compounds through the adjustment of pressure and temperature. The technique uses minimal solvent and is environmentally friendly, producing non-toxic and residue-free extracts suitable for pharmaceutical and food applications. Additionally, SFE operates under moderate temperatures, which helps preserve thermolabile compounds. It also enables rapid extraction with high yield and purity, making it highly efficient for high-value phytochemicals (Jan *et al*., 2021; Xu *et al*., 2023).

7.3.3 Limitations of SFE

Despite its advantages, SFE has certain limitations. The technique requires high initial investment due to expensive equipment and operational costs. Process optimization can be complex, as extraction conditions must be carefully adjusted for different plant materials and target compounds. Furthermore, SFE is less effective for highly polar compounds unless co-solvents are used to enhance solubility. There are also challenges associated with scaling up the process for large-scale industrial applications (Yang *et al*., 2023; Salam *et al*., 2023).

7.3.4 Applications in Phytochemical Research

SFE is widely used for the extraction of essential oils, carotenoids, polyphenols, alkaloids, and flavonoids. Its ability to produce high-purity, solvent-free extracts make it particularly suitable for applications in pharmaceuticals, nutraceuticals, cosmetics, and functional foods (Kumari *et al*., 2024; Jan *et al*., 2021). Moreover, SFE is increasingly applied in analytical and quality control laboratories, where selective extraction of bioactive compounds is essential for accurate chromatographic and spectroscopic analyses (Xu *et al*., 2023).

7.4 Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), is an advanced technique that employs elevated temperature and pressure to enhance the recovery of plant secondary metabolites. By maintaining solvents in the liquid state above their normal boiling points, PLE improves solubility, desorption, and mass transfer processes, leading to rapid and efficient extraction (Jan *et al*., 2021; Xu *et al*., 2023). This technique has gained significant

importance in phytochemical and analytical chemistry due to its high efficiency, reduced extraction time, and lower solvent consumption. As a result, PLE is considered a sustainable alternative to conventional methods such as Soxhlet extraction and maceration (Kumari *et al*., 2024).

7.4.1 Principle of PLE

The principle of PLE is based on the combined effects of high temperature (typically 50–200°C) and high pressure (10–15 MPa), which enhance the extraction capability of solvents. Elevated temperature increases the solubility of analytes, improves diffusion rates, and reduces solvent viscosity and surface tension, thereby facilitating penetration into plant matrices (Yang *et al*., 2023). At the same time, high pressure ensures that the solvent remains in the liquid state even above its boiling point, maintaining continuous interaction between the solvent and the plant material. This combination of conditions results in improved extraction efficiency and faster recovery of secondary metabolites (Jan *et al*., 2021).

7.4.2 Advantages of PLE

Pressurized liquid extraction (PLE) offers several notable advantages:

- Rapid extraction kinetics, significantly reducing processing time
- Lower solvent consumption, contributing to sustainability
- High extraction efficiency and reproducibility
- Automation with reduced human intervention
- Compatibility with a wide range of compounds and solvents (Jan *et al*., 2021)

In addition, PLE aligns with green extraction principles by minimizing both solvent use and energy consumption while maintaining high yields, making it an environmentally friendly approach to phytochemical extraction (Usman *et al*., 2023).

7.4.3 Limitations of PLE

Despite its advantages, PLE presents some limitations:

- Thermal degradation of heat-sensitive compounds at elevated temperatures
- High initial equipment and operational costs
- Optimization challenges for different plant matrices and analytes
- Potential co-extraction of interfering substances under harsh conditions (Yang *et al*., 2023)

7.4.4 Applications in Phytochemical Research

PLE is widely applied for the extraction of phenolics, flavonoids, alkaloids, terpenoids, and glycosides from plant materials. Its high efficiency, reproducibility, and compatibility with diverse solvents make it particularly suitable for analytical applications, including chromatographic and spectroscopic analyses (Xu *et al*., 2023; Jan *et al*., 2021). Furthermore, PLE is increasingly adopted in pharmaceutical, nutraceutical, and food industries, where rapid, efficient, and reproducible extraction processes are essential for producing high-quality plant-based products (Kumari *et al*., 2024).

7.4.5 Comparison with Other Extraction Methods

Compared to conventional methods such as maceration and Soxhlet extraction, PLE provides shorter extraction times, improved efficiency, and reduced solvent usage. Unlike supercritical fluid extraction, which relies on supercritical fluids, PLE uses liquid solvents under pressure, making it

more versatile for the extraction of both polar and moderately non-polar compounds (Jan *et al*., 2021).

8. Solvent Selection in Extraction

Solvent selection is a critical determinant of extraction efficiency, selectivity, and overall quality when recovering plant secondary metabolites. The choice of solvent directly influences the types and yields of compounds extracted, as well as the effectiveness of the process (Jan *et a*l., 2021). An appropriate solvent should be selected based on the physicochemical properties of target compounds, including polarity, solubility, stability, and molecular structure. Proper solvent selection ensures maximum recovery of bioactive compounds while maintaining their chemical integrity and biological activity (Xu *et al*., 2023).

8.1 Role of Solvent Polarity

The principle of "like dissolves like" governs solvent selection in phytochemical extraction. Polar solvents, such as water, methanol, and ethanol, are effective for extracting polar compounds, including phenolics, flavonoids, glycosides, and tannins. Non-polar solvents, such as hexane and petroleum ether, are suitable for extracting lipophilic compounds like terpenoids, steroids, and essential oils (Yang *et al*., 2023; Kumari *et al*., 2024). Solvents of intermediate polarity, including ethyl acetate and acetone, are often employed to extract moderately polar compounds, enabling selective fractionation of complex plant matrices (Jan *et al*., 2021; Xu *et al*., 2023).

8.2 Physicochemical Properties of Solvents

In addition to polarity, several physicochemical properties must be considered when selecting an extraction solvent:

- Solubility: The solvent must effectively dissolve the target compounds (Coltescu *et al*., 2020).
- Viscosity and Surface Tension: Lower viscosity enhances solvent penetration into plant tissues, improving mass transfer (Cannavacciuolo *et al*., 2022).
- Boiling Point: Solvents with lower boiling points are easier to remove after extraction, reducing energy consumption and preventing thermal degradation (Xu *et al*., 2023).
- Chemical Stability: The solvent should not react with the target compounds or degrade during extraction (Yang *et al*., 2023).

