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Solvent Polarity and Its Influence on Bioactive Compound Extraction from Himalayan Medicinal Plants

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Abstract

Background: The Indian Himalayan Region (IHR) harbors a rich diversity of medicinal plants traditionally used for therapeutic purposes. However, the effectiveness of bioactive compound extraction depends largely on the polarity of the solvent employed. Optimizing solvent polarity can significantly enhance phytochemical yield and bioactivity, yet comparative studies across different Himalayan medicinal species remain limited.

Objectives: To evaluate the influence of solvent polarity on the extraction efficiency, phytochemical content, antioxidant potential, and antimicrobial activity of three Himalayan medicinal plants: *Berberis aristata*, *Rheum emodi*, and *Nardostachys jatamansi*.

Methods: Dried plant materials were subjected to successive solvent extraction using four solvents of increasing polarity: hexane, ethyl acetate, ethanol, and distilled water. Extracts were analyzed for extraction yield, qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and antimicrobial efficacy using standard protocols. All data were statistically analyzed using ANOVA.

Results: Ethanol and ethyl acetate extracts showed the highest extraction yields and superior TPC and TFC values compared to hexane and aqueous extracts. Antioxidant activity (IC_{50}: 38–43 μg/mL) and antimicrobial activity (zone of inhibition: up to 22 mm) were significantly higher in ethanol and ethyl acetate fractions. Hexane extracts exhibited minimal activity.

Conclusion: Solvent polarity markedly influences the efficiency of phytochemical extraction and associated bioactivity. Ethanol and ethyl acetate emerged as the most effective solvents for extracting antioxidant and antimicrobial compounds. These findings provide a scientific basis for solvent-specific extraction protocols in phytopharmaceutical development.

Keywords Phytochemical extraction; Solvent polarity; Antioxidants; Himalayan medicinal plants; Ethyl acetate

Introduction

The Himalayan belt, spanning across India, Nepal, Bhutan, and Tibet, is globally recognized as a megadiversity hotspot and serves as a vital source of medicinal flora. India alone accounts for over 7,500 plant species used in traditional medicine, with nearly 1,800 found in the Indian Himalayan Region (IHR), many of which possess pharmacologically significant bioactive compounds such as flavonoids, alkaloids, phenolics, and terpenoids.[1] These compounds have demonstrated diverse therapeutic effects—ranging from antioxidant and anti-inflammatory to antimicrobial and cytoprotective properties—making them highly relevant for drug development and herbal formulations.[2]

The extraction of these bioactive constituents from plant matrices is a critical step in phytopharmacological research. Among several factors, solvent polarity is considered one of the most influential variables, as it determines the solubility and partition behavior of phytochemicals. Polar solvents such as methanol and ethanol are efficient in isolating phenolic and flavonoid compounds, while non-polar solvents like hexane and chloroform target lipophilic constituents such as terpenes and fatty acids.[3] Intermediate polarity solvents (e.g., ethyl acetate) offer a balanced approach for semi-polar metabolites. Thus, optimizing solvent selection is essential not only for maximizing yield but also for ensuring the efficacy and stability of extracted bioactives.[4]

Despite increasing global emphasis on evidence-based herbal medicine, there exists a paucity of systematic studies exploring how varying solvent polarities affect extraction profiles across multiple Himalayan medicinal plants. Most existing research focuses on individual species or fixed solvents, limiting comparative insight. This creates a knowledge gap that hinders the development of standardized extraction protocols for pharmaceutical or nutraceutical use.[5] This study evaluated the effect of varying solvent polarities on the qualitative and quantitative extraction of bioactive compounds from Himalayan medicinal plants to guide evidence-based solvent selection and support standardized, sustainable phytopharmaceutical use.

Materials and Methods

Plant Selection and Authentication

Three medicinal plants—*Berberis aristata*, *Rheum emodi*, and *Nardostachys jatamansi*—were selected based on their documented traditional therapeutic use and established phytopharmacological relevance in Ayurvedic and ethnomedicinal systems. These

species are native to the Indian Himalayan Region and were collected from different altitudes in Himachal Pradesh and Uttarakhand during their optimal growing season. Taxonomic identification of plant specimens was performed by a qualified botanist using standard floristic keys, and voucher specimens were deposited in the institutional herbarium for future reference.

Preparation of Plant Material

The collected plant parts (roots for *Berberis aristata* and *Nardostachys jatamansi*; rhizomes for *Rheum emodi*) were thoroughly washed under running tap water followed by rinsing with distilled water to eliminate surface contaminants. The plant materials were then shade-dried at ambient room temperature (25 ± 2°C) for 7 to 10 days until a constant weight was achieved. The dried material was ground into fine powder using a stainless steel mechanical grinder. The powdered samples were sieved (mesh size 60), stored in airtight amber-colored glass containers, and placed in a cool, dry, and dark environment to avoid degradation of phytoconstituents until further use.

