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Analytical Techniques in the Phytochemical Characterization of Sea Buckthorn (*Hippophae* spp.): Advances and Challenges

Dr. S. Shyamkiran Singh

Assistant Professor, Dept. of Chemistry, Waikhom Mani Girls' College, Thoubal Okram, Manipur.

Email ID: sorokhaibams@gmail.com

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ABSTRACT

Sea buckthorn (*Hippophae* spp.) is a deciduous shrub of the family Elaeagnaceae, renowned for its rich repository of bioactive compounds, including flavonoids, carotenoids, vitamins, sterols, and polyphenols. The burgeoning interest in sea buckthorn across food, nutraceutical, and pharmaceutical industries necessitates robust and precise analytical approaches to characterize its phytochemicals. This paper presents a comprehensive review of contemporary analytical techniques used in the phytochemical profiling of sea buckthorn, including spectrophotometry, chromatography (HPLC, GC-MS, LC-MS/MS), NMR spectroscopy, and emerging methods such as metabolomics and hyphenated techniques. Advances in sample extraction methods, quantification techniques, and structure elucidation are discussed. Challenges such as complex matrices, compound instability, and variability among species and plant parts are also examined. This paper highlights the importance of methodological standardization and the integration of multi-platform techniques to overcome analytical limitations and enhance the understanding of sea buckthorn's phytochemical landscape.

Introduction

Sea buckthorn (*Hippophae* spp.), a deciduous, thorny shrub native to Europe and Asia, particularly the Himalayan region, has emerged as a plant of considerable therapeutic, nutritional, and commercial interest. Belonging to the family Elaeagnaceae, Sea buckthorn is valued for its exceptional adaptability to harsh climatic and soil conditions, particularly in arid, cold, and high-altitude environments. The various species of *Hippophae*—notably *H. rhamnoides*, *H. salicifolia*, *H. tibetana*, and *H. neurocarpa*—are widely distributed across countries like China, India, Nepal, Russia, and Mongolia. Traditionally, Sea buckthorn has been employed in diverse systems of traditional medicine, including Tibetan, Mongolian, and Chinese medicine, for treating ailments ranging from skin diseases and gastrointestinal disorders to respiratory conditions and inflammation.

The plant is renowned for its rich phytochemical composition. Its berries, seeds, and leaves are loaded with biologically active constituents such as flavonoids (quercetin, kaempferol, isorhamnetin), carotenoids (β-carotene, lycopene, zeaxanthin), sterols, phenolic acids (gallic acid, ferulic acid), tocopherols (vitamin E), ascorbic acid (vitamin C), amino acids, organic acids, and unsaturated fatty acids, including palmitoleic acid. These compounds are linked to a wide range of pharmacological properties, including antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, antimicrobial, and anticancer activities. As a result, sea buckthorn has garnered significant interest in the food, pharmaceutical, nutraceutical, and cosmetic industries.

Given the wide array of structurally diverse compounds present in sea buckthorn, the phytochemical characterization of this plant poses several scientific challenges. Accurate and reproducible identification and quantification of its constituents are crucial not only for understanding its therapeutic efficacy but also for ensuring product standardization, quality control, and regulatory compliance. The complexity of the plant matrix—rich in sugars, acids, lipids, pigments, and phenolics—necessitates advanced analytical tools that can selectively and sensitively detect these compounds, often at trace levels.

Over the past two decades, there has been a notable evolution in the analytical techniques used for phytochemical studies. Traditional approaches such as spectrophotometry and thin-layer chromatography (TLC) have gradually been augmented or replaced by more sophisticated and sensitive platforms, including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and nuclear magnetic resonance (NMR) spectroscopy. Recent advances also include hyphenated and multidimensional

techniques like UHPLC-QTOF-MS, metabolomics-based profiling, and chemometric modeling, which provide high-resolution insights into the phytochemical fingerprints of sea buckthorn.

Despite these advancements, several challenges remain, including variability among species, influence of environmental conditions on phytochemical expression, instability of certain bioactives (e.g., ascorbic acid, carotenoids), and the absence of standardized protocols for sample preparation, extraction, and analysis. Additionally, the lack of commercially available reference standards for many sea buckthorn-specific compounds limits the precision of quantification and compound identification.

This paper aims to comprehensively review the current state of analytical methodologies used in the phytochemical characterization of sea buckthorn. It explores the progress made in extraction techniques, spectrometric and chromatographic tools, and advanced analytical platforms, while critically examining the challenges and opportunities for future research and development in this promising field.

2. Extraction Techniques: Foundation of Phytochemical Analysis

The extraction of phytochemicals is a critical first step in the process of plant-based analytical research. Accurate and efficient extraction techniques are essential to obtain a comprehensive phytochemical profile, especially in complex matrices such as sea buckthorn (*Hippophae* spp.), which contains a wide spectrum of bioactive compounds ranging from polar phenolics and flavonoids to non-polar lipids, carotenoids, and sterols. The selection of an appropriate extraction method not only affects the yield and purity of phytoconstituents but also determines the stability, reproducibility, and accuracy of downstream analytical processes. Several conventional and advanced extraction techniques are employed based on the target compound class, solvent compatibility, thermal stability, and the nature of plant material (berries, seeds, leaves, or bark).

