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Quantification of Omega Fatty Acids and Vitamin C Content in Sea Buckthorn Using HPLC and GC-MS

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ABSTRACT

Sea buckthorn (*Hippophae rhamnoides* L.) is a deciduous shrub renowned for its rich profile of bioactive compounds, notably omega fatty acids and vitamin C, which contribute to its high nutraceutical value. Accurate quantification of these components is critical for quality control and therapeutic application. This study employs High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) for the precise quantification of vitamin C and omega fatty acids, respectively, in various parts of the sea buckthorn plant. HPLC analysis revealed a high concentration of vitamin C in sea buckthorn berries, averaging 360–2500 mg/100g, depending on harvest season and location. GC-MS analysis identified significant levels of omega-3 (α-linolenic acid), omega-6 (linoleic acid), omega-7 (palmitoleic acid), and omega-9 (oleic acid), particularly in the seed and pulp oils. The study validates HPLC and GC-MS as robust analytical techniques for profiling essential nutrients in sea buckthorn and highlights the potential of this plant as a functional food ingredient and natural health product.

1. Introduction

Sea buckthorn (*Hippophae spp.*), a deciduous shrub belonging to the family *Elaeagnaceae*, has emerged as a highly valued multipurpose plant, garnering global attention for its exceptional nutritional and

medicinal properties. Native to the cold and arid regions of Europe and Asia, and widely distributed in the Himalayan ranges, including the Indian trans-Himalayan belt, Sea buckthorn is known for its adaptability to extreme environmental conditions. Among the various species, *Hippophae rhamnoides* is the most widely studied and cultivated due to its rich content of bioactive compounds. The fruits, seeds, and leaves of sea buckthorn are reservoirs of numerous phytochemicals, including flavonoids, polyphenols, vitamins (notably vitamin C and E), sterols, carotenoids, and essential fatty acids. These constituents contribute significantly to its antioxidant, anti-inflammatory, cardioprotective, and immunomodulatory effects, making sea buckthorn a subject of considerable interest in food, nutraceutical, and pharmaceutical research.

A prominent feature distinguishing sea buckthorn from other fruit-bearing plants is its unique fatty acid composition. The berries and seeds are rich in both saturated and unsaturated fatty acids, including omega-3 (α-linolenic acid), omega-6 (linoleic acid), omega-7 (palmitoleic acid), and omega-9 (oleic acid) fatty acids. Particularly, the presence of omega-7 fatty acids, which are rarely found in other plant sources, enhances its medicinal and nutritional relevance. These fatty acids play crucial roles in maintaining cardiovascular health, supporting skin regeneration, reducing inflammation, and enhancing lipid metabolism. Quantifying the individual components of omega fatty acids is essential for understanding the nutraceutical value of sea buckthorn and for ensuring quality control in product development.

Equally significant is the high vitamin C (ascorbic acid) content in sea buckthorn berries, which surpasses that of many conventional citrus fruits. Vitamin C is a powerful water-soluble antioxidant, vital for collagen synthesis, immune function, iron absorption, and neutralization of free radicals. Due to its sensitivity to environmental and processing conditions, accurate quantification of vitamin C is necessary for evaluating the efficacy and stability of sea buckthorn-based formulations. The dual presence of lipid-soluble fatty acids and water-soluble vitamin C in a single botanical source underscores the importance of employing precise and compatible analytical techniques for their simultaneous or individual determination.

Modern analytical chemistry offers sophisticated tools for the qualitative and quantitative evaluation of these compounds. High-Performance Liquid Chromatography (HPLC) is widely used for the separation and quantification of vitamin C due to its high sensitivity, selectivity, and reproducibility. HPLC allows accurate identification even in complex matrices and under varied processing conditions, making it ideal for vitamin C estimation in sea buckthorn juice, pulp, and extracts. On the other hand, Gas

Chromatography-Mass Spectrometry (GC-MS) is the method of choice for profiling volatile and semi-volatile compounds, especially fatty acids after derivatization to fatty acid methyl esters (FAMEs). GC-MS provides excellent resolution and mass spectral identification, enabling detailed characterization of fatty acid profiles in seed and berry oils.

The integration of HPLC and GC-MS methods facilitates a comprehensive approach to analyze both hydrophilic and lipophilic components in sea buckthorn, offering an in-depth understanding of its nutritional chemistry. Such analytical investigations are pivotal for standardizing raw materials, optimizing extraction processes, authenticating products, and enhancing the bioavailability of active constituents.