8.3 Green Solvents and Sustainability

Recent advances in extraction technologies emphasize the use of green solvents, such as water, ethanol, and natural deep eutectic solvents (NADES), to reduce environmental and health risks (Cannavacciuolo *et al*., 2022). These solvents are biodegradable, non-toxic, and sustainable, aligning with the principles of green chemistry. The adoption of green solvents not only minimizes environmental impact but also improves the safety and quality of extracts, particularly in pharmaceutical and food applications (Jan *et al*., 2021).

8.4 Solvent Mixtures and Optimization

In many cases, a single solvent may not be sufficient to extract all target compounds. Therefore, binary or ternary solvent mixtures (e.g., ethanol–water, methanol–water) are often used to enhance extraction efficiency by adjusting polarity (Xu *et al*., 2023; Yang *et al*., 2023). Optimization of

solvent composition, along with extraction parameters such as temperature and time, is essential to achieve maximum yield and selectivity (Cannavacciuolo *et al*., 2023).

8.5 Challenge in Solvent Selection

The wide range of polarity, molecular size, and structural complexity of compounds such as phenolics, alkaloids, and terpenoids makes it difficult for a single solvent to achieve optimal extraction efficiency and selectivity (Jan *et al*., 2021; Xu *et al*., 2023). A major limitation is the trade-off between selectivity and yield, where highly selective solvents may result in low recovery, while less selective solvents often co-extract unwanted components such as chlorophyll, lipids, and waxes (Yang *et al*., 2023). This necessitates careful optimization or the use of solvent mixtures to balance extraction performance (Kumari *et al*., 2024). Toxicity and safety concerns further complicate solvent selection, as commonly used organic solvents (e.g., methanol, chloroform, hexane) may pose health risks and leave harmful residues in extracts intended for pharmaceutical or food applications (Xu *et al*., 2023; Jan *et al*., 2021). In parallel, increasing environmental concerns regarding solvent volatility, non-biodegradability, and pollution have driven the shift toward green solvents, although these may sometimes exhibit lower extraction efficiency (Cannavacciuolo *et al*., 2022). Additionally, solvent–matrix interactions and plant structural characteristics can limit solvent penetration and compound solubilization, reducing extraction efficiency (Kumari *et al*., 2024; Yang *et al*., 2023). Economic factors, including solvent cost, availability, and recovery requirements, also influence solvent choice, particularly in large-scale applications (Jan *et al*., 2021). Finally, solvent selection must be compatible with the chosen extraction technique, as factors such as microwave absorption, cavitation efficiency, and supercritical behaviour vary depending on solvent properties (Xu *et al*., 2023). Regulatory constraints further restrict solvent use, especially in food and pharmaceutical industries, where strict limits on residual solvents must be met (Jan *et al*., 2021).

8.6. Factors Affecting Extraction Efficiency

The efficiency of extracting plant secondary metabolites is governed by a complex interplay of solvent properties, extraction conditions, and plant matrix characteristics. Optimization of these parameters is essential to achieve maximum yield, selectivity, and reproducibility in phytochemical extraction processes (Jan *et al*., 2021; Xu *et al*., 2023).

8.6.1 Solvent Properties

Solvent characteristics such as polarity, viscosity, dielectric constant, and solubility significantly influence extraction efficiency. The principle of "like dissolves like" dictates that polar solvents (e.g., water, methanol, ethanol) are suitable for extracting phenolics, flavonoids, and glycosides, whereas non-polar solvents (e.g., hexane) are effective for lipophilic compounds (Yang *et al*., 2023; Kumari *et al*., 2024). Additionally, lower viscosity and surface tension improve solvent penetration and mass transfer, enhancing extraction yield (Cannavacciuolo *et al*., 2022).

8.6.2 Temperature

Temperature plays a crucial role in enhancing solute solubility, diffusion rate, and mass transfer kinetics. Elevated temperatures reduce solvent viscosity and improve extraction efficiency; however, excessive heat may lead to thermal degradation of sensitive compounds, such as flavonoids and essential oils (Xu *et al*., 2023; Jan *et al*., 2021). Therefore, maintaining an optimal temperature is essential to balance extraction efficiency with compound stability (Cannavacciuolo *et al*., 2022).

8.6.3 Extraction Time

Extraction time affects the extent of solute diffusion from plant matrices into the solvent. Increasing extraction time generally enhances yield until equilibrium is reached, beyond which no significant improvement occurs (Ameer *et al*., 2017; Ramesh *et al*., 2024). Prolonged extraction may also result in compound degradation and co-extraction of impurities, negatively impacting extract quality (Jan *et al*., 2021).

8.6.4 Particle Size and Surface Area

Reduction of particle size increases the surface area available for solvent interaction, thereby improving extraction efficiency (Kumari *et al*., 2024). Smaller particles enhance solvent penetration and diffusion rates; however, excessively fine particles may cause agglomeration, clogging, or reduced solvent flow, limiting extraction performance (Xu *et al*., 2023).

8.6.5 Solvent-to-Sample Ratio

The solvent-to-sample ratio significantly influences solute concentration gradients and mass transfer efficiency. Higher solvent volumes improve extraction yield but may increase solvent consumption and processing costs (Jan *et al*., 2021). Optimization is therefore necessary to achieve maximum efficiency with minimal resource use.

8.6.6 pH of the Extraction Medium

The pH of the extraction medium affects the ionization, solubility, and stability of certain secondary metabolites, particularly alkaloids and phenolic compounds (Yang *et al*., 2023). Appropriate pH adjustment can enhance extraction efficiency; however, extreme pH conditions may cause chemical degradation or structural modification of target compounds (Xu *et al*., 2023).

8.6.7 Extraction Technique

The extraction method significantly influences efficiency. Advanced techniques, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE), improve extraction through enhanced cell disruption, solvent penetration, and mass transfer (Jan *et al*., 2021). Compared to conventional methods, these techniques provide higher yields, shorter extraction times, and improved reproducibility (Kumari *et al*., 2024).

8.6.8 Plant Matrix Characteristics

The structural and chemical properties of the plant matrix including cell wall composition, moisture content, and metabolite binding interactions significantly affect extraction efficiency (Xu *et al*., 2023; Yang et al., 2023). Strong interactions between metabolites and plant components may hinder extraction, necessitating pretreatment methods or optimized extraction conditions (Krakowska-Sieprawska *et al*., 2022).