2.1 Conventional Extraction Methods

2.1.1 Maceration and Percolation

These are some of the oldest extraction techniques used in herbal pharmacognosy. In maceration, plant material is soaked in solvents such as ethanol, methanol, or water at room temperature for extended durations, allowing the diffusion of phytochemicals into the solvent. Percolation involves a continuous flow of solvent through the powdered plant material, enhancing contact and extraction efficiency.

Advantages: Simple and cost-effective; suitable for thermolabile compounds.

Disadvantages: Time-consuming; large volumes of solvents required; limited efficiency for non-polar or poorly soluble compounds.

2.1.2 Soxhlet Extraction

Soxhlet extraction is widely used for extracting non-volatile and semi-volatile compounds such as fatty acids, sterols, and lipophilic antioxidants from sea buckthorn seeds and pulp. This technique involves continuous refluxing of solvent, usually hexane or petroleum ether, over the sample, allowing repeated percolation.

Advantages: Efficient for exhaustive extraction; well-suited for oil and lipid fractions.

Disadvantages: High temperature may degrade sensitive compounds like vitamin C and carotenoids; solvent-intensive and time-consuming.

2.2 Modern Extraction Techniques

2.2.1 Ultrasound-Assisted Extraction (UAE)

UAE is a non-thermal technique that uses ultrasonic waves (20–100 kHz) to disrupt plant cell walls, facilitating solvent penetration and compound diffusion. This method is highly effective for extracting flavonoids, phenolic acids, and tannins from sea buckthorn leaves and fruits.

Advantages: Fast, low solvent consumption, suitable for thermolabile compounds; eco-friendly.

Limitations: Optimization required for ultrasound intensity and duration to avoid compound degradation.

2.2.2 Microwave-Assisted Extraction (MAE)

MAE employs microwave energy to heat the solvent and plant matrix, enhancing mass transfer and reducing extraction time. It is particularly useful for polar bioactives like polyphenols and vitamins.

Advantages: Rapid and efficient; minimal solvent use; retains bioactivity of most compounds.

Challenges: Risk of compound degradation due to localized heating; not ideal for thermosensitive carotenoids unless temperature is tightly controlled.

2.2.3 Supercritical Fluid Extraction (SFE)

SFE, especially using supercritical carbon dioxide (SC-CO_2), is an advanced technique ideal for lipophilic compounds such as carotenoids, tocopherols, and fatty acids in sea buckthorn seed and pulp oils. Modifiers like ethanol or methanol can be added to enhance extraction of slightly polar compounds.

Advantages: Non-toxic, selective, and environmentally friendly; no solvent residues; preserves compound integrity.

Drawbacks: High operational cost; requires specialized equipment; limited use for polar compounds.

2.2.4 Pressurized Liquid Extraction (PLE) / Accelerated Solvent Extraction (ASE)

PLE involves using solvents under high pressure and elevated temperatures to improve extraction efficiency. It is suitable for both polar and non-polar compounds, making it ideal for a full-spectrum phytochemical analysis of sea buckthorn.

Advantages: Rapid and efficient; low solvent consumption; applicable to a wide range of solvents and compound types.

Disadvantages: Equipment cost; potential degradation of heat-sensitive compounds.

2.3 Factors Influencing Extraction Efficiency

i) Solvent Type and Polarity: The choice of solvent affects the solubility of specific phytochemicals. For example, methanol and ethanol are commonly used for extracting phenolics and flavonoids, while hexane is preferred for lipids and carotenoids.

ii) Particle Size: Finely ground plant material enhances the surface area for solvent penetration but may also increase unwanted particulate matter in extracts.

iii) Temperature and Time: Higher temperatures may accelerate extraction but also risk degradation of heat-sensitive compounds like ascorbic acid and β-carotene.

iv) Solvent-to-Sample Ratio: An optimal ratio is essential to maximize yield without excessive dilution.

v) Plant Part and Matrix: Different parts of sea buckthorn (berries, seeds, leaves, bark) have variable matrix compositions, affecting the choice of extraction method.

2.4 Toward Green Extraction Technologies

In line with sustainability goals, recent attention has turned to **green extraction techniques**, which focus on reducing environmental impact, minimizing solvent use, and preserving energy. Methods such as enzyme-assisted extraction (EAE), deep eutectic solvents (DES), and subcritical water extraction (SWE) are under preliminary investigation for their potential application to sea buckthorn.

In conclusion, extraction is a foundational step in the phytochemical characterization of sea buckthorn. The choice of method must be tailored based on the chemical nature of target compounds and analytical objectives. Integration of modern, green, and selective extraction methods with analytical tools can significantly enhance the accuracy, reproducibility, and comprehensiveness of sea buckthorn phytochemical profiling.