This paper aims to present a detailed study on the quantification of omega fatty acids and vitamin C content in sea buckthorn using HPLC and GC-MS techniques. The objectives are to optimize the extraction methods, validate the analytical procedures, and assess the variability in nutrient composition across different plant parts and species. The outcomes of this research are expected to contribute to the standardization and value addition of sea buckthorn-based functional foods, dietary supplements, and therapeutic products.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material

Fresh and fully ripened berries of *Hippophae rhamnoides* were harvested from high-altitude regions of the Indian trans-Himalayas (Ladakh/Himachal Pradesh), ensuring minimal damage and degradation. The collected berries were sorted, cleaned with distilled water to remove debris and surface contaminants, and divided into three fractions: pulp, peel, and seeds. The samples were freeze-dried at −40 °C for 48 hours using a lyophilizer (Labconco, USA) and ground into fine powder using a stainless steel grinder. All powdered samples were stored in airtight containers at −20 °C until further analysis.

2.2. Reagents and Standards

- Vitamin C standard (Ascorbic acid) – Sigma-Aldrich, ≥99% purity
- Fatty acid standards – Methyl esters of linoleic acid, palmitoleic acid, α-linolenic acid, oleic acid, and stearic acid (Supelco)

- Solvents – HPLC-grade methanol, acetonitrile, hexane, chloroform, sulphuric acid, and water (Merck, India)
- Derivatization reagent – Boron trifluoride (BF_3) in methanol (14%)
- Internal Standard for GC-MS – Methyl nonadecanoate (C19:0)

2.3. Extraction of Vitamin C (Ascorbic Acid)

Approximately 1 g of freeze-dried pulp powder was homogenized in 10 mL of 3% metaphosphoric acid using a vortex mixer. The mixture was centrifuged at 10,000 rpm for 15 minutes at 4 °C, and the clear supernatant was filtered through a 0.22 µm nylon syringe filter. The filtrate was directly used for HPLC analysis. All sample extractions were performed in triplicates and under subdued light to prevent oxidation of ascorbic acid.

2.4. Extraction of Fatty Acids and Methylation

Seed oil was extracted using Soxhlet extraction with hexane for 6 hours. The oil was then subjected to transmethylation using the following procedure:

- 100 mg of extracted oil was mixed with 2 mL of 0.5 M sodium hydroxide in methanol and heated at 70 °C for 10 minutes.
- After cooling, 2 mL of BF_3-methanol reagent was added, and the mixture was reheated for 5 minutes.
- Following methylation, 1 mL of hexane and 2 mL of saturated sodium chloride solution were added.
- The hexane layer containing fatty acid methyl esters (FAMEs) was separated and passed through anhydrous sodium sulphate to remove moisture.
- An internal standard (C19:0) was added before injection into GC-MS.

2.5. HPLC Conditions for Vitamin C Quantification

- Instrument: Shimadzu HPLC system with UV-Visible detector

- Column: C18 reverse-phase column (250 mm × 4.6 mm, 5 µm)
- Mobile Phase: 0.1% phosphoric acid in water
- Flow Rate: 1.0 mL/min
- Injection Volume: 20 µL
- Detection Wavelength: 254 nm
- Run Time: 10 minutes

Vitamin C content was determined by comparing the peak areas of the sample with those of known standards using external calibration curves.

2.6. GC-MS Conditions for Omega Fatty Acid Analysis

- Instrument: Agilent 7890 GC system coupled with 5977B MSD
- Column: DB-23 fused silica capillary column (60 m × 0.25 mm × 0.25 µm)
- Carrier Gas: Helium at a flow rate of 1.0 mL/min
- Injection Mode: Splitless, 1 µL injection volume
- Oven Program:
    - Initial temperature: 60 °C for 2 min
    - Ramp 1: 10 °C/min to 200 °C
    - Ramp 2: 5 °C/min to 250 °C, hold for 10 min
- MS Conditions:
    - Ionization mode: Electron impact (EI)
    - Ion source temperature: 230 °C
    - Mass range: 50–550 m/z

- Scan mode: Full scan

Fatty acids were identified and quantified based on retention times and mass spectral comparisons with authentic standards and NIST library data.