9. Fractionation and Purification

Following extraction, crude plant extracts often contain complex mixtures of compounds, including both target secondary metabolites and non-bioactive constituents. To obtain pure, structurally defined, and biologically active compounds, subsequent fractionation and purification are essential. These processes are critical for structural characterization, bioactivity assessment, and

quality standardization in pharmacognosy, phytochemistry, and drug discovery (Yang *et al*., 2020; Wang *et al*., 2022).

9.1 Liquid–Liquid Extraction (LLE)

Liquid–liquid extraction (LLE), also known as solvent partitioning, is a fundamental separation technique widely used in phytochemical research for fractionating crude plant extracts. It relies on the differential distribution of compounds between two immiscible liquid phases, typically an aqueous phase and an organic solvent, allowing selective enrichment of secondary metabolites based on polarity and solubility (Tuhanioglu & Ubeyitogullari, 2025; Yang *et al*., 2020).

The principle of LLE is governed by the partition coefficient (K), which describes the equilibrium distribution of a solute between two immiscible solvents. Compounds preferentially dissolve in the solvent in which they are more soluble, following the "like dissolves like" principle (Zhang *et al*., 2022; Yang *et al*., 2023). Typically, polar compounds remain in the aqueous phase, while non-polar or moderately polar compounds partition into the organic phase (Xu *et al*., 2023; Jan et al., 2021). LLE remains a critical step in natural product isolation due to its simplicity, scalability, and effectiveness in reducing matrix complexity prior to chromatographic purification (Liko *et al*., 2016; Wang *et al*., 2022).

9.2. Column Chromatography in the Purification

Column chromatography is one of the most widely used techniques for the separation and purification of plant secondary metabolites. It operates on the principle of differential adsorption and partitioning of compounds between a stationary phase and a mobile phase, enabling the isolation of individual constituents from complex mixtures (Yang *et al*., 2020; Wang *et al*., 2022). Separation in column chromatography is determined by differences in the affinity of compounds for the stationary phase (adsorbent) and their solubility in the mobile phase (eluent). Compounds with stronger interactions with the stationary phase move more slowly, whereas those with weaker interactions elute faster (Zhang *et al*., 2022; Yang *et al*., 2023). The process is governed by adsorption, partitioning, and polarity differences, allowing effective separation of compounds with varying chemical properties (Xu *et al*., 2023; Jan *et al*., 2021). Due to its versatility, simplicity, and scalability, column chromatography remains a cornerstone method in phytochemical research and natural product isolation (Li *et al*., 2023; Kumari *et al*., 2024).

9.3 Thin-Layer Chromatography (TLC) in Analysis

Thin-layer chromatography (TLC) is a rapid, simple, and cost-effective analytical technique widely employed for the qualitative analysis, monitoring, and preliminary separation of plant secondary metabolites. TLC relies on the differential migration of compounds on a stationary phase under the influence of a mobile phase, allowing separation according to polarity and adsorption characteristics (Yang *et al*., 2020; Li *et al*., 2023). The principle of TLC involves adsorption of compounds onto a stationary phase typically silica gel or alumina coated on a plate and their movement with a solvent (mobile phase) by capillary action. Separation occurs due to differences in affinity for the stationary phase and solubility in the mobile phase (Yang *et al*., 2023; Xu *et al*., 2023). The degree of separation is expressed as the retardation factor (Rf value), defined as the ratio of the distance travelled by the compound to that of the solvent front (Zhang *et al*., 2022). TLC plays a crucial role in phytochemical research, particularly for screening extracts, monitoring

fractionation processes, and assessing purity prior to advanced chromatographic techniques (Wang *et al*., 2022; Kumari *et al*., 2024).

9.4 High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a highly efficient analytical and preparative technique widely used for the separation, identification, and quantification of plant secondary metabolites. HPLC operates under high pressure to pass liquid solvents through a column packed with a stationary phase, enabling high-resolution separation of complex mixtures (Yang *et al*., 2020; Li *et al*., 2023). The principle of HPLC is based on the differential partitioning of compounds between a stationary phase and a mobile phase under high pressure. Separation occurs due to variations in polarity, molecular size, and interactions with the stationary phase (Zhang *et al*., 2022; Yang *et al*., 2023). Compounds with stronger interactions with the stationary phase exhibit longer retention times, while those with weaker interactions elute faster. Separation efficiency is influenced by parameters such as flow rate, column composition, and solvent system (Xu *et al*., 2023; Jan *et al*., 2021). HPLC is considered a gold-standard technique in phytochemistry due to its sensitivity, precision, and reproducibility, making it indispensable in natural product research, quality control, and drug development (Liko *et al*., 2016; Kumari *et al*., 2024).

10. Qualitative and Quantitative Analysis

The analysis of plant secondary metabolites involves both qualitative and quantitative approaches to identify, characterize, and determine the concentration of bioactive compounds. These analytical strategies are essential for phytochemical profiling, quality control, pharmacological evaluation, and standardization of herbal products (Yang *et al*., 2020; Jan *et al*., 2021).

10.1 Phytochemical Screening

Phytochemical screening is a fundamental step in natural product research, involving the qualitative identification of bioactive secondary metabolites present in plant extracts. It provides preliminary information on the chemical composition of plant materials and serves as a basis for further isolation, characterization, and pharmacological evaluation (Yang *et al*., 2020; Jan *et al*., 2021; Kumari *et al*., 2024). The presence of phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolics contributes to the biological activities of plants, including antioxidant, antimicrobial, and anti-inflammatory properties (Yang *et al*., 2023; Xu *et al*., 2023). Phytochemical screening relies on specific chemical reactions between plant constituents and reagents, resulting in observable changes such as colour formation, precipitation, or frothing, indicative of particular classes of compounds. Although qualitative in nature, these tests are essential for rapid screening and classification of plant extracts, especially during preliminary investigations (Kumari *et al*., 2024; Yang *et al*., 2020).

10.1.1 Common Phytochemical Tests

Alkaloids – Detected using:

- Dragendorff's test: orange or reddish-brown precipitate
- Mayer's test: cream or white precipitate
- Wagner's test: reddish-brown precipitate. These reactions involve the formation of insoluble complexes with alkaloid salts (Yang *et al*., 2023; Lawan *et al*., 2026).

Flavonoids – Detected using:

- Shinoda test: pink or red coloration
- Alkaline reagent test: yellow coloration that disappears upon acidification. These reactions indicate flavonoid structures capable of reduction and complex formation (Kumari *et al*., 2024).

