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QUALITY CONTROL AND ANTIOXIDENT PROFILING OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*) CHURNA USING HPLC MARKER ANALYSIS AND DPPH ASSAY

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Abstract:

The present study focuses on the quality control and antioxidant profiling of Sea Buckthorn Churna prepared from Hippophae rhamnoides using High Performance Liquid Chromatography (HPLC) marker analysis and DPPH free radical scavenging assay. Sea Buckthorn is a medicinal plant widely recognized for its rich content of bioactive compounds such as flavonoids, phenolic acids, vitamins, carotenoids, and antioxidants, which contribute to its therapeutic potential in various oxidative stress-related disorders. The increasing use of herbal formulations necessitates proper standardization and quality evaluation to ensure safety, efficacy, and consistency.

In the present investigation, Sea Buckthorn fruits were processed into churna form and subjected to physicochemical evaluation including organoleptic characteristics, ash values, moisture content, extractive values, and powder flow properties according to standard pharmacopoeial guidelines. HPLC marker analysis was performed for the identification and quantification of selected phytochemical markers to establish the chemical fingerprint profile of the formulation. The chromatographic study confirmed the presence of important phenolic and flavonoid constituents responsible for antioxidant activity.

The antioxidant potential of the prepared churna was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The formulation exhibited significant free radical scavenging activity in a concentration-dependent manner, indicating strong antioxidant potential due to the presence of natural phytoconstituents. The results suggest that Sea Buckthorn churna possesses appreciable antioxidant properties and can serve as a valuable herbal formulation for combating oxidative stress.

The study concludes that HPLC marker analysis combined with DPPH assay provides an effective approach for the standardization, quality control, and antioxidant evaluation of Sea Buckthorn churna. The developed analytical profile may be useful for ensuring batch-to-batch consistency and promoting the scientific validation of herbal formulations.

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INTRODUCTION:

Herbal medicines have gained significant importance worldwide because of their therapeutic benefits and minimal side effects. The increasing demand for herbal formulations has created the need for proper quality control and standardization methods. Herbal products contain multiple bioactive constituents whose concentration may vary depending on geographical source, cultivation conditions, harvesting, processing, and storage.

To ensure safety and efficacy, analytical techniques such as HPLC marker analysis and antioxidant assays are extensively used. HPLC is one of the most reliable chromatographic methods for qualitative and quantitative analysis of phytoconstituents. Marker compounds serve as chemical indicators for authentication and quality assessment of herbal products.

Oxidative stress caused by free radicals is associated with various diseases including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Antioxidants neutralize free radicals and protect biological systems from oxidative damage. Among different antioxidant assays, the DPPH assay is widely accepted due to its simplicity and rapidity.

High-Performance Liquid Chromatography (HPLC) is a vital analytical tool in the pharmaceutical industry, offering high sensitivity and precision for the detection and quantification of a wide range of pharmaceutical compounds, including active ingredients, impurities, and degradation products [1,2]. It plays a crucial role in impurity profiling, quality control, and regulatory compliance by enabling the identification of trace-level contaminants such as genotoxic impurities and nitrosamines in drug substances and formulations [5].HPLC operates on the principles of liquid-phase separation, utilizing a high-pressure pump to deliver the mobile phase through a column packed with stationary phase particles, facilitating effective analyte resolution and detection [3]. Since its introduction in the mid-20th century, HPLC has undergone continuous advancements, enhancing its efficiency, resolution, and applicability across various fields, including pharmaceuticals, environmental analysis, and food safety [6]. Key technological innovations, such as ultra-high-performance liquid chromatography (UHPLC), advanced detection systems (e.g., diode-array detection, fluorescence detection, and mass spectrometry integration), and automation, have significantly improved method sensitivity, throughput, and reproducibility [16]. Additionally, developments in sample preparation techniques, including solidphase extraction (SPE) and liquid-liquid extraction (LLE), have further optimized analytical workflows..