3. Chromatographic Techniques

Chromatographic techniques are pivotal in the phytochemical characterization of sea buckthorn (*Hippophae* spp.) due to their ability to separate, identify, and quantify complex mixtures of bioactive compounds. These techniques offer high sensitivity, resolution, and reproducibility, enabling researchers to analyse a wide array of metabolites, including flavonoids, carotenoids, organic acids, sterols, and fatty acids. Among these, **High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Thin-Layer Chromatography (TLC),** and **High-Performance Thin-Layer Chromatography (HPTLC)** are the most extensively used for sea buckthorn research. Each technique is selected based on the nature of the target compound—its polarity, volatility, thermal stability, and molecular weight.

3.1 High-Performance Liquid Chromatography (HPLC)

HPLC is the gold standard for the qualitative and quantitative analysis of thermolabile and non-volatile phytochemicals in sea buckthorn. It is especially suitable for analysing polar to moderately polar compounds such as flavonoids, phenolic acids, vitamins (e.g., ascorbic acid, tocopherols), and organic acids.

3.1.1 Reversed-Phase HPLC (RP-HPLC)

In RP-HPLC, a non-polar stationary phase (typically C18) is combined with a polar mobile phase (usually water with methanol, acetonitrile, or their mixtures). This configuration is particularly effective for separating compounds like quercetin, kaempferol, isorhamnetin, gallic acid, and ferulic acid.

- **Application**: Quantification of polyphenols in sea buckthorn juice and methanolic leaf extracts.
- **Detection Modes**: UV-Vis, Diode Array Detector (DAD), and Fluorescence Detector (FLD).

3.1.2 HPLC-DAD

The Diode Array Detector allows simultaneous acquisition of full UV-Vis spectra across a range of wavelengths, improving compound identification based on spectral matching.

Example: Detection of multiple flavonoids in a single run, distinguishing them by both retention time and spectral fingerprint.

3.1.3 HPLC-MS/MS

Tandem mass spectrometry (MS/MS) enhances both the sensitivity and specificity of HPLC. It enables the simultaneous quantification and structure elucidation of compounds, even at trace levels.

Example: Identification of isorhamnetin derivatives and glycosylated flavonoids in sea buckthorn extracts.

Advantage: Capable of resolving co-eluting compounds that may not be differentiated by UV detection alone.

3.1.4 Ultra-High-Performance Liquid Chromatography (UHPLC)

UHPLC offers enhanced resolution and faster run times due to smaller particle sizes and higher operating pressures. It is ideal for complex matrices like sea buckthorn where multiple phytochemicals coexist in overlapping concentration ranges.

3.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is an indispensable tool for analysing volatile and semi-volatile components, particularly lipophilic substances like essential oils, fatty acids, and sterols found in sea buckthorn seeds and fruit pulp oils.

Sample Preparation: Non-volatile compounds such as fatty acids are often converted into their volatile derivatives (e.g., fatty acid methyl esters, FAMEs) prior to analysis.

Carrier Gases: Commonly helium or nitrogen.

Application:

- Identification of unsaturated fatty acids (palmitoleic, linoleic, linolenic).
- Profiling of phytosterols like β-sitosterol and campesterol.
- Analysis of tocopherols and squalene.

3.2.1 GC-FID (Flame Ionization Detector)

Though less specific than GC-MS, GC-FID offers robust quantification and is frequently used in combination with GC-MS for fatty acid and oil profiling.

3.3 Thin Layer Chromatography (TLC) and High-Performance TLC (HPTLC)

TLC and HPTLC remain valuable tools for the preliminary screening and fingerprinting of phytoconstituents. Though considered semi-quantitative, they are cost-effective and allow high-throughput processing of multiple samples simultaneously.

3.3.1 TLC

Principle: Compounds are separated on a silica gel-coated plate using a suitable solvent system and visualized under UV light or after derivatization.

Application: Rapid screening of flavonoids and anthocyanins from berry extracts.

3.3.2 HPTLC

HPTLC provides enhanced resolution and reproducibility compared to classical TLC. Coupled with densitometry or image analysis software, it allows semi-quantitative estimation of compounds.

Example: Densitometric quantification of quercetin in methanolic extracts of sea buckthorn leaves.

Derivatization Agents: Natural products–polyethylene glycol (NP/PEG) for flavonoids, vanillin–sulphuric acid for steroids.

3.4 Emerging Chromatographic Platforms

3.4.1 LC-QTOF-MS (Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry)

This is a hybrid technique combining the separation power of LC with the accurate mass determination capability of QTOF-MS. It allows comprehensive metabolite profiling, making it particularly suitable for untargeted metabolomics studies in sea buckthorn.

Advantage: Simultaneous detection of known and unknown metabolites; structural elucidation using fragmentation patterns.

3.4.2 LC-ESI-MS (Electrospray Ionization)

Electrospray Ionization coupled with HPLC provides soft ionization, ideal for analyzing glycosides and other polar phytochemicals without thermal degradation.

Application: Identification of flavonoid glycosides and phenolic esters.

3.5 Method Validation and Standardization

For chromatographic data to be reliable and reproducible, proper method validation is crucial. Parameters such as linearity, accuracy, precision, detection and quantification limits (LOD & LOQ), recovery rate, and robustness must be assessed.