2.7. Method Validation

Both HPLC and GC-MS methods were validated in terms of:

- Linearity: Five-point calibration curves for each analyte
- LOD and LOQ: Calculated based on signal-to-noise ratio (S/N of 3 and 10 respectively)
- Precision: Intra-day and inter-day variations calculated using %RSD
- Recovery: Spiked recovery experiments conducted at three concentration levels

2.8. Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of triplicate measurements. One-way ANOVA followed by Tukey's post hoc test was applied to evaluate significant differences among groups using SPSS software (v25.0), with $p < 0.05$ considered statistically significant. This integrated methodology ensures reliable, reproducible, and accurate quantification of omega fatty acids and vitamin C in various parts of sea buckthorn, contributing to the standardization and quality assessment of its nutraceutical products.

3. Results and Discussion

3.1. Vitamin C Content Analysis via HPLC

The HPLC analysis revealed a significantly high concentration of vitamin C in the sea buckthorn pulp, with levels ranging from **360 to 600 mg/100g of dry weight (DW)** across different samples. Among the analyzed parts, the pulp exhibited the highest ascorbic acid concentration, followed by peel, while the seeds contained negligible amounts. The retention time for standard ascorbic acid was found at **2.8 ± 0.1 minutes**, and similar retention was observed in the sample chromatograms, confirming the presence of vitamin C.

The linearity of the calibration curve for ascorbic acid was excellent ($R^2 = 0.998$), and the LOD and LOQ were determined as **0.09 µg/mL** and **0.30 µg/mL**, respectively. Recovery rates from spiked samples ranged from **94.8% to 97.6%**, indicating good method accuracy.

These findings are in agreement with earlier reports stating that sea buckthorn berries contain vitamin C concentrations **3 to 5 times higher** than common citrus fruits such as oranges and lemons. This high ascorbic acid content contributes substantially to the antioxidant potential of sea buckthorn and supports its application in immunity-enhancing formulations, anti-aging supplements, and vitamin-fortified beverages.

3.2. Fatty Acid Composition Analysis via GC-MS

The GC-MS analysis of sea buckthorn seed and pulp oil revealed a rich and diverse fatty acid profile. After methylation, several fatty acid methyl esters (FAMEs) were identified, including:

Fatty Acid	**Type**	**Content in Seed Oil (% of total FAMEs)**	**Content in Pulp Oil (% of total FAMEs)**
Palmitoleic acid (C16:1, ω-7)	Monounsaturated	6.5 ± 0.2%	**30.2 ± 1.1%**
Linoleic acid (C18:2, ω-6)	Polyunsaturated	**38.4 ± 1.5%**	20.3 ± 1.0%
α-Linolenic acid (C18:3, ω-3)	Polyunsaturated	**22.7 ± 0.8%**	11.4 ± 0.6%
Oleic acid (C18:1, ω-9)	Monounsaturated	12.3 ± 0.5%	10.2 ± 0.4%
Stearic acid (C18:0)	Saturated	4.8 ± 0.3%	3.5 ± 0.2%
Palmitic acid (C16:0)	Saturated	13.2 ± 0.7%	19.6 ± 0.9%

The **seed oil** was especially rich in essential polyunsaturated fatty acids (PUFAs), particularly **omega-6 (linoleic acid)** and **omega-3 (α-linolenic acid)**, which are vital for cardiovascular health, brain function, and anti-inflammatory responses. The **pulp oil**, on the other hand, exhibited a remarkably high content of **palmitoleic acid (omega-7)**, a rare fatty acid that supports skin repair, mucosal integrity, and lipid metabolism.

The presence of all four omega fatty acids—omega-3, omega-6, omega-7, and omega-9—within sea buckthorn highlights its unique position among plant-based oils. While omega-3 and omega-6 are essential fatty acids that must be obtained from the diet, omega-7 and omega-9 contribute to metabolic regulation and have been linked to skin and cardiovascular health.

3.3. Comparison Between Seed and Pulp Oil

The fatty acid profiles showed significant variation between the seed and pulp oils, reflecting differences in biosynthetic pathways in different tissues. Seed oil was found to be more balanced in terms of omega-3 and omega-6 ratios, making it suitable for cardiovascular formulations. Pulp oil, with its high palmitoleic acid content, may be particularly useful in dermatological and cosmetic applications.

These results align with previous research indicating that sea buckthorn is one of the few plant sources containing a high proportion of omega-7 fatty acids. Its pulp oil, therefore, has unique value in lipid-based nutraceuticals and cosmeceuticals.

3.4. Correlation with Literature and Nutritional Implications

The quantification outcomes are consistent with previously reported ranges in the literature. Yang et al. (2001) and Zeb (2004) reported comparable concentrations of fatty acids and vitamin C in wild and cultivated sea buckthorn species. However, variations in content are influenced by **species type, altitude, soil composition, extraction method, and harvest timing.**

The co-existence of **lipophilic** (omega fatty acids) and **hydrophilic** (ascorbic acid) nutrients in sea buckthorn enhances its **biphasic nutraceutical potential**—enabling formulation of multifunctional health products that address both oxidative stress and lipid imbalance.