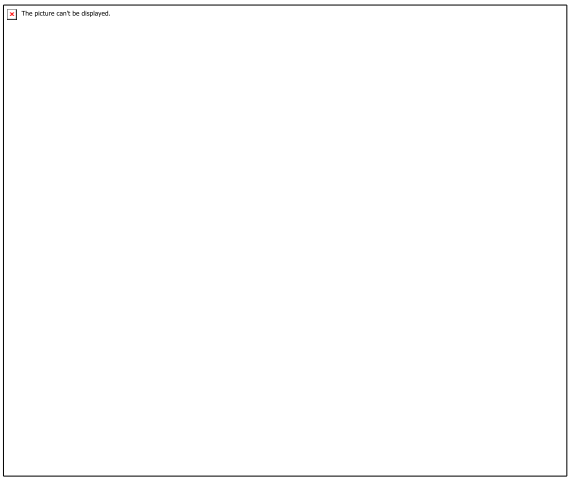

Fig no 1 :High performance thin layer chromatography

HPLC, a shortcut name for the term highpressure liquid chromatography or highperformance liquid chromatography can be useful for identifying sample composition. HPLC with UV-Vis detector can be selective and the use of a DAD (diode array detector for UV measurements) can provide UV spectra of the analytes, which in certain cases can be diagnostic in the sense that once the UV spectrum of a compound is known, it can be useful for positive identification. However (with a few exceptions), this detector is not useful for the identification of unknown compounds. HPLC is a versatile, robust, and widely used technique for the isolation and quantitation of analytes in samples such as pharmaceuticals, environmental samples, pollutants, biological samples, food and agricultural products, and many other materials and/or processes. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components such as polyphenols and bioactive compounds..

HPLC is a specific kind of column chromatography used to identify, separate, and quantify the active compounds [2]. Three main parts make up an HPLC system: a stationary phase (column filled with packing material), a pump that moves one or more mobile phases through the column, and a detector that shows the molecules retention times. Retention time is influenced by the interactions that occur between the stationary phase, the molecules under investigation, and the solvent used [3]. The sample for analysis is delayed by specific chemical or physical interactions with the stationary phase after being added in small quantities to the stream of

mobile phase. The kind of analyte and the composition of the mobile and stationary phases affect the retardation. A given analyte's retention time is the length of time it takes for it to elute, or leave, the column. Most miscible combinations of organic liquids or water are used as solvents (acetonitrile and methanol are the most popular ones) [2, 3]. The separation procedure called gradient elution is employed to alter the composition of the mobile phase in the course of the analysis [4]. The gradient divides the mixtures of analytes according to their affinity for the current mobile phase. The gradient, solvents, and additives selected depend on the characteristics of the analyte and stationary phase.

Instrumentation of HPLC

The major components of HPLC include:

1. Solvent reservoir
2. Pump system
3. Injector
4. Column
5. Detector (UV, PDA, Fluorescence)
6. Data acquisition system

Marker Compounds in Herbal Analysis

Marker compounds are chemically defined constituents used for quality evaluation of herbal drugs.

Types of Markers

- Active markers
- Analytical markers
- Negative markers

Table no 1 :Common Herbal Markers

Herbal Drug	Marker Compound
Turmeric	Curcumin
Green tea	Catechin
Ashwagandha	Withanolides
Sea buckthorn	Quercetin
Aloe vera	Aloin

Applications of HPLC Marker Analysis

- Standardization of herbal formulations
- Quantification of phytoconstituents
- Detection of adulteration
- Stability studies
- Quality control of nutraceuticals
- Pharmacokinetic studies

Advantages of HPLC

- High sensitivity
- Accurate quantification
- Rapid analysis
- Good reproducibility
- Suitable for complex mixtures

Limitations of HPLC

- Expensive instrumentation
- Requirement of skilled operators
- Costly solvents and columns
- Time-consuming sample preparation

Methodology of HPLC Marker Analysis

Sample Preparation

- Drying and powdering of plant material
- Extraction using solvents like methanol, ethanol, or water
- Filtration and sonication

Chromatographic Conditions

- Column: C18 reverse-phase column
- Mobile phase: Methanol:Water or Acetonitrile:Water
- Flow rate: 1 mL/min
- Detection wavelength: 254–370 nm

Validation Parameters

According to International Council for Harmonisation guidelines:

- Accuracy
- Precision
- Linearity
- Specificity
- Robustness
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)

DPPH Assay

Introduction

The DPPH assay is a spectrophotometric method used to evaluate antioxidant activity of plant extracts and herbal formulations.