Tannins – Detected using:

- Ferric chloride test: blue-black or green coloration
- Lead acetate test: formation of precipitate. These reactions result from complex formation between tannins and metal ions (Yang *et al*., 2023; Mustapha *et al*. 2025).

Saponins – Detected using:

- Frothing test: formation of stable foam upon shaking. This reflects the surfactant properties of saponins, reducing surface tension (Kumari *et al*., 2024).

Terpenoids and Steroids – Detected using:

- Salkowski test: reddish-brown coloration
- Liebermann–Burchard test: green or blue coloration. These reactions indicate unsaturated ring structures via interaction with concentrated acids (Yang *et al*., 2023; Kabir & Lawan 2026).

Glycosides – Detected using:

- Keller–Killiani test: brown ring formation
- Borntrager's test: pink or red coloration for anthraquinone glycosides. These tests indicate sugar-bound secondary metabolites (Mustapha *et al*., 2025; Kabir & Lawan 2026).

Phenolic Compounds – Detected using:

- Ferric chloride test: blue, green, or violet coloration. This reaction is based on complex formation between phenolic hydroxyl groups and iron ions (Yang *et al*., 2023).

10.1.2 Recent Advances in Phytochemical Screening

Modern techniques have enhanced traditional screening methods through:

- High-performance thin-layer chromatography (HPTLC) for higher resolution
- Hyphenated techniques (LC–MS, GC–MS) for simultaneous identification
- Metabolomics-based profiling for comprehensive analysis
- Digital image analysis for colorimetric assays.

These advancements improve accuracy, reproducibility, and efficiency in phytochemical investigations (Liko *et al*., 2016; Wolfender *et al*., 2018).

10.2 Total Phenolic Content (TPC) Determination

Total phenolic content (TPC) is a widely used parameter for evaluating the overall concentration of phenolic compounds in plant extracts, which contribute to antioxidant, antimicrobial, and anti-inflammatory activities. Phenolic compounds, including phenolic acids, flavonoids, tannins, and lignans, significantly influence the biological properties of plants (Kumari *et al*., 2024; Yang *et al*., 2023). TPC determination is commonly performed using the Folin–

Ciocalteu (F–C) colorimetric assay, a standard method valued for its simplicity, sensitivity, and reproducibility (Yang *et al*., 2020).

10.3 Total Flavonoid Content (TFC)

Flavonoids are polyphenolic secondary metabolites widely distributed in plants and studied for antioxidant, anti-inflammatory, antimicrobial, antiviral, and cardioprotective activities (Li *et al*., 2024; Yang *et al*., 2023; Kumari *et al*., 2024). These compounds play vital roles in plant physiology, including UV protection, pigmentation, and defence against biotic and abiotic stress, making them critical targets in phytochemical and pharmacological research (Yang *et al*., 2020; Jan *et al*., 2021; Xu *et al*., 2023). TFC determination provides a rapid, cost-effective preliminary tool for estimating flavonoid levels in plant extracts, preceding advanced profiling techniques such as HPLC, UHPLC, and LC–MS/MS (Li *et al*., 2023; Liko *et al*., 2016; Yang *et al*., 2020; Nicolescu *et al*., 2025). While simple and affordable, TFC assays are ideally complemented by more selective techniques for accurate quantification (Nicolescu *et al*., 2025).

10.4 Spectroscopic Methods

Spectroscopic methods are fundamental analytical tools used for identification, characterization, and quantification of plant secondary metabolites, including flavonoids, alkaloids, phenolics, and terpenoids. These techniques rely on interactions between electromagnetic radiation and matter, providing detailed information on molecular structure, functional groups, and compound concentration (Dunnivant & Ginsbach, 2024; Li *et al*., 2023; Liko *et al*., 2016). Due to their high sensitivity, rapid analysis, minimal sample preparation, and non-destructive nature, spectroscopic methods are extensively applied in phytochemistry, pharmacognosy, and natural product research (Yang *et al*., 2020; Xu *et al*., 2023).

10.4.1. Ultraviolet–Visible (UV–Vis) Spectroscopy

UV–Vis Spectroscopy is one of the most commonly used techniques for the quantitative determination of phytochemicals, particularly phenolics and flavonoids, based on their ability to absorb light in the 200–800 nm range (Dunnivant & Ginsbach, 2024; Yang *et al*., 2023; Li *et al*., 2024). The method relies on electronic transitions ($\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$) within chromophoric groups in bioactive compounds. It is widely applied in assays such as:

- Total phenolic content (Folin–Ciocalteu method)
- Total flavonoid content ($AlCl_3$ method)
- DPPH and other antioxidant assays

UV–Vis Spectroscopy is favored for its simplicity, rapidity, and cost-effectiveness, although it lacks specificity and cannot distinguish individual compounds (Kumari *et al*., 2024; Yang *et al*., 2020).

10.4.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy identifies functional groups in phytochemicals by measuring absorption of infrared radiation, which induces vibrational transitions in molecular bonds (Li *et al*., 2023). Characteristic absorption bands allow identification of functional groups such as:

- O–H (phenols and alcohols)
- C=O (carbonyl compounds)
- C–H (alkanes and aromatics)
- C–O (ethers and esters)

FTIR is widely applied for qualitative analysis and chemical fingerprinting of plant extracts, enabling rapid screening of chemical composition (Xu *et al*., 2023; Liko *et al*., 2016).

10.4.3Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy provides detailed structural information and chemical environment analysis of atoms within phytochemicals, typically using ^{1}H and ^{13}C nuclei (Claridge, 2016; Li *et al*., 2024). NMR reveals:

- Molecular structure and connectivity
- Functional groups and stereochemistry
- Purity and composition of compounds

It is regarded as a highly reliable method for structure elucidation of natural products, particularly when combined with chromatographic techniques (Yang *et al*., 2020; Liko *et al*., 2016).

10.4.4 Mass Spectrometry (MS)

Mass spectrometry identifies and determines the molecular weight of phytochemicals by measuring the mass-to-charge ratio (m/z) of ionized compounds (Gross, 2017; Liko *et al*., 2016). When coupled with separation techniques such as GC–MS or LC–MS, it provides:

- High sensitivity and selectivity
- Structural fragmentation patterns
- Accurate identification of complex mixtures

MS-based approaches are widely used in metabolomics and phytochemical profiling due to their precision and ability to analyse diverse compounds (Li *et al*., 2023; Xu *et al*., 2023; Jan *et al*., 2021).