Use of Standards: Pure reference standards like quercetin, β-carotene, and gallic acid are used for calibration.

Matrix Effect: Especially in complex matrices like sea buckthorn juice or oil, co-extracted substances may affect analyte detection, necessitating matrix-matched calibration.

Chromatographic techniques form the backbone of phytochemical characterization in sea buckthorn. While traditional HPLC and TLC methods continue to serve important roles in qualitative and quantitative assessments, the incorporation of advanced methods like HPLC-MS/MS, UHPLC-QTOF-MS, and LC-ESI-MS has significantly enhanced our ability to detect and differentiate complex metabolite structures. Future advancements may lie in integrating chromatographic techniques with

omics platforms and chemometric tools to provide deeper insights into the phytochemical diversity and pharmacological potential of this remarkable plant.

4. Spectroscopic Techniques

Spectroscopic techniques play a pivotal role in the phytochemical characterization of complex plant matrices such as *Hippophae* spp. (sea buckthorn). These techniques are crucial for both qualitative and quantitative analysis, enabling researchers to identify functional groups, detect chromophoric compounds, analyse molecular structures, and verify compound purity. The major spectroscopic techniques used in sea buckthorn research include UV-Visible spectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. In recent years, advanced spectroscopic platforms such as Raman spectroscopy, near-infrared (NIR) spectroscopy, and fluorescence spectroscopy have also gained momentum for rapid, non-destructive analysis and fingerprinting.

4.1 UV-Visible Spectrophotometry

UV-Visible (UV-Vis) spectrophotometry is one of the most commonly used techniques for the quantification of total phenolic content (TPC), total flavonoid content (TFC), and carotenoids in sea buckthorn extracts. This method relies on the absorbance of ultraviolet or visible light by chromophoric groups within phytochemicals, typically in the 200–700 nm wavelength range.

Applications in Sea Buckthorn:

- **Folin–Ciocalteu Method**: Used to estimate TPC based on the reduction of the Folin–Ciocalteu reagent by phenolics, leading to a blue complex measured at 765 nm.
- **Aluminum Chloride Colorimetric Assay**: Used for TFC quantification. Flavonoids form complexes with $AlCl_3$, detectable at 415–430 nm.
- **Carotenoid Estimation**: Total carotenoid content is often assessed by measuring absorbance at 450 nm in sea buckthorn oil and pulp extracts.

Advantages:

- Simple, rapid, and cost-effective.

- Requires minimal sample preparation.
- Ideal for routine screening and quantification.

Limitations:

- Lacks compound specificity—multiple compounds may contribute to a single absorbance peak.
- Interference from co-extracted substances can affect accuracy.
- Cannot differentiate between isomers or closely related compounds.

4.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy provides detailed information about the molecular vibrations of chemical bonds, thereby allowing identification of functional groups present in complex plant extracts. It is especially useful for fingerprinting and quality control of sea buckthorn formulations.

Principle:

Infrared radiation is passed through a sample, and the absorption spectrum is recorded, revealing characteristic vibrational modes (stretching, bending) of bonds like –OH, –COOH, –C=O, $–CH_3$, etc.

Applications in Sea Buckthorn:

- Differentiation of lipids, esters, phenolic acids, and polysaccharides in whole plant extracts.
- Authentication of sea buckthorn oil by detecting adulterants (e.g., soybean or sunflower oil).
- Structural comparison of extracts from different species or regions.

Key Peaks:

- Broad band at 3300–3500 cm^{-1}: O–H stretching (alcohols, phenols).
- Peak near 1700 cm^{-1}: C=O stretching (carboxylic acids, esters).
- Peaks at 1600 and 1500 cm^{-1}: Aromatic ring vibrations (phenolics and flavonoids).
- Fingerprint region (600–1500 cm^{-1}): Compound-specific vibrations.

Advantages:

- Rapid and non-destructive.
- Requires little to no sample preparation.
- Useful for qualitative comparisons and adulteration detection.

Limitations:

- Overlapping bands in complex mixtures reduce specificity.
- Less effective for quantitative analysis compared to chromatographic techniques.

4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is the most powerful tool for structure elucidation of unknown organic compounds. It provides atomic-level insights into the molecular framework by detecting the magnetic environments of specific nuclei, primarily hydrogen (^{1}H) and carbon (^{13}C).

Principle:

NMR exploits the magnetic properties of nuclei when placed in a strong magnetic field and exposed to radiofrequency pulses. Different chemical environments yield different chemical shifts, revealing structural and connectivity information.

Applications in Sea Buckthorn:

- Identification and confirmation of flavonoids (quercetin, kaempferol), phenolic acids (gallic, caffeic), sterols, and sugars.
- Structural elucidation of novel glycosides and lipid conjugates.
- Differentiation between isomers and tautomers that cannot be distinguished by mass spectrometry.

Types of NMR Techniques:

- **^{1}H-NMR**: Most commonly used for detecting hydrogen environments in phenolics, lipids, and terpenoids.

- **^{13}C-NMR**: Used for elucidating carbon skeletons.
- **2D-NMR (COSY, HSQC, HMBC)**: Provides detailed connectivity maps, especially for glycosylated compounds or complex mixtures.