Principle

% DPPH Radical Scavenging Activity=A0−A1A0×100\%\,\text{DPPH Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100%DPPH Radical Scavenging Activity=A0A0 −A1×100

DPPH is a stable free radical having deep violet color. Antioxidants donate hydrogen atoms or electrons to DPPH radicals, converting them into a yellow-colored reduced form. Reduction in absorbance at 517 nm indicates antioxidant activity.

Procedure of DPPH Assay

Prepare DPPH solution in methanol.

Add different concentrations of plant extract.

Incubate the mixture in dark for 30 minutes.

Measure absorbance at 517 nm using UV-visible spectrophotometer.

Calculate percentage inhibition.

Table no 2 :Interpretation of DPPH Results

% Inhibition	Antioxidant Activity
Below 30%	Weak
30–60%	Moderate
Above 60%	Strong

Advantages of HPLC and DPPH Assay

HPLC

- High sensitivity

- Accurate quantification
- Excellent reproducibility
- Suitable for complex mixtures

DPPH Assay

- Simple and rapid
- Cost-effective
- Requires small sample quantity
- Reliable antioxidant screening method

Limitations

HPLC

- Expensive instrumentation
- Requires skilled operators
- High solvent consumption

DPPH Assay

- Limited to in vitro antioxidant evaluation
- Color interference from plant extracts
- Does not represent biological conditions completely

Recent Advances

Recent developments include:

- HPLC coupled with mass spectrometry (HPLC-MS)
- Ultra-performance liquid chromatography (UPLC)
- Bioautography-assisted antioxidant analysis
- Chemometric fingerprinting techniques

These approaches improve sensitivity, selectivity, and rapid identification of phytoconstituents.

Sea buckthorn (Hippophae rhamnoides L., family: Elaeagnaceae) is a thorny, deciduous shrub that grows widely at high altitudes of 7,000–15,000 foot of the northwest Himalayan region, native to Eurasia. It is also been domesticated and used in traditional medicine in several countries, which has long been used for relieving cough, aiding digestion, invigorating blood circulation, and alleviating pain since ancient time (Rousi, 1971; Zheng, Dong, & Yu, 1997). Recently, sea buckthorn has been planted as a new berry crop for obtaining important bioactive compounds. Its good adaptability,rapid growth, ability to act as protection against wind and sand drift, assistance in soil and water conservation, and improvement of soil by efficient nitrogen fixation allow sea buckthorn to be widely used in vegetation and restoration of degenerated ecosystems (Chen & Chen, 2003; Hou, Bai, & Cao, 1995; Li, 2004; Ruan & Li, 2002; Ruan, Xie, & Li, 2000; Wei, Yu, & Zhu, 1998). Sea buckthorn with diverse uses such as, controlling soil erosion, a source of horse fodder, nutritious foods, drugs, and skin-care products, also contains bioactive compounds with antioxidant properties that are preferred over the synthetic antioxidants simply because the latter have quite often been found to be carcinogenic (Fan, Ding, & Gu, 2007; Rodríguez-Meizoso et al., 2006). Oil from sea buckthorn has shown effectiveness in skin therapy for sunburns, chemical burns, radiation burns, and eczema (Goel et al., 2002; Seven et al., 2009; Yang et al., 2000; Zeb, 2004b). Furthermore, sea buckthorn oil has shown positive results in treating health problems related to damaged mucous membranes of the gastrointestinal tract including mouth ulcers, gastric ulcers, and stress ulcers (Suleyman, Buyukokuroglu, et al., 2001; Suleyman, Demirezer, et al., 2001; Xu et al., 2007). Of particular interest, the berries, the oil, and the seeds of sea buckthorn have been shown to possess antiatherogenic, hypocholesteromic, hypotensive, and anti-inflammatory properties (Eccleston et al., 2002; Ganju et al., 2005; Wu, Yu, Ma, Li, & Liu, 1994; Yang, 1995; Zhang, 1987) and could therefore be successfully exploited to prevent or treat cardiovascular disease. Sea buckthorn has been used mostly in the Tibetian and Chinese traditional medicines for so long, which is supported by a lot of both ancient and recent literature on the use of different parts of sea buckthorn, such that the plant is also commonly known as Chinese medicinal plant. The fact that all parts of the plant possess bioactive compounds with antioxidant properties, the literature on its antioxidant activity, impact on the medical lines, and its safety evaluation are reviewed in the present manuscript.