10.4.5 Fluorescence Spectroscopy

Fluorescence spectroscopy measures emission of light from excited molecules and is highly sensitive for naturally fluorescent compounds, such as certain flavonoids and alkaloids (Yang *et al*., 2023).

Advantages include:

- High sensitivity and low detection limits
- Selectivity for fluorescent compounds
- Rapid analysis with minimal sample preparation

Its application is limited to compounds with intrinsic fluorescence or those derivatized to become fluorescent (Kumari *et al.*, 2024*;* Li *et al.*, 2024).

11. Future Perspectives in the Extraction and Application of Secondary Metabolites

The study and application of plant secondary metabolites continue to expand due to their diverse biological activities and industrial relevance. Future research is expected to focus on:

- Innovative extraction technologies
- Advanced analytical tools for rapid and precise characterization
- Sustainable production systems and green solvent adoption
- Integration of interdisciplinary approaches to overcome current limitations. These efforts aim to enhance efficiency, selectivity, and scalability in phytochemical extraction and application, ultimately facilitating natural product research, pharmaceutical development, and functional food production (Liko *et al*., 2016; Jan *et al*., 2021; Li *et al*., 2024).

11.1. Development of Green and Sustainable Extraction Technologies

Future directions in the extraction and application of plant secondary metabolites are increasingly guided by the principles of green chemistry, emphasizing reduced solvent consumption, energy efficiency, and environmental sustainability (Li *et al*., 2024). Emerging extraction approaches focus on improving efficiency, selectivity, and safety while minimizing environmental impact. Key strategies include:

- Green solvents: Utilization of environmentally friendly solvents such as deep eutectic solvents (DES) and natural deep eutectic solvents (NADES) for enhanced solubility and sustainability.
- Supercritical fluid extraction (SFE): Use of CO_2 as a solvent-free and eco-friendly alternative, yielding high-quality extracts without harmful residues.
- Assisted extraction techniques: Ultrasound- and microwave-assisted extraction methods reduce processing time, energy consumption, and improve mass transfer efficiency. These innovations collectively aim to advance sustainable, efficient, and selective extraction of bioactive compounds (Jan *et al*., 2021; Yang *et al*., 2020).

11.2 Integration of Advanced Analytical Techniques

The future of phytochemical research also emphasizes the integration of high-resolution analytical tools with computational methods to achieve comprehensive profiling and accurate quantification. Emerging trends include:

High-resolution separation and detection: Techniques such as LC–MS/MS, UHPLC–HRMS, and hyphenated methods (LC–NMR, GC–MS) enable detailed structural elucidation and simultaneous analysis of complex metabolite mixtures.

NMR-based metabolomics: Provides holistic insights into metabolite composition and dynamics in plant extracts.

Data analytics and computational approaches: Application of chemometrics and artificial intelligence (AI) enhances data interpretation, pattern recognition, predictive modeling, and informed decision-making in phytochemical investigations. These integrated approaches are expected to improve the efficiency, accuracy, and reproducibility of phytochemical analysis, facilitating natural product research, drug discovery, and functional food development (Liko *et al*., 2016; Li *et al*., 2023; Xu *et al*., 2023; Jan *et al*., 2021).

11.3. Biotechnological and Synthetic Biology Approaches

Advances in biotechnology and synthetic biology are opening new avenues for controlled and scalable production of secondary metabolites, reducing reliance on natural plant sources and addressing low-yield limitations. Strategies include:

- Metabolic engineering of microorganisms and plants to enhance biosynthesis of target compounds.
- In vitro plant cell and tissue culture for sustainable metabolite production.
- Microbial biosynthesis for high-value secondary metabolites. These approaches allow precise control over production conditions and contribute to sustainable natural product development (Liko *et al*., 2016; Jan *et al*., 2021; Xu *et al*., 2023).

11.4 Expansion in Functional Foods and Nutraceuticals

Future research is anticipated to expand the application of secondary metabolites in functional foods and nutraceuticals, driven by increasing consumer demand for natural, health-promoting products (Li *et al*., 2024; Yang *et al*., 2023). Key developments in this area include:

- Fortification of foods with bioactive compounds to enhance nutritional and therapeutic value.
- Development of plant-based dietary supplements to provide convenient and standardized sources of phytochemicals.
- Use in active packaging and food preservation systems to extend shelf life while providing additional health benefits. These applications are expected to contribute significantly to disease prevention and health promotion, particularly in managing chronic conditions such as cardiovascular diseases and oxidative stress-related disorders (Yang *et al*., 2020; Jan *et al*., 2021).

11.5 Precision Agriculture and Sustainable Crop Protection

Secondary metabolites are poised to play a major role in precision agriculture and sustainable crop protection through:

Development of eco-friendly biopesticides and bioherbicides, reducing environmental impact compared to conventional agrochemicals.

Integration into smart farming systems for optimized resource use and crop management.

Enhancement of plant stress tolerance, improving resilience to biotic and abiotic stressors. These strategies are expected to decrease reliance on synthetic chemicals and promote environmentally sustainable agricultural practices (Kumari *et al*., 2024; Jan *et al*., 2021; Xu *et al*., 2023).

11.6 Standardization and Regulatory Harmonization

Future efforts will emphasize:

- Establishing standardized extraction and analytical protocols to ensure reproducibility across laboratories.
- Developing validated methods for quantification and quality control of bioactive compounds.
- Harmonizing regulatory frameworks to facilitate global acceptance of plant-derived products. Such initiatives will improve the safety, reliability, and commercialization potential of phytochemicals, ensuring consistent quality for research, therapeutic, and commercial applications (Yang *et al*., 2020; Liko *et al*., 2016; Li *et al*., 2024).

11.7 Digitalization and Artificial Intelligence

The integration of digital tools and artificial intelligence (AI) is expected to transform phytochemical research by enabling:

- High-throughput data analysis, allowing rapid processing of large datasets from metabolomic and phytochemical studies.
- Prediction of bioactive compounds and their biological activities, improving the efficiency of natural product discovery and targeted applications. The adoption of AI and digital technologies will enhance data-driven decision-making, optimize extraction and analysis processes, and accelerate innovation in natural product research (Jan *et al*., 2021).

11.8 Optimization of extraction processes

Machine learning models hold significant potential to enhance the efficiency, accuracy, and innovation in natural product discovery, enabling predictive modeling, optimization of extraction processes, and identification of novel bioactive compounds (Jan *et al*., 2021; Xu *et al*., 2023).