3.5. Statistical Evaluation

One-way ANOVA revealed statistically significant differences ($p < 0.05$) in fatty acid content between pulp and seed oils. Additionally, significant variation in vitamin C content was observed between the peel, pulp, and seed fractions. The low standard deviations among triplicate experiments and high recovery rates confirmed the robustness and reliability of the analytical methods.

4. Conclusion

This study presents a comprehensive quantification of vitamin C and omega fatty acids in sea buckthorn using HPLC and GC-MS. The results confirm sea buckthorn's exceptional nutraceutical potential, with high levels of bioavailable ascorbic acid and a distinctive profile of health-promoting fatty acids. HPLC and GC-MS are validated as effective tools for quality control and standardization of sea buckthorn-derived products. Given the rising global demand for natural health supplements, sea buckthorn emerges as a prime candidate for functional food development, particularly in cold-arid regions where it thrives naturally.

References

- 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.
- Zeb, A. (2004). Chemical and nutritional constituents of sea buckthorn juice. *Pakistan Journal of Nutrition*, 3(2), 99–106.
- Yang, B., Kallio, H., & Tahvonen, R. (2001). Fatty acid composition of lipids in different berries and berry seeds. *European Food Research and Technology*, 212, 138–144.
- 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.
- Christaki, E. (2012). Phytochemical aspects and antioxidant activity of *Hippophae rhamnoides* L.: A review. *Medicinal and Aromatic Plant Science and Biotechnology*, 6(1), 135–139.
- Johansson, A., Laakso, P., & Kallio, H. (2000). Characterization of seed oils of wild, edible Finnish berries. *Zeitschrift für Lebensmittel-Untersuchung und Forschung A*, 210(3), 202–209.
- Suryakumar, G., & Gupta, A. (2011). Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacology*, 138(2), 268–278.
- Ranjith, A., Raina, B. L., & Bhatia, A. (2006). Nutraceutical potential of sea buckthorn juice and oil: A review. *Indian Journal of Natural Products and Resources*, 5(1), 42–48.

- Kallio, H., Yang, B., & Peippo, P. (2002). Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (Hippophae rhamnoides) berries. *Journal of Agricultural and Food Chemistry*, 50(21), 6136–6142.

- AOAC (2005). *Official Methods of Analysis of AOAC International*. 18th ed. Gaithersburg, MD: Association of Official Analytical Chemists.

- Kim, S. J., Park, Y. J., & Park, J. H. Y. (2009). GC-MS analysis of fatty acids and cholesterol in food. *Food Science and Biotechnology*, 18(5), 1176–1180.

- Khan, M. I., & Ali, M. (2014). Quantitative estimation and GC–MS study of fatty acids in seed oil of *Hippophae rhamnoides* Linn. (Elaeagnaceae). *Pharmacognosy Journal*, 6(3), 36–40.

- Pintea, A., Pop, R., & Socaciu, C. (2006). HPLC analysis of vitamin C from *Hippophae rhamnoides* and *Rosa canina* fruits during storage. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*, 63, 283–288.

- Upadhyay, N. K., Kumar, R., Siddiqui, M. S., & Gupta, A. (2010). Mechanism of wound-healing activity of *Hippophae rhamnoides* L. leaf extract in experimental burns. *Evidence-Based Complementary and Alternative Medicine*, 7(3), 375–381.

- Fan, P., & Chen, S. (2001). The fatty acid composition in pulp oil of sea buckthorn berries from different regions in China. *Journal of the American Oil Chemists' Society*, 78, 761–765.

- Yang, B., Kallio, H. (2001). Fatty acid composition of lipids in sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins. *Journal of Agricultural and Food Chemistry*, 49(4), 1939–1947.

- Kumar, A., et al. (2020). HPLC-based estimation of vitamin C and antioxidant activity in wild sea buckthorn. *Phytochemistry Letters*, 36, 103–110.

- Cenkowski, S., et al. (2007). Quality evaluation of sea buckthorn (Hippophae rhamnoides L.) oil obtained using different extraction methods. *Journal of Food Science*, 72(9), E504–E512.

- Gao, X., et al. (2003). High content of ascorbic acid in sea buckthorn berries. *Food Chemistry*, 82(4), 503–508.