Fig no 2 :*Hippophae rhamnoides L*

Bioactive compounds and antioxidant properties :Bioactive substances like vitamins (A, C, E, riboflavin, folic acid, and K), carotenoids (α, β, ð-carotene, and lycopene), flavonoids, organic acids (malic acid and oxalic acid), sterols (ergosterol, stigmasterol, lanosterol, and amyrins) and some essential amino acids have been found in all parts of the plant (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999; Upendra et al., 2008). In general, the major components of the seed are vitamin C, large amount of carotenoids and vitamin E, flavonoids and kaempferol, fatty acids, triacylglycerol, phytosterols, sugar, organic acids, proanthocyanidins, and phenolic compounds (Fan et al., 2007; Li, Beveridge, & Drover, 2007). The ripe fruit has been reported to be a source of exceptionally high contents of vitamins (A, C, E, and K), carotenoids, flavonoids, and organic acids (Geetha, Sai Ram, Singh, Ilavazhagan, & Sawhney,

2002). Oil from sea buckthorn contains several bioactive components such as vitamin E, vitamin K, carotenoids, and β-70 sitosterol (Zeb, 2004a). Oil extracts obtained from the berries of sea buckthorn in recent studies have been found to be rich in monounsaturated fatty acids (MUFA) (Yang & Kallio, 2001), tocopherols, tocotrienols (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002), carotenoids, and other bioactive compounds (Guliyev, Gul, & Yildirim, 2004). The leaves of sea buckthorn are rich in kaempferol-3-Oβ-D-(6″-O-coumaryl) glycoside, 1-feruloyl-β-D-glucopyranoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-D glucopyranoside, quercetin-3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, and isorhamnetin-3-O-rutinoside. Nine fractions, four monomeric flavan-3-ols, catechin, epicatechin, gallocatechin, and epigallocatechin, along with two dimeric procyanidins, catechin(4α-8)catechin and catechin(4α-8)epicatechin, have been reported from the extracts of sea buckthorn seeds.

The assays like 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP), which are often used to test the antioxidant activity, have revealed that the antioxidant activity of seed and root extracts is better than that of leaf and stem extracts (Michel, Destandau, Le Floch, Lucchesi, & Elfakir, 2012; Nitin et al., 2010). Gallic acid, which is also present in sea buckthorn, has been reported to be the most effective antioxidant (Pandurangan, Bose, & Banerji, 2011). The antioxidant potential of aqueous extract of sea buckthorn leaves varies within the range of 76.44–88.82% while as the total polyphenols vary in the range of 67.91–88.69 GAE/g (Wani, Wani, Shah, & Masoodi, 2013). Sea buckthorn leaf evaluation using maceration, soxhlet, and subcritical water extraction techniques showed the antioxidant potential of 86.35, 133.31–255.87, and 164.03–343.86 Trolox equivalents per gram (TE/g), respectively, while as the respective total phenolic content was reported to be 28.35, 43.77– 77.85, and 60.22–86.70 mg/g (Kumar, Dutta, Prasad, & Misra, 2011). The phenolic rich fraction (PRF) of sea buckthorn leaves showed a total phenolic content of 319.33 mg gallic acid equivalents (GAE) per gram while as in the berries, it ranged from 21.31 to 55.38 mg GAE/g on dry weight basis. It showed the highest antioxidant activity of 93.54% and the lowest of 80.38% with no correlation between the total phenolic content and the antioxidant activity. The DPPH radical scavenging activity of sea buckthorn leaf extract (50% effective concentration (EC50) = 1.81 μg/mL) is higher than the butanol fraction (EC50 = 1.86 μg/mL), and quercetin-3-O-β-D-glucopyranoside. It showed stronger reducing power (OD700 = 1.83, and 1.78, respectively), with the highest amount of phenolic compounds (477 mg GAE/g) contained in the butanol fraction (Ercisli, Orhan, Ozdemir, & Sengul, 2007; Kim et al., 2011; Maheshwari, Yogendra Kumar, Verma, Singh, & Singh, 2011). The EC50 values of sea buckthorn seed oil from the hydrogen peroxide, superoxide radical, and hydroxyl radical scavenging assays were 2.63, 2.16, and 0.77 mg/ml, respectively (Ting et al., 2011). Taken together, sea buckthorn seed oil, leaf, branches, and root extracts have significant potential as natural antioxidants and could be used potentially for food additives and the development of useful natural compounds.

