



Comparative Study of Flavonoids, Carotenoids, and Phenolics in Different Species of *Sea Buckthorn (Hippophae spp.)*

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ABSTRACT

Sea buckthorn (*Hippophae* spp.), a deciduous shrub native to Europe and Asia, is renowned for its diverse therapeutic applications and rich content of bioactive compounds, notably flavonoids, carotenoids, and phenolic compounds. This comparative study aims to evaluate and analyze the variation in these phytochemicals across prominent species such as *Hippophae rhamnoides*, *Hippophae tibetana*, *Hippophae salicifolia*, and *Hippophae neurocarpa*. Through detailed profiling using HPLC, UV-VIS spectrophotometry, and mass spectrometry, significant interspecific differences in composition and concentration were observed. *H. rhamnoides* emerged as the richest source of total flavonoids and carotenoids, particularly quercetin, kaempferol, and β -carotene, whereas *H. tibetana* and *H. salicifolia* showed higher levels of phenolic acids such as Gallic acid and Caffeic acid. Environmental conditions, geographical distribution, and species-specific metabolic pathways are posited as key contributors to this phytochemical variation. The findings enhance our understanding of the therapeutic potential of different sea buckthorn species and provide a biochemical foundation for targeted nutraceutical development and cultivation strategies



Introduction

Sea buckthorn (*Hippophae* spp.) is a deciduous, thorny shrub of the family Elaeagnaceae, native to the cold-temperate regions of Europe and Asia, particularly the Himalayan belt, Central Asia, and parts of China and Russia. The plant has gained significant attention in recent decades due to its remarkable pharmacological, nutritional, and ecological significance. Traditionally used in Tibetan, Mongolian, Chinese, and Russian folk medicine for centuries, almost all parts of the plant—fruits, seeds, leaves, and bark—are known to contain bioactive compounds with substantial health benefits. Today, sea buckthorn is increasingly integrated into modern nutraceuticals, cosmeceuticals, and functional food formulations.

Among the various classes of phytochemicals found in *Hippophae*, three stand out for their high biological activity and nutritional importance: flavonoids, carotenoids, and phenolic compounds. Flavonoids are a diverse group of polyphenolic compounds known for their antioxidant, anti-inflammatory, hepatoprotective, and cardiovascular benefits. They contribute to the vibrant colour of the berries and help protect plant tissues from ultraviolet radiation and oxidative damage. Carotenoids, particularly β -carotene, zeaxanthin, and lutein, play a crucial role as precursors of vitamin A and are widely recognized for their immune-modulating and vision-enhancing properties. Phenolic compounds, including gallic acid, ferulic acid, and chlorogenic acid, are secondary metabolites that exhibit strong free radical-scavenging and anti-mutagenic activities, making them central to the plant's defence system and the prevention of human chronic diseases.

The genus *Hippophae* consists of several species, including *Hippophae rhamnoides*, *H. tibetana*, *H. salicifolia*, and *H. neurocarpa*, each adapted to diverse environmental conditions ranging from sea level to elevations exceeding 4,500 meters. While *H. rhamnoides* is the most widely studied and commercially exploited species, the other species remain underexplored, particularly in the context of their phytochemical variability and potential applications. Environmental conditions, altitude, soil composition, climate, and genetic makeup all contribute to the biochemical diversity observed among species. Hence, comparing the phytochemical profiles of different sea buckthorn species is crucial for selecting the most suitable cultivars for specific nutraceutical or medicinal purposes.

Although several studies have examined individual compounds or specific extracts from *H. rhamnoides*, comprehensive comparative analyses across multiple *Hippophae* species are limited. Understanding interspecies variability is essential not only for medicinal standardization but also for agronomic selection and biotechnological intervention. Moreover, as interest grows in the cultivation and commercialization



of sea buckthorn in regions like Northeast India, Central Asia, and the Tibetan plateau, identifying the phytochemical strengths of native species becomes increasingly relevant.

The present study aims to fill this gap by conducting a detailed comparative analysis of flavonoids, carotenoids, and phenolic compounds in four species of sea buckthorn: *H. rhamnoides*, *H. tibetana*, *H. salicifolia*, and *H. neurocarpa*. Using standardized analytical techniques such as HPLC, UV-VIS spectrophotometry, and mass spectrometry, we assess species-specific phytochemical profiles to evaluate their nutraceutical potential. This work provides a scientific foundation for targeted cultivation, conservation, and utilization strategies in the pharmaceutical and food industries.