12. Conclusion

Plant secondary metabolites are a diverse and valuable class of bioactive compounds with extensive applications in pharmaceutical, agricultural, and industrial sectors. Their effective utilization relies on the careful selection of extraction methods, appropriate solvent systems, and the application of reliable analytical techniques for accurate identification and quantification. Despite substantial advancements in extraction efficiency and analytical precision, challenges remain, including variability in plant composition, low metabolite yields, compound instability, and difficulties in scaling up production. Future research focusing on sustainable extraction technologies, biotechnological production strategies, advanced analytical methods, and computational approaches including machine learning is expected to overcome these limitations, thereby expanding the practical applications of secondary metabolites. Continued methodological innovation is essential to fully realize the therapeutic, nutritional, and industrial potential of these plant-derived compounds.

References

Abdulhussain N., Nawada S., & Schoenmakers P. (2021). Latest trends on the future of three-dimensional separations in chromatography, *Chemical reviews*, *121*(19), 12016-12034.

Abu-Reidah I. M. (2023). Special Issue on "Secondary Metabolites: Extraction, Optimization, Identification and Applications in Food, Nutraceutical, and Pharmaceutical Industries", Processes, *11*(9), 2801. https://doi.org/10.3390/pr11092801.

Adamski Z., Blythe L. L., Milella L., & Bufo S. A. (2020). Biological activities of alkaloids: from toxicology to pharmacology. *Toxins*, *12*(4), 210. doi.org/10.3390/toxins12040210.

Akbar R., Sun J., Bo Y., Khattak W. A., Khan A. A., Jin C., Zeb U., Ullah N., Abbas A., Liu W., Wang X., Khan S. M. & Du D. (2024). Understanding the influence of secondary metabolites in plant invasion strategies: A comprehensive review, *Plants*, *13*(22), 3162. https://doi.org/10.3390/plants13223162.

Ameer K., Shahbaz H. M., & Kwon J. H. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review, *Comprehensive reviews in food science and food safety*, *16*(2), 295-315.

Aourabi S., Driouch M., Sfaira M., Mahjoubi F., *et al*. (2021), Phenolic fraction of Ammi visnaga extract as environmentally friendly antioxidant and corrosion inhibitor for mild steel in acidic medium, *Journal of Molecular Liquids*, 323, 114950. https://doi.org/10.1016/j.molliq.2020.114950

Ashraf M. A., Iqbal M., Rasheed R., Hussain I., Riaz M., & Arif M. S. (2018). Environmental stress and secondary metabolites in plants: an overview, *Plant metabolites and regulation under environmental stress*, 153-167. doi.org/10.1016/B978-0-12-812689-9.00008-X

Atanasov A.G., Zotchev S.B., Dirsch V.M., Supuran C.T. (2021). Natural products in drug discovery: advances and opportunities, *Nature reviews Drug discovery*, *20*(3), 200-216. https://doi.org/10.1038/s41573-020-00114-z.

Barthwal R., & Mahar R. (2024). Exploring the significance, extraction, and characterization of plant-derived secondary metabolites in complex mixtures, *Metabolites*, *14*(2), 119. https://doi.org/10.3390/metabo14020119.

Bhadange Y. A., Carpenter J., & Saharan V. K. (2024). A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources, *ACS omega*, *9*(29), 31274-31297. https://doi.org/10.1021/acsomega.4c02718.

Bhambhani S., Kondhare K. R., & Giri A. P. (2021). Diversity in chemical structures and biological properties of plant alkaloids, *Molecules*, *26*(11), 3374. https://doi.org/10.3390/molecules26113374.

Bhatti M. Z., Ismail H., & Kayani W. K. (2022). Plant secondary metabolites: therapeutic potential and pharmacological properties. In Secondary metabolites-trends and reviews. *IntechOpen*.

Bocso N. S., & Butnariu M. (2022). The biological role of primary and secondary plants metabolites. *Journal of Nutrition and Food Processing*, *5*(3), 1-7. https://doi.org./10.31579/2637-8914/094.

Bribi N. (2018). Pharmacological activity of alkaloids: a review, *Asian J. Bot*, *1*(1), 1-6. https://doi.org/10.63019/ajb.v1i2.467.

Brizzolara S., Manganaris G.A., Fotopoulos V., Watkins C.B., Tonutti P. (2020). Primary metabolism in fresh fruits during storage, *Frontiers in plant science*, *11*, 80. https://doi.org/10.3389/fpls.2020.00080.

Cannavacciuolo C., Pagliari S., Frigerio J., Giustra C. M., Labra M., & Campone L. (2022). Natural deep eutectic solvents (NADESs) combined with sustainable extraction techniques: A review of the green chemistry approach in food analysis, *Foods*, *12*(1), 56. https://doi.org/10.3390/foods12010056.

Claridge, T. D. (2016). High-resolution NMR techniques in organic chemistry (27). Elsevier.

Coltescu A. R., Butnariu M., & Sarac I. (2020). The importance of solubility for new drug molecules, *Biomedical and Pharmacology Journal*, *13*(2), 577-583. https://dx.doi.org/10.13005/bpj/1920.

Debnath S., Das M., Mondal S., Sarkar B. K., & Babu G. (2025). Advances in chromatography: contemporary techniques and applications, *Essential Chem*, *2*(1), 1-27.

Devi A. M., Devi K. K., Devi P. P., Devi M. L., & Das S. (2023). Metabolic engineering of plant secondary metabolites: prospects and its technological challenges. *Frontiers in Plant Science*, *14*, 1171154. https://doi.org/10.3389/fpls.2023.1171154.

Diass K., Brahmi F., Mokhtari O., Abdellaoui S., Hammouti B. (2021). Biological and pharmaceutical properties of essential oils of Rosmarinus officinalis L. and Lavandula officinalis L., *Materials Today: Proceedings*, 45(8), 7768-7773. https://doi.org/10.1016/j.matpr.2021.03.495

Dunnivant F. M., & Ginsbach J. W. (2024). Essential Methods of Instrumental Analysis. John Wiley & Sons.

El Amri, N., Chabir, R., Kadda, S., Kachkoul, R., & Bour, A. (2025). Optimization of Phenolic Compounds Extraction from Capparis spinosa Flowers Using Response Surface Methodology. *Mor. J. Chem.*, 13(4), 1798–1807. https://doi.org/10.48317/IMIST.PRSM/morjchem-v13i4.54053

El-Readi M. Z., Al-Abd A. M., Althubiti M. A., Almaimani R. A., Al-Amoodi H. S., Ashour M. L., Wink M. & Eid S. Y. (2021). Multiple molecular mechanisms to overcome multidrug resistance in cancer by natural secondary metabolites, *Frontiers in pharmacology*, *12*, 658513.