General medical properties :The branches of sea buckthorn contain (-)-epigallocatechin and ursolic acid that exhibit anti-inflammatory effects and the leaves are used in the treatment of diarrhea, gastrointestinal, and dermatologic disorders (Yasukawa, Kitanaka, Kawata, & Goto, 2009). The leaf extracts of sea buckthorn are rich in flavonoids, tannins, and triterpenes (Kallio et al., 2002), which as well as the extracts of its branches are used to treat colitis and enterocolitis in humans and animals in Mongolia . The leaves have also been applied as compress form in rheumatoid arthritis in the Middle Asia. Flowers of sea buckthorn have been used as skin softener in Tajikistan. The extracts of whole fruit, fruit pulp, pulp oil, and seed oil of ripe fruits have been reported to be useful in treating various diseases such as gastric ulcers, coronary heart disease, radiation-induced oxidative damage , wound healing), thrombosis, and platelet aggregation in Indian and Tibetian medicine . Sea buckthorn can increase the production of plasma leptin and of neuropeptide Y in children with functional dyspepsia. The overall effect of sea buckthorn is improvement of gastric emptying, gastric mobility, gastrointestinal digestive function, and promotion of children's growth (Xiao, Qiu, Yue, Cai, & Mo, 2013). Sea buckthorn berry oil is reported to play a potential role in treating atopic dermatitis and thrombosis (Cheng et al., 2003; Yang et al., 2000). Juice, syrup, and oil of the fruits have been used as pain killer, to heal wounds, in ulcer and other diseases of the stomach, disantheria, cancer, and as a metabolism regulator in traditional medicine. The freshly pressed juice is used in the treatment of colds, febrile conditions, and exhaustion (Yang et al., 2000). Oil from seeds and fruits is used in the treatment of eczema, lupus erythematosus, chronic wounds that are difficult to heal, inflammatory diseases, erosion of the cervix uteri, in the treatment of burns and frozen parts of the body, and in ophthalmology. Besides, they are used in the treatment of keratitis, trachoma, injuries or burns of eyelid, and conjunctivitis (Guliyev et al., 2004). Isorhamnetin isolated from sea buckthorn has been investigated for its cytotoxicity and its influence on human hepatocellular carcinoma cells. The cytotoxic effects of isorhamnetin showed dose

and time dependency against hepatocellular carcinoma cells (IC50 = 74.4 µg/ml) after treatment with isorhamnetin for 72 h. The cytotoxicity of isorhamnetin on tumor cells depends on cellular accumulation of the drug that permeates the cell membrane into the cell.

Effects on adverse stressful situations Alcoholic leaf extract of sea buckthorn (70% ethanol) has been analyzed to inhibit hypoxia-induced cytotoxicity, mitochondrial integrity, reactive oxygen species (ROS) production, and DNA damage better than vitamin C (Narayanan et al., 2005). In a study of effect of dry sea buckthorn leaf (aqueous lyophilized) extract, untreated rats exposed to cold-hypoxia-restraint (C–H–R) up to fall of rectal temperature (Trec) 23°C, blood hexokinase (HK) activity decreased, liver phosphofructokinase (PFK) activity increased, and muscle PFK activity decreased. During cold exposure of 20°C for 8 h in rat, liver glycolysis activated at the regulatory step catalyzed by PFK/fructose-1,6- biphosphatase.

Table no 3 :Parts of plants used in treatments

Parts of plants	Use of treatments
Leaves	Rheumatoid arthritis
	Overweight,viseral fat ,triglyceride
	Inflammation ,Hypoxia induced cytotoxicity
	Cytotoxicity
Branches	Inflammation and dirrehea ,coilities
Fruits	Gastric ulcers ,skin disorders ,cardiovascular disease
Seeds and berry oil	Dermatitis and thrombiosis

Sea Buckthorn churna is a powdered herbal preparation prepared from dried berries or plant parts and is traditionally used as a nutritional supplement and antioxidant remedy. Due to increasing commercialization of herbal formulations, scientific quality control and standardization have become necessary.