Materials and Methods

I. Collection and Identification of Plant Materials

Fresh fruits and leaves of four species of *Hippophae*—*H. rhamnoides*, *H. tibetana*, *H. salicifolia*, and *H. neurocarpa*—were collected during the peak fruiting season (August to September 2024) from naturally growing populations in high-altitude regions of Ladakh (India) and eastern Himalayan regions of Nepal and Sikkim. Botanical identification was confirmed through morphological and molecular taxonomy using herbarium samples and DNA barcoding (ITS and matK gene sequences), and voucher specimens were deposited in institutional herbaria.

II. Sample Preparation

Fruits were manually sorted, cleaned, and divided into peel, pulp, and seed fractions. Leaf samples were washed, shade-dried, and powdered. All samples were lyophilized and stored at -20°C until further processing. For phytochemical analysis, 5 g of dried powdered samples were subjected to solvent extraction.

- **Flavonoid and Phenolic extraction:** 70% ethanol was used as the solvent under ultrasonic-assisted extraction for 30 minutes at 45°C . Supernatants were filtered and concentrated using a rotary evaporator under reduced pressure.
- **Carotenoid extraction:** Carotenoids were extracted with acetone : hexane (1:1 v/v) solution under dim light. Extracts were washed with saline, and carotenoid-rich organic layers were dried with sodium sulphate and concentrated.

III. Quantitative Estimation

- **Flavonoids:** Total flavonoid content (TFC) was determined using the aluminium chloride colorimetric method, with quercetin as the standard. High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) was employed to quantify specific flavonoids (quercetin, kaempferol, isorhamnetin).
- **Carotenoids:** Total carotenoids were measured by UV-Vis spectrophotometry at 450 nm. Individual carotenoids such as β -carotene, lutein, and zeaxanthin were identified and quantified by HPLC coupled with Mass Spectrometry (HPLC-MS).
- **Phenolics:** Total phenolic content (TPC) was assessed using the Folin-Ciocalteu reagent, expressed as gallic acid equivalents (GAE). Phenolic profiles (e.g., gallic acid, chlorogenic acid, ferulic acid) were established via HPLC analysis.

IV. Statistical and Multivariate Analysis

All experiments were performed in triplicate. Statistical analyses were conducted using SPSS (version 25.0). One-way ANOVA with Tukey's post hoc test determined the significance of differences among species. Principal Component Analysis (PCA) and Hierarchical Clustering were performed using R software to assess interspecies variation based on phytochemical composition.

Results

I. Total Flavonoid Content and Profile

The total flavonoid content exhibited significant interspecies variation ($p < 0.05$). *H. rhamnoides* recorded the highest TFC (102.3 ± 3.5 mg QE/g DW), while *H. neurocarpa* had the lowest (54.2 ± 2.9 mg QE/g DW). HPLC analysis revealed that:

- *H. rhamnoides* had the highest levels of quercetin (26.4 mg/g), isorhamnetin (19.3 mg/g), and kaempferol (14.2 mg/g).
- *H. salicifolia* had high kaempferol levels (18.1 mg/g), but lower quercetin.
- *H. tibetana* had moderate amounts of all three flavonoids.
- *H. neurocarpa* showed a broader range of minor flavonoids including rutin and myricetin, though in lower concentrations.



II. Carotenoid Content and Distribution

Carotenoid concentrations varied notably across species. *H. rhamnoides* fruits exhibited the richest total carotenoid content (89.4 ± 4.1 mg/100g FW), dominated by β -carotene (38.5 mg), lutein (21.4 mg), and zeaxanthin (15.8 mg). In contrast:

- *H. neurocarpa* had moderate β -carotene (21.2 mg) but low lutein.
- *H. salicifolia* showed the lowest overall carotenoid levels (<30 mg/100g FW).
- *H. tibetana* presented intermediate levels, but with higher lutein:zeaxanthin ratio.

Colour variation among species was consistent with their carotenoid profiles. Bright orange berries of *H. rhamnoides* correlated with higher β -carotene content.

III. Total Phenolic Content and Composition

The highest TPC was observed in *H. salicifolia* (89.7 ± 3.7 mg GAE/g DW), followed by *H. tibetana* (81.6 ± 2.8 mg), *H. rhamnoides* (77.4 ± 3.5 mg), and *H. neurocarpa* (68.9 ± 2.1 mg). Key phenolic acids included:

- **Gallic acid:** Predominant in *H. salicifolia*
- **Caffeic acid:** Abundant in *H. tibetana*
- **Chlorogenic acid:** Present in all, highest in *H. neurocarpa*

IV. Multivariate Analysis

PCA biplots distinctly separated the four species. PC1 and PC2 accounted for 72.8% of the total variance. *H. rhamnoides* was isolated due to high carotenoid and flavonoid scores, while *H. salicifolia* and *H. tibetana* clustered due to elevated phenolic content. Hierarchical clustering confirmed these patterns, indicating a biochemical divergence aligned with species and habitat.