Elshafie H. S., Camele I., & Mohamed A. A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin, *International journal of molecular sciences*, *24*(4), 3266. https://doi.org/10.3390/ijms24043266.

Elshafie H. S., Camele I., & Mohamed A. A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of plant-based secondary metabolites. *International Journal of Molecular Sciences*, *24*(4), 3266. https://doi.org/10.3390/ijms24043266.

Hammad M. (2025). Antimicrobial Potential, Mechanisms, and Biosynthesis Pathways of Lichen-Derived Bioactive Compounds; A Systematic Review, *Journal of Agriculture and Biology*, *03*(2), 202-218

Hilal B., Khan M. M., & Fariduddin Q. (2024). Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: Phenolics, terpenes, and alkaloids, *Plant Physiology and Biochemistry*, *211*, 108674. https://doi.org/10.1016/j.plaphy.2024.108674.

Husaini M., Lawan I., Hamza M., Kabir D., Mu'azu J. B. (2023). Traditional and Advanced Extraction Methods of Bioactive Compounds: A Review, *J. Appl. Sci. Envir. Stud*., *6*(3), 253-267.

Jan R., Asaf S., Numan M., Lubna & Kim K. M. (2021). Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*, *11*(5), 968. https://doi.org/10.3390/agronomy11050968.

Kabir D., Lawan I. (2026) Qualitative Phytochemical Screening Methods of Bioactive Compounds from Medicinal Plants, *J. Mater. Environ. Sci.*, *17*(2), 251-266

Kabir D., Mohammed A.S., Hamza M., and Lawan I. (2025) Natural Products and Their Applications: A Review, *J. Mater. Environ. Sci.*, *16*(11), 2105-2125.

Kadda, S., Ouahhoud, S., Hadda, T. B., Bouhrim, M., El Hajjaji, F., Azzaoui, K., *et al*. (2026). Opuntia Ficus-indica l seed oil: Phytochemistry, Biological Activities, Cosmetic Applications, Good Health and Well-Being- A POM-Based Hybrid review. *Mor. J. Chem.*, 14(1), 152–172. https://doi.org/10.48317/IMIST.PRSM/morjchem-v14i1.62112

Kaushik B., Sharma J., Kumar P., & Shourie A. (2021). Phytochemical properties and pharmacological role of plants: secondary metabolites, *Biosciences Biotechnology Research Asia*, *18*(1), 23-35. doi.org/10.13005/bbra/2894.

Kowalski R., & Baj T. (2025). Secondary Metabolites from Natural Products: Extraction, Isolation and Biological Activities, *Molecules*, *30*(21), 4233.

Kowsalya K., Vidya N., Halka J., Preetha J. S. Y., Saradhadevi M., Sahayarayan J. J., Gurusaravanan P. & Arun M. (2025). Plant glycosides and glycosidases: classification, sources, and therapeutic insights in current medicine, *Glycoconjugate journal*, *42*(2), 107-124.

Krakowska-Sieprawska A., Kiełbasa A., Rafińska K., Ligor M., & Buszewski B. (2022). Modern methods of pre-treatment of plant material for the extraction of bioactive compounds, *Molecules*, *27*(3), 730. https://doi.org/10.3390/molecules27030730.

Kumari S., Nazir F., Maheshwari C., Kaur H., Gupta R., Siddique K. H., & Khan M. I. R. (2024). Plant hormones and secondary metabolites under environmental stresses: Enlightening defence molecules. *Plant Physiology and Biochemistry*, *206*, 108238.

Lawan I., Ismail S. A., Baita A. & Baba Z. A. (2026). Phytochemical Screening and Antimicrobial Activity of *Phyllanthus Amarus* Leaves (Stone Breaker) Against Some Selected Microorganisms. *Indonesian Journal of Health Research and Development*, *4*(1), 42–51. https://doi.org/10.58723/ijhrd.v4i1.535.

Li W., Zhang X., Wang S., Gao X., & Zhang X. (2024). Research progress on extraction and detection technologies of flavonoid compounds in foods, *Foods*, *13*(4), 628. https://doi.org/10.3390/foods13040628.

Liko I., Allison T. M., Hopper J. T., & Robinson C. V. (2016). Mass spectrometry guided structural biology, *Current opinion in structural biology*, *40*, 136-144. https://doi.org/10.1016/j.sbi.2016.09.008.

Marks B. B., Nogueira M. A., & Hungria M. (2025). Microbial secondary metabolites and their use in achieving sustainable agriculture: Present achievements and future challenges, *Agronomy*, *15*(6), 1350. https://doi.org/10.3390/agronomy15061350.

Mustapha R. K., Adoum O. A. and Rabil A. K. (2022). Qualitative, Quantitative Phytochemical Screening and Antioxidant Study of Leaf and Stem bark Extracts of *Lannea microcarpa* and *Terminalia aviccinniodes*, *Bayero Journal of Pure and Applied Sciences*, *13*(1), 582-588.

Mustapha R.K., Baita A., Sani F.F., Lawan I., Kabir D. (2025). Exploring the Antimicrobial Properties of *Bryophyllum pinnatum* Leaf Through Phytochemical Investigation and a bibliometric analysis, *J.Appl.Sci. Envir. Stud*, *8*(1).1-18

Naji, E. F., Abdulfatah, H. F., & Hashim, K. S. (2024). Plant Secondary Metabolites, Their Classification and Biological Roles: A Review. Journal of University of Anbar for Pure Science, *18*(1). 106-115. https://doi.org/10.37652/juaps.2023.144549.1164.

Newman D. J., & Cragg G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019, *Journal of natural products*, *83*(3), 770-803. https://doi.org/10.1021/acs.jnatprod.9b01285.

Nicolescu A., Bunea C. I., & Mocan A. (2025). Total Flavonoid content revised: An overview of past, present, and future determinations in phytochemical analysis, *Analytical Biochemistry*, 115794. https://doi.org /10.1016/j.ab.2025.115794.