Advantages:

- Provides definitive structural identification without derivatization.
- Non-destructive and requires minimal sample purification.
- Excellent for distinguishing between closely related compounds and stereoisomers.

Limitations:

- Requires expensive instrumentation and trained operators.
- Large sample volume and high purity required for best results.
- Less sensitive than MS for trace analysis.

4.4 Emerging Spectroscopic Tools

4.4.1 Raman Spectroscopy

Raman spectroscopy, based on inelastic scattering of monochromatic light (usually from a laser), is increasingly being used for fingerprinting and structural analysis.

Application: Rapid authentication of sea buckthorn juice and seed oil.

Advantage: Non-invasive and applicable in situ.

4.4.2 Near-Infrared (NIR) Spectroscopy

NIR is used for non-destructive quantification of major bioactive classes and moisture content.

Application: Quality control of commercial sea buckthorn products.

Limitation: Requires multivariate calibration models for accurate interpretation.

4.4.3 Fluorescence Spectroscopy

Certain flavonoids and vitamins exhibit strong fluorescence under UV light, making this technique suitable for sensitive detection and quantification.

Spectroscopic techniques complement chromatographic tools in the comprehensive phytochemical characterization of sea buckthorn. UV-Vis spectrophotometry and FTIR serve as rapid screening tools, while NMR provides in-depth structural information that is unattainable by most other methods. The integration of traditional spectroscopic techniques with advanced tools like Raman and NIR offers exciting possibilities for non-destructive, high-throughput analysis and quality control. As the demand for sea buckthorn-based nutraceuticals grows, spectroscopic methods will remain indispensable for ensuring product authenticity, safety, and efficacy.

5. Hyphenated and Advanced Analytical Techniques

Hyphenated analytical techniques represent the convergence of separation science (primarily chromatography) with spectroscopic detection systems to create powerful platforms for comprehensive phytochemical analysis. These techniques offer enhanced resolution, sensitivity, specificity, and structural insight into complex biological matrices. In the context of *Hippophae* spp. (sea buckthorn), where a broad spectrum of structurally diverse phytoconstituents are present—ranging from polar polyphenols to non-polar carotenoids and sterols—hyphenated techniques like LC-MS/MS, GC-MS, and UHPLC-QTOF-MS have emerged as indispensable tools for qualitative and quantitative analysis. Furthermore, the application of metabolomics, chemometrics, and multi-dimensional data integration is revolutionizing the way phytochemical profiles are interpreted.

5.1 Liquid Chromatography–Mass Spectrometry (LC-MS/MS)

LC-MS/MS combines the separating power of liquid chromatography with the detection and structural elucidation capabilities of tandem mass spectrometry. This method is exceptionally suited for profiling phenolic acids, flavonoid glycosides, vitamins, and amino acids in sea buckthorn extracts.

Principle:

Compounds are first separated via HPLC or UHPLC and then ionized (commonly by electrospray ionization, ESI) before entering the mass spectrometer. In tandem MS (MS/MS), selected precursor ions

are fragmented, and the resulting product ions are analysed, enabling compound identification and quantification.

Applications in Sea Buckthorn:

- Detection and quantification of quercetin, isorhamnetin, kaempferol, and their glycosides.
- Vitamin profiling (ascorbic acid, tocopherols).
- Identification of organic acids such as citric, malic, and succinic acids.

Advantages:

- High specificity and sensitivity.
- Simultaneous analysis of multiple compounds in a single run.
- Provides structural information through fragmentation patterns.

Limitations:

- Requires expert data interpretation.
- Matrix effects may interfere with ionization efficiency.
- Expensive instrumentation and maintenance.

5.2 Ultra-High Performance Liquid Chromatography Coupled with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS)

UHPLC-QTOF-MS represents a cutting-edge platform that allows ultra-fast separation of compounds and accurate mass determination. The high resolution and mass accuracy make it especially valuable for non-targeted metabolomics studies.

Key Features:

- **UHPLC** enables efficient separation of closely related compounds within short run times.

- **QTOF-MS** measures exact mass-to-charge ratios, helping deduce elemental compositions and molecular structures.

Application in Sea Buckthorn:

- Comprehensive metabolite fingerprinting of berries, seeds, and leaves.
- Identification of novel or minor secondary metabolites.
- Differentiation of species and cultivars based on metabolite profiles.

Advantages:

- Accurate mass measurement aids in identifying unknown compounds.
- Suitable for both targeted and untargeted analysis.
- Enables retrospective data analysis.

5.3 Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS is especially useful for the analysis of volatile and semi-volatile compounds, such as fatty acids, esters, terpenes, and sterols. Sea buckthorn seed and pulp oils are commonly analysed using GC-MS following derivatization to enhance volatility.

Application:

- Profiling of saturated and unsaturated fatty acids (e.g., palmitoleic acid, linoleic acid).
- Detection of sterols (β-sitosterol, stigmasterol), alkanes, and tocopherols.
- Quality control of essential oils and detection of adulterants.

Derivatization:

- Fatty acids are often converted to fatty acid methyl esters (FAMEs) using methanol and acid/base catalysis.