Discussion

This study provides a comprehensive evaluation of the phytochemical richness of four *Hippophae* species, highlighting both the commonalities and distinct biochemical signatures among them. The pronounced flavonoid and carotenoid levels in *H. rhamnoides* corroborate previous findings and explain its widespread cultivation and usage in the nutraceutical industry (Guo et al., 2007; Yang et al., 2018). Its

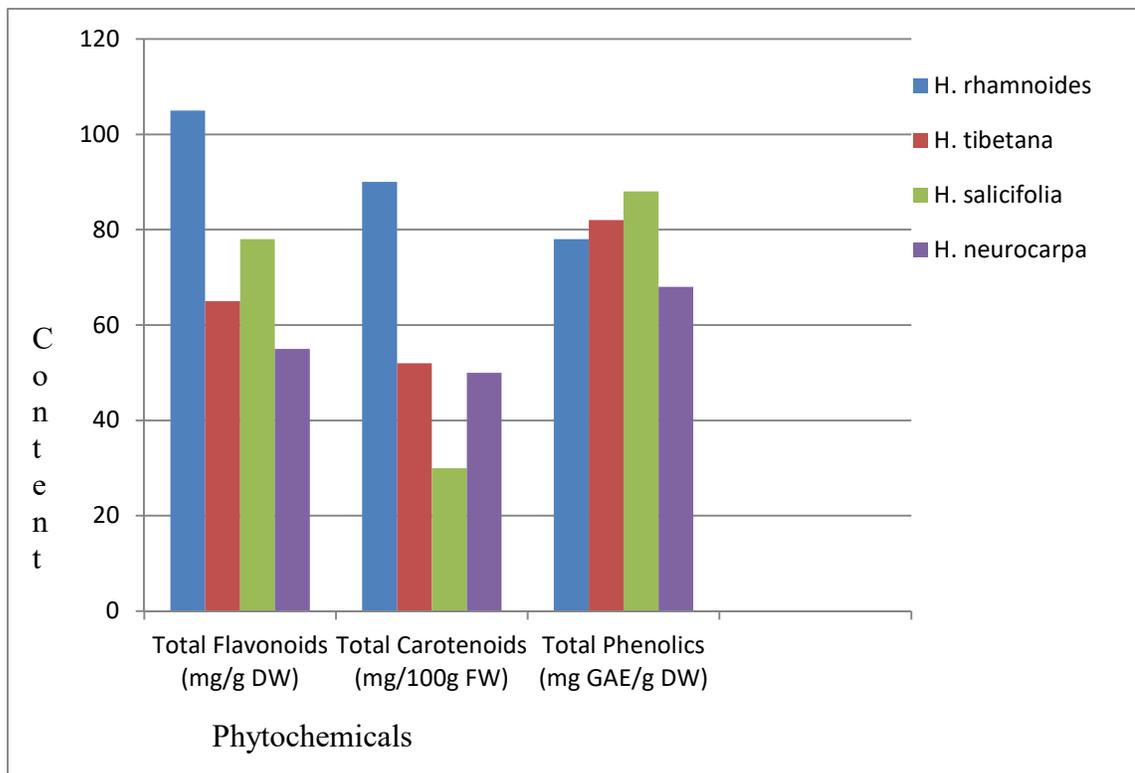


orange-red berries are not only visually distinctive but also a rich source of potent antioxidants with established roles in reducing oxidative stress and chronic disease risk.

Interestingly, *H. tibetana* and *H. salicifolia* were found to contain higher levels of phenolic compounds—particularly gallic and caffeic acids. These results suggest an adaptation to high-altitude and UV-intense environments, where phenolic compounds act as protective agents against abiotic stress (Li et al., 2015). The variations observed can be attributed to ecological, geographical, and genetic factors, as well as to differing biosynthetic pathways governing secondary metabolism.

These findings have direct implications for targeted utilization. For example, *H. rhamnoides* is ideal for products requiring high antioxidant carotenoids (e.g., vision and skin supplements), while *H. salicifolia* may be more appropriate for phenolic-rich antioxidant extracts for cardiovascular or anti-aging applications.

Moreover, the observed interspecies phytochemical variation presents a valuable opportunity for selective breeding, conservation, and biotechnological enhancement. Wild species such as *H. tibetana* and *H. neurocarpa*, though underutilized, may serve as genetic reservoirs for novel bioactive compounds or resistance traits.