Ouahabi, S., Loukili, E.H., Elbouzidi, A., Taibi, M., Bouslamti, M.; Nafidi, H-A., Salamatullah, A.M.; Saidi, N.; Bellaouchi, R.; Addi, M., Ramdani M., Bourhia M. and Hammouti B. (2023). Pharmacological Properties of Chemically Characterized Extracts from Mastic Tree: In vitro and in silico assays. *Life*, 12, 1393; https://doi.org/10.3390/life13061393

Ozyigit I. I., Dogan I., Hocaoglu-Ozyigit A., Yalcin B., Erdogan A., Yalcin I. E., Cabi E. & Kaya Y. (2023). Production of secondary metabolites using tissue culture-based biotechnological applications, *Frontiers in Plant Science*, *14*, 1132555. https://doi.org/10.3389/fpls.2023.1132555.

Poole C. F. (2018). Jürgen H. Gross: Mass Spectrometry. A Textbook, 3rd Edn.

Pramanik J., Kumar A., Rustagi S., Katyal M., Thakur S., Bora J., Sumira M., Anchal T., Nayan T. & Slama P. (2024). A perspective review on the biosynthesis of plant-based secondary metabolites and their application as potent drugs. *Biocell*, *48*(4). https://doi.org/10.32604/biocell.2023.029031.

Rahman A., Albadrani G. M., Waraich E. A., Awan T. H., Yavaş İ., & Hussain S. (2023). Plant secondary metabolites and abiotic stress tolerance: overview and implications. Plant abiotic stress responses and tolerance mechanisms, *IntechOpen*. https://doi.org/10.5772/intechopen.111696.

Ramesh M. M., Shankar N. S., & Venkatappa A. H. (2024). Driving/Critical Factors Considered During Extraction to Obtain Bioactive Enriched Extracts, Pharmacognosy Reviews, *18*(35). 68. https://doi.org/10.5530/phrev.2024.18.7.

Sadgrove N. J., Mitaine-Offer A. C., Khumalo G., & Van Wyk B. E. (2025). An overview of the phytochemistry of medicinal bark (trunk, stem or root) from the most popular southern African species, *Phytochemistry reviews*, 1-21.

Salam U., Ullah S., Tang Z. H., Elateeq A. A., Khan Y., Khan J., Khan A. & Ali S. (2023). Plant metabolomics: an overview of the role of primary and secondary metabolites against different environmental stress factors, *Life*, *13*(3), 706. https://doi.org/10.3390/life13030706.

Sana., Aftab T., Naeem M., Jha P. K., & Prasad P. V. (2025). Production of secondary metabolites under challenging environments: understanding functions and mechanisms of signalling molecules, *Frontiers in Plant Science*, *16*, 1569014. https://doi.org/10.3389/fpls.2025.1569014.

Simsek M., & Whitney K. (2024). Examination of primary and secondary metabolites associated with a plant-based diet and their impact on human health, *Foods*, *13*(7), 1020. https://doi.org/10.3390/foods13071020.

Tang H., Wang Q., Xie H., & Li W. (2024). The function of secondary metabolites in resisting stresses in horticultural plants, *Fruit Research*, *4*(1), e021. https://doi.org/10.48130/frures-0024-0014.

Thirumurugan D., Cholarajan A., Raja S. S., & Vijayakumar R. (2018). An introductory chapter: secondary metabolites. Secondary metabolites-sources and applications, *Intechopen*, *1*, 13. https://doi.org/10.5772/intechopen.79766.

Tuhanioglu, A., & Ubeyitogullari, A. (2025). Recent Advances in Eco-Friendly Supercritical Carbon Dioxide Fractionation Techniques for Food Applications, *Comprehensive Reviews in Food Science and Food Safety*, *24*(4), e70240. https://doi.org/10.1111/1541-4337.70240.

Usman M., Nakagawa M., & Cheng S. (2023). Emerging trends in green extraction techniques for bioactive natural products. *Processes*, *11*(12), 3444. https://doi.org/10.3390/pr11123444.

Wang Y., Ma Y., Tao L., Zhang X., Hao F., Zhao S., Han L. & Bai C. (2022). Recent advances in separation and analysis of saponins in natural products, *Separations*, 9(7), 163. https://doi.org/10.3390/separations9070163.

Wolfender J. L., Nuzillard J. M., Van Der Hooft J. J., Renault J. H., & Bertrand S. (2018). Accelerating metabolite identification in natural product research: toward an ideal combination of liquid chromatography–high-resolution tandem mass spectrometry and NMR profiling, in silico databases, and chemometrics, *Analytical Chemistry*, *91*(1), 704-742.

Xie M., Wang J., Wang F., Wang J., Yan Y., Feng K., & Chen B. (2025). A review of genomic, transcriptomic, and proteomic applications in edible fungi biology: current status and future directions. *Journal of Fungi*, *11*(6), 422.

Xu Z., Ullah N., Duan Y., Hou Z., Liu A., & Xu L. (2023). Plant secondary metabolites and their effects on environmental adaptation based on functional genomics, *Frontiers in Genetics*, *14*, 1211639. https://doi.org/10.3389/fgene.2023.1211639.

Yang L., Wen K. S., Ruan X., Zhao Y. X., Wei F., & Wang Q. (2018). Response of plant secondary metabolites to environmental factors, *Molecules*, *23*(4), 762. https://doi.org/10.3390/molecules23040762.

Yang Y., Wang Y., Zeng W., Tian J., Zhao X., Han J., Dezhi H & Gu D. (2020). A strategy based on liquid-liquid-refining extraction and high-speed counter-current chromatography for the bioassay-guided separation of active compound from *Taraxacum mongolicum*. *Journal of Chromatography A*, 1614, 460727. doi.org/10.1016/j.chroma.2019.460727.

Zhan X., Chen Z., Chen R., & Shen C. (2022). Environmental and genetic factors involved in plant protection-associated secondary metabolite biosynthesis pathways. *Frontiers in Plant Science*, *13*, 877304. https://doi.org/10.3389/fpls.2022.877304.

Zhang Q. F., Wang W. J., Cai S., & Zheng G. D. (2024). Plant secondary metabolites in food: Absorption, metabolism and effects on glucolipid metabolism, *Frontiers in Nutrition*, *11*, 1382677. https://doi.org/10.3389/fnut.2024.1382677.

Zhao Y., Liu G., Yang F., Liang Y., Gao Q., Xiang C., Li X., Yang R., Zhang G., Jiang H., & Yang S. (2023). Multilayered regulation of secondary metabolism in medicinal plants, *Molecular Horticulture*, *3*(1), 11. https://doi.org/10.1186/s43897-023-00059-y.