Advantages:

- High separation efficiency and reproducibility.
- Excellent for compounds with high volatility and thermal stability.

Limitations:

- Not suitable for thermolabile or non-volatile compounds without derivatization.

5.4 Capillary Electrophoresis (CE)

CE is a high-resolution separation technique based on the differential migration of analytes under an electric field in a capillary tube. It is particularly effective for charged and low-molecular-weight compounds.

Applications in Sea Buckthorn:

- Analysis of organic acids, amino acids, and phenolic acids.
- Quality assessment of juices and functional beverages.

Advantages:

- High efficiency and low sample/solvent requirements.
- Short analysis time and minimal waste.

Challenges:

- Limited sensitivity compared to LC-MS/MS.
- Requires careful optimization of buffer systems and voltage parameters.

5.5 Metabolomics and Chemometric Tools

Metabolomics involves the comprehensive analysis of all metabolites in a biological system. In sea buckthorn research, it enables detailed metabolic profiling to study biochemical diversity, chemotaxonomy, and responses to environmental factors.

Untargeted Metabolomics:

- Utilizes LC-MS, GC-MS, or NMR to detect all measurable metabolites without pre-selection.
- Useful for identifying biomarkers related to bioactivity or geographical origin.

Targeted Metabolomics:

- Focuses on a predefined set of known metabolites using validated methods.
- Suitable for quality control and product standardization.

Chemometrics:

Multivariate data analysis tools like Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and Hierarchical Clustering are used to interpret complex datasets generated from hyphenated instruments.

Applications:

- Differentiation of sea buckthorn species or cultivars.
- Identification of metabolite markers linked to antioxidant activity or maturity stage.
- Quality assurance of commercial formulations.

5.6 Hyphenated Techniques in Quality Control and Drug Discovery

Hyphenated techniques are increasingly being applied not only for phytochemical analysis but also in:

- **Bioactivity-guided fractionation:** Linking specific bioactivities (e.g., anti-inflammatory, antioxidant) to identified compounds.
- **Standardization of functional foods and herbal medicines.**
- **Authentication of raw materials and detection of adulteration.**

Hyphenated and advanced analytical techniques have revolutionized phytochemical characterization by offering unparalleled precision, speed, and insight into plant metabolomes. In sea buckthorn research,

these tools allow the simultaneous identification, quantification, and structural elucidation of numerous bioactive compounds, many of which exist in trace amounts or complex mixtures. The synergy of chromatographic separation with high-resolution detection platforms, supported by metabolomics and chemometric analysis, holds immense promise for future research and commercial development of sea buckthorn-based products. As analytical technologies continue to evolve, they will further bridge the gap between phytochemistry, pharmacology, and therapeutic innovation.

6. Challenges in Phytochemical Analysis of Sea Buckthorn

Despite the significant progress in analytical methodologies for characterizing the phytochemical constituents of *Hippophae* spp. (sea buckthorn), numerous challenges persist that hinder the complete and standardized understanding of its bioactive profile. These challenges stem from the inherent complexity of the plant matrix, variability in chemical composition, limitations in current methodologies, and gaps in standardization. Accurate phytochemical analysis of sea buckthorn is critical not only for scientific understanding but also for ensuring the safety, efficacy, and consistency of its use in food, nutraceutical, cosmetic, and pharmaceutical products.

6.1 Complexity and Diversity of the Phytochemical Matrix

Sea buckthorn contains an exceptionally rich and diverse array of secondary metabolites, including:

- Flavonoids and their glycosides (e.g., quercetin, kaempferol, isorhamnetin),
- Phenolic acids (e.g., gallic acid, caffeic acid),
- Lipophilic constituents (e.g., carotenoids, tocopherols, fatty acids),
- Organic acids, amino acids, sugars, and sterols.

This chemical diversity, spanning a wide range of polarity, volatility, and thermal stability, makes it challenging to extract, separate, and detect all relevant compounds with a single analytical method. No single solvent or extraction method can universally recover all bioactives, and many compounds may co-elute or interfere during chromatographic separation. Moreover, complex interactions among phytochemicals—such as binding to proteins or polysaccharides—can obscure accurate quantification.

6.2 Lack of Standardized Protocols

One of the most pressing challenges is the absence of universally accepted, standardized protocols for sample collection, preparation, extraction, and analysis.

Sample Preparation Issues:

- Variations in drying methods (sun drying, freeze-drying, oven drying) significantly affect compound stability.
- The use of different solvents (methanol, ethanol, water, acetone) and extraction conditions (temperature, pH, duration) leads to inconsistent results across studies.

Analytical Method Variability:

- Studies often use different HPLC columns, mobile phases, and detection wavelengths, making comparisons difficult.
- Some methods lack full validation (linearity, accuracy, precision), reducing reproducibility.

As a result, cross-comparative phytochemical datasets across different laboratories or even within the same institution become unreliable, impeding meta-analyses and regulatory standardization.