Need for Quality Control of Herbal Churna

Quality control of herbal formulations is essential to:

- Ensure identity and purity
- Detect adulteration and contamination
- Maintain therapeutic efficacy
- Achieve batch-to-batch consistency
- Standardize active phytoconstituents

Quality Control Parameters for Sea Buckthorn Churna

Organoleptic Evaluation

- Color
- Odor
- Taste
- Texture

Physicochemical Parameters

- Ash value
- Extractive value
- Moisture content
- pH determination
- Bulk density
- Particle size

Microbial Load Testing

Evaluation of:

- Total bacterial count
- Fungal count
- Pathogenic microorganisms

Heavy Metal Analysis

Determination of:

- Lead
- Arsenic
- Mercury
- Cadmium

HPLC Marker Analysis

Principle of HPLC

High-Performance Liquid Chromatography (HPLC) is an analytical technique used for separation, identification, and quantification of phytoconstituents in herbal formulations.

Common HPLC Markers in Sea Buckthorn

- Quercetin
- Rutin
- Gallic acid
- Kaempferol
- Isorhamnetin

Table no 4 :Typical HPLC Conditions

Parameter	Condition
Column	C18 reverse phase column
Mobile Phase	Acetonitrile: Water
Detector	UV detector
Wavelength	254–370 nm
Flow Rate	1 mL/min
Injection Volume	20 µL

Advantages of HPLC

- High sensitivity
- Accurate quantification
- Rapid analysis
- Reliable fingerprint profiling

Antioxidant Profiling Using DPPH Assay

Principle of DPPH Assay

The DPPH assay measures the free radical scavenging activity of antioxidants present in herbal extracts. DPPH is a stable free radical with deep violet color which becomes yellow upon reduction by antioxidants.

The percentage inhibition is calculated using the formula:

% Inhibition=A0−A1A0×100\%\ Inhibition = \frac{A_0 - A_1}{A_0} \times 100% Inhibition=A0A0−A1×100

Where:

- A0A_0A0 = Absorbance of control
- A1A_1A1 = Absorbance of sample

Procedure

1. Prepare methanolic DPPH solution.
2. Mix sample extract with DPPH solution.
3. Incubate in dark for 30 minutes.
4. Measure absorbance at 517 nm using UV spectrophotometer.

Significance

- Determines antioxidant potential
- Evaluates free radical scavenging activity
- Useful for herbal standardization

Pharmacological Activities of Sea Buckthorn

Sea Buckthorn exhibits several biological activities:

- Antioxidant
- Anti-inflammatory
- Hepatoprotective
- Cardioprotective
- Immunomodulatory
- Antimicrobial
- Anti-aging activity

Applications of Sea Buckthorn Churna

- Nutraceutical formulations
- Herbal supplements
- Functional foods
- Antioxidant therapy
- Skin care products

CONCLUSION:

Hippophae rhamnoides churna is a valuable herbal formulation rich in flavonoids, phenolic compounds, vitamins, and other bioactive constituents that contribute significantly to its antioxidant potential and therapeutic efficacy. The present study on quality control and antioxidant profiling demonstrated the importance of scientific standardization of herbal formulations to ensure safety, purity, consistency, and effectiveness.

Physicochemical evaluation, organoleptic characteristics, and microbial quality assessment confirmed the acceptable quality parameters of the churna formulation. HPLC marker analysis proved to be a reliable and precise analytical technique for the identification and quantification of important phytochemical markers such as quercetin, rutin, gallic acid, and kaempferol, thereby establishing the authenticity and batch-to-batch consistency of the formulation.

Furthermore, antioxidant profiling using the DPPH free radical scavenging assay revealed significant antioxidant activity of Sea Buckthorn churna, indicating its potential role in combating oxidative stress and related disorders. The presence of high levels of phenolic and flavonoid compounds may be responsible for its strong free radical scavenging effect.

Overall, the integration of HPLC marker analysis with DPPH antioxidant assay provides an effective approach for comprehensive quality assessment and standardization of Sea Buckthorn churna. These findings support the therapeutic and nutraceutical importance of Sea Buckthorn and promote its future application in herbal medicine, functional foods, and antioxidant-based pharmaceutical formulations.

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