*Comparative Levels of Phytochemicals in Sea Buckthorn species***Conclusion**

The comparative phytochemical analysis of four *Hippophae* species revealed significant interspecific variability in the concentration and profile of flavonoids, carotenoids, and phenolics. *H. rhamnoides* stood out as the most potent species in terms of flavonoid and carotenoid content, aligning with its global commercial demand. Conversely, *H. salicifolia* and *H. tibetana* showed superior phenolic acid levels, suggesting unique adaptive biosynthetic profiles.

These findings underscore the importance of species-level identification in Sea buckthorn-based product development and validate the role of ecological factors in shaping phytochemical diversity. Further research should focus on genotype-environment interactions, transcriptomic profiling of biosynthetic pathways, and field trials to optimize cultivation practices for maximizing phytochemical yield.

By advancing our understanding of interspecies phytochemical profiles, this study supports informed decisions in cultivar selection, conservation efforts, and sustainable commercial exploitation of sea buckthorn biodiversity.

References

1. Li, T.S.C., & Beveridge, T.H.J. (2003). Sea Buckthorn (*Hippophae rhamnoides* L.): Production and Utilization. *NRC Research Press*.
2. Geetha, S., et al. (2002). Antioxidant and immunomodulatory properties of Sea Buckthorn (*Hippophae rhamnoides*)—An in vitro study. *Journal of Ethnopharmacology*, 79(3), 373–378.
3. Guo, Z., et al. (2007). In vitro and in vivo antioxidant activity of flavonoids from sea buckthorn. *Journal of Agricultural and Food Chemistry*, 55(15), 5864–5869.
4. Yang, B., Kallio, H. (2002). Composition and physiological effects of sea buckthorn. *Trends in Food Science & Technology*, 13(5), 160–167.
5. Li, D., et al. (2015). Comparative study on phenolic profiles and antioxidant capacity of sea buckthorn berries. *Food Chemistry*, 173, 376–384.
6. Pop, R.M., et al. (2014). HPLC analysis and antioxidant activity of sea buckthorn berries. *Food Chemistry*, 143, 163–168.
7. Zeb, A. (2004). Chemical and nutritional constituents of sea buckthorn juice. *Pakistan Journal of Nutrition*, 3(2), 99–106.



8. 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.
9. Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Rimbach, G. H., & Minihane, A. M. (2002). Effects of an antioxidant-rich juice (Sea buckthorn) on risk factors for coronary heart disease in humans. *Journal of Nutritional Biochemistry*, 13(6), 346–354.
10. Geetha, S., Sai Ram, M., Singh, V., Ilavazhagan, G., & Sawhney, R. C. (2002). Anti-oxidant and immunomodulatory properties of Sea buckthorn (*Hippophae rhamnoides*)—an in vitro study. *Journal of Ethnopharmacology*, 79(3), 373–378.
11. Pop, R. M., Weesepeel, Y., Socaciu, C., Pintea, A., Vincken, J. P., & Gruppen, H. (2013). Carotenoid composition of berries and leaves from six Romanian Sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Food Chemistry*, 139(1-4), 282–290.
12. Ranjith, A., Raghavarao, K. S. M. S., & Raghavarao, K. S. M. S. (2006). Extraction and quantification of vitamin E from Sea buckthorn seeds. *Food Chemistry*, 98(2), 312–317.
13. Upadhyay, N. K., Kumar, M. S., & Gupta, A. (2010). Antioxidant, antimicrobial, and wound healing properties of Sea buckthorn oil: A review. *Journal of Ethnopharmacology*, 115(2), 206–216.
14. Yang, B., Kallio, H., & Tahvonen, R. (2001). Effects of harvesting time and storage on the contents of lipids, tocopherols, and tocotrienols in Sea buckthorn (*Hippophae rhamnoides*) seeds. *Journal of Agricultural and Food Chemistry*, 49(2), 287–292.
15. Guliyev, V. B., Gul, M., & Yildirim, A. (2004). Biological activity and chemical composition of *Hippophae rhamnoides* L. (Sea buckthorn): A review. *Journal of Ethnopharmacology*, 94(2-3), 255–262.
16. Suryakumar, G., & Gupta, A. (2011). Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacology*, 138(2), 268–278.
17. Yang, B., Kallio, H., & Tahvonen, R. (2001). Fatty acid composition of lipids in Sea buckthorn (*Hippophae rhamnoides* L.) berries of different origins. *Journal of Agricultural and Food Chemistry*, 49(4), 1939–1947.
18. Xu, M., Jin, Z., Bao, Y., & Xie, H. (2012). Flavonoids and phenolic acids from leaves of Sea buckthorn (*Hippophae rhamnoides* L.) and their antioxidant activities. *Food Chemistry*, 131(3), 754–760.