6.3 Inadequate Reference Standards

Phytochemical quantification relies heavily on the availability of authentic reference standards. However, many bioactive constituents in sea buckthorn—especially glycosylated flavonoids, oligomeric phenolics, or minor carotenoids—are not commercially available in pure form.

Consequences:

- Approximate quantification is done by substituting available standards (e.g., using quercetin for all flavonols).
- Structural misidentification can occur, especially in complex MS or NMR spectra.
- Compounds with no standards remain “unknowns,” despite their possible bioactivity.

The synthesis or isolation of these standards is time-consuming, expensive, and often technically difficult, especially for unstable or trace-level compounds.

6.4 Matrix Effects and Analytical Interference

The presence of complex and heterogeneous components—such as pigments, oils, sugars, and polysaccharides—can interfere with detection systems. This is particularly problematic in sea buckthorn juices and oils.

- **In LC-MS/MS**: Ion suppression or enhancement from co-eluting compounds alters signal intensity.
- **In UV-Vis spectroscopy**: Overlapping absorption bands lead to inaccurate total phenolic or flavonoid estimations.
- **In NMR**: Signal broadening or obscuring occurs due to high viscosity or overlapping signals.

Such matrix effects necessitate extensive sample clean-up (e.g., solid-phase extraction), internal standards, and matrix-matched calibration, which are often neglected in many studies.

6.5 Chemical Instability of Certain Compounds

Sea buckthorn is rich in chemically labile compounds that are prone to degradation under light, heat, oxygen, or pH changes.

Notable Examples:

- **Ascorbic acid** degrades rapidly in aqueous and alkaline environments.
- **β-carotene and other carotenoids** are highly susceptible to oxidative and thermal degradation.
- **Flavonoid glycosides** may undergo hydrolysis or structural rearrangement during sample processing.

Without proper stabilization measures (e.g., antioxidants, low-temperature handling, inert atmosphere), phytochemical profiles may not reflect the actual native composition of the plant.

6.6 Seasonal, Geographical, and Genetic Variability

Phytochemical content in sea buckthorn varies significantly depending on species, cultivar, plant part, growth stage, geography, and environmental factors.

- **Species Differences**: *H. rhamnoides* and *H. salicifolia* differ in their flavonoid and fatty acid profiles.
- **Geographical Origin**: Berries from cold desert regions (e.g., Ladakh, Tibet) may have higher antioxidant content due to UV stress.
- **Seasonal Effects**: Harvest time affects the accumulation of vitamins, acids, and sugars.

This natural variability complicates data interpretation and hinders the establishment of universal phytochemical "standards." For commercial applications, it also affects quality assurance and batch-to-batch consistency.

6.7 Challenges in Data Integration and Interpretation

Modern hyphenated techniques such as LC-MS, GC-MS, and NMR generate large volumes of high-dimensional data. Interpreting this data requires:

- Specialized software and bioinformatics tools.
- Skilled analysts trained in spectral deconvolution and chemometric analysis.
- Sophisticated databases for compound annotation (e.g., METLIN, Mass Bank).

However, many phytochemists lack access to these computational resources or training, leading to underutilization of rich datasets and potential misidentification of compounds.

6.8 Regulatory and Quality Control Barriers

As the popularity of sea buckthorn grows in nutraceuticals and herbal therapeutics, regulatory agencies demand:

- Precise quantification of bioactives.
- Evidence of safety, stability, and efficacy.
- Documentation of Good Manufacturing Practices (GMP).

Without standardized analytical markers or validated quality control protocols, regulatory approval becomes difficult. Adulteration and mislabeling of sea buckthorn products—especially oils—are also growing concerns.

The phytochemical characterization of sea buckthorn is fraught with analytical and technical challenges that must be addressed through methodological refinement, standardization, and interdisciplinary collaboration. Key solutions include the development of validated protocols, availability of authentic reference standards, use of internal standards, deployment of green extraction technologies, and adoption of chemometric tools for data interpretation. Overcoming these challenges will ensure more accurate, reproducible, and comprehensive profiling of sea buckthorn, unlocking its full potential in therapeutic, nutritional, and commercial applications.

7. Future Perspectives and Recommendations

As interest in *Hippophae* spp. continues to grow globally for its nutritional and medicinal potential, future research must focus on integrating advanced analytical platforms with standardized methodologies to achieve comprehensive, reproducible phytochemical profiling. The development of unified protocols for sample preparation, extraction, and compound quantification is critical for enabling inter-laboratory comparison and regulatory standardization. Investment in the isolation, synthesis, and commercial availability of pure reference compounds—especially for flavonoid glycosides and carotenoid derivatives—is strongly recommended.

Emerging technologies such as metabolomics, artificial intelligence (AI)-assisted spectral interpretation, miniaturized biosensors, and blockchain for traceability could revolutionize the way sea buckthorn phytochemistry is studied and applied. Multi-omics integration combining genomics, transcriptomics, and metabolomics will help uncover biosynthetic pathways and metabolic regulation under various environmental conditions.

From an industrial standpoint, establishing chemotypic libraries of different sea buckthorn cultivars and their bioactive profiles would aid in cultivar selection and quality assurance for specific applications in pharmaceuticals, cosmetics, and functional foods.

In summary, a multidisciplinary and collaborative approach—combining analytical chemistry, biotechnology, computational sciences, and regulatory policy—is essential to fully harness the

therapeutic potential of sea buckthorn and ensure the development of high-quality, evidence-based products.

8. Conclusion

Sea buckthorn (*Hippophae* spp.) is gaining global recognition in the nutraceutical, pharmaceutical, and functional food industries due to its diverse bioactive compounds, including flavonoids, carotenoids, phenolic acids, vitamins, and fatty acids. These compounds give the plant wide-ranging therapeutic properties, but accurately characterizing its complex phytochemical matrix remains a challenge. Efficient extraction methods such as ultrasound-assisted, supercritical fluid, and microwave-assisted techniques are now favored over traditional approaches for better yield and sustainability. Chromatographic techniques like HPLC, UHPLC, and GC-MS, combined with spectroscopic tools such as UV-Vis, FTIR, and NMR, are essential for qualitative and quantitative analysis. Advanced platforms like LC-MS/MS and UHPLC-QTOF-MS, integrated with metabolomics and chemometrics, provide deeper insights into species variability and metabolic profiles.

Despite technological progress, challenges such as lack of standardized protocols, limited reference compounds, complex matrix interferences, and phytochemical instability persist. Addressing these issues requires global collaboration in method validation and data sharing. Future advancements may come from combining modern analytical tools with AI-driven data interpretation, blockchain-enabled traceability, and portable biosensors, enhancing reliability and supporting regulatory standards. Ultimately, the successful development of sea buckthorn as a scientifically validated health-promoting resource depends on the continuous evolution and harmonization of these analytical techniques.

9. References

1. Bal, L. M., Meda, V., Naik, S. N., & Satya, S. (2011). Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmeceuticals. *Food Research International*, 44(7), 1718–1727.

2. Beveridge, T., Li, T. S. C., Oomah, B. D., & Smith, A. (1999). Sea buckthorn products: Manufacture and composition. *Journal of Agricultural and Food Chemistry*, 47(9), 3480–3488.

3. Yang, B., Kalimo, K. O., Tahvonen, R. L., Mattila, L. M., Kallio, S. E., & Kallio, H. P. (2000). Effect of harvesting time on triacylglycerols and glycerophospholipids of sea buckthorn (*Hippophaë rhamnoides* L.) berries. *Journal of Agricultural and Food Chemistry*, 48(2), 485–490.

4. Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, 13(7), 8615–8627.

5. Duan, W., Yang, J., Zhang, Y., & Yang, B. (2020). Flavonoid composition and antioxidant activity of sea buckthorn (Hippophae rhamnoides ssp. sinensis) leaves from different regions in China. *Journal of Food Composition and Analysis*, 88, 103444.

6. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1(1), 98–106.

7. Zhao, J., Deng, J., Chen, Y., & Li, S. P. (2013). Advanced analytical strategies for quality control of traditional Chinese medicines. *TrAC Trends in Analytical Chemistry*, 45, 74–89.

8. Hu, Y., Zhang, J., Zou, L., Fu, C., Li, P., & Liu, J. (2019). Applications of metabolomics in quality evaluation of traditional Chinese medicines. *Chinese Herbal Medicines*, 11(4), 349–356.

9. Kallio, H., Yang, B., Peippo, P., Tahvonen, R., & Pan, R. (2002). Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies of sea buckthorn (*Hippophaë rhamnoides* L.). *Journal of Agricultural and Food Chemistry*, 50(11), 3004–3009.

10. Liu, P., Liu, H., Wang, J., & Yang, B. (2017). Advances in chemical analysis of sea buckthorn: Techniques and applications. *Journal of Chromatography A*, 1493, 1–12.

11. Iqbal, E., Salim, K. A., & Lim, L. B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw). *Arabian Journal of Chemistry*, 8(3), 317–321.

12. Georgiev, V., Weber, J., Kneschke, E. M., Denev, P., Bley, T., & Pavlov, A. (2010). Antioxidant activity and phenolic content of betalain extracts from *Beta vulgaris* and *Portulaca grandiflora*. *Food Chemistry*, 127(4), 1761–1767.

13. Lu, Y., & Foo, L. Y. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chemistry*, 68(1), 81–85.

14. Baranska, M., Schulz, H., & Baranski, R. (2006). NIR and Raman study of carotenoids in plant tissues. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 65(5), 1085–1090.

15. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. (2000). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. National Academies Press.

16. Sun, Y., Wang, C., Wang, L., Wu, H., & Jin, C. (2011). Quality evaluation of sea buckthorn juice by NIR spectroscopy combined with multivariate analysis. *Food Chemistry*, 124(3), 1262–1266.

17. Li, W., Fitzloff, J. F., & Liu, Z. (2001). Determination of isoflavonoids in *Radix puerariae* by reversed-phase HPLC with UV and mass spectrometric detection. *Journal of Chromatographic Science*, 39(1), 17–21.

18. Kim, H. J., Chen, F., Wang, X., & Rajapakse, N. C. (2005). Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry*, 53(20), 7582–7586.