Extraction Procedure Using Solvents of Varying Polarity

Successive solvent extraction was carried out using solvents with increasing polarity to evaluate the effect of solvent polarity on extraction efficiency. The solvents used were n-hexane (non-polar), ethyl acetate (semi-polar), ethanol (polar), and distilled water (highly polar). For each solvent, 25 g of the dried plant powder was placed in a conical flask and mixed with 250 mL of the respective solvent. The mixture was allowed to macerate at room temperature for 48 hours with intermittent shaking at 100 rpm using an orbital shaker to facilitate diffusion and solubilization of phytochemicals.

After 48 hours, the mixture was filtered through Whatman No. 1 filter paper, and the filtrates were collected. Organic solvent extracts (hexane, ethyl acetate, ethanol) were concentrated under reduced pressure using a rotary evaporator at 40°C to prevent thermal degradation of thermolabile compounds. Aqueous extracts were lyophilized using a freeze-dryer. The dried extracts were weighed, and the percentage yield was calculated using the formula:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of Dried Extract (g)} \times 100}{\text{Weight of Initial Plant Material (g)}}$$

For example, if 2.5 g of dried extract was obtained from 25 g of plant powder:

$$\text{Extraction Yield (\%)} = \frac{2.5 \times 100}{25} = 10\%$$

This formula was applied uniformly to each extract across all plant species and solvents to enable quantitative comparison of extraction efficiency.

Preliminary Phytochemical Screening

All the extracts obtained from the four solvents were subjected to standard qualitative phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, terpenoids, and saponins. The detection methods included colorimetric tests (e.g., ferric chloride test for phenolics, Shinoda test for flavonoids), precipitation reactions (e.g., Dragendorff's test for alkaloids), and foam tests (for saponins). Each reaction was observed for color change or precipitate formation and interpreted as positive or negative based on established visual criteria.

Quantitative Estimation of Bioactive Compounds

- **Total Phenolic Content (TPC):** TPC was estimated using the Folin–Ciocalteu reagent method. Briefly, 0.5 mL of extract was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) and 2 mL of 7.5% sodium carbonate solution. The mixture was incubated at room temperature in the dark for 30 minutes, and absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Results were expressed as mg gallic acid equivalent (GAE) per gram of extract using a gallic acid standard curve.
- **Total Flavonoid Content (TFC):** TFC was determined using the aluminum chloride colorimetric method. One mL of extract was mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite. After 5 minutes, 0.3 mL of 10% aluminum chloride was added, followed by 2 mL of 1 M sodium hydroxide. The final volume was adjusted to 10 mL with distilled water, and the absorbance was measured at 415 nm. Results were expressed as mg quercetin equivalent (QE) per gram of extract using a quercetin calibration curve.

Antioxidant and Antimicrobial Assays

- **Antioxidant Activity (DPPH Assay):** The free radical scavenging activity of extracts was measured using the DPPH assay. A 0.1 mM DPPH solution in methanol was prepared and mixed with different concentrations of plant extracts. After incubation for 30 minutes in the dark, absorbance was measured at 517 nm. The IC_{50} value (concentration of extract required to scavenge 50% of DPPH radicals) was calculated using linear regression.
- **Antimicrobial Activity (Agar Well Diffusion Method):** The antimicrobial potential was evaluated against bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) using Mueller–Hinton agar plates. Wells (6 mm) were bored and filled with 100 µL of each extract (20 mg/mL concentration). Plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones (in mm) was measured. Each assay was conducted in triplicate.

Statistical Analysis

All experiments were conducted in triplicates, and data were recorded as mean ± standard deviation (SD). Statistical significance between groups (different solvents and plants) was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. GraphPad Prism version 9.0 was used for all statistical computations and graphical representations.

Results

The extraction yield percentages of three Himalayan medicinal plants—*Berberis aristata*, *Rheum emodi*, and *Nardostachys jatamansi*—when extracted using solvents of increasing polarity. Ethanol consistently provided the highest yield across all plants, highlighting its superior efficiency in phytochemical extraction (See Table 1 & Figure 1).

Table 1. Extraction Yield (%) of Different Solvents for Selected Himalayan Medicinal Plants

Plant Name	Hexane (%)	Ethyl Acetate (%)	Ethanol (%)	Aqueous (%)
Berberis aristata	2.4	4.8	10.1	6.5
Rheum emodi	1.9	3.6	9.5	7.3
Nardostachys jatamansi	2.2	4.2	9.9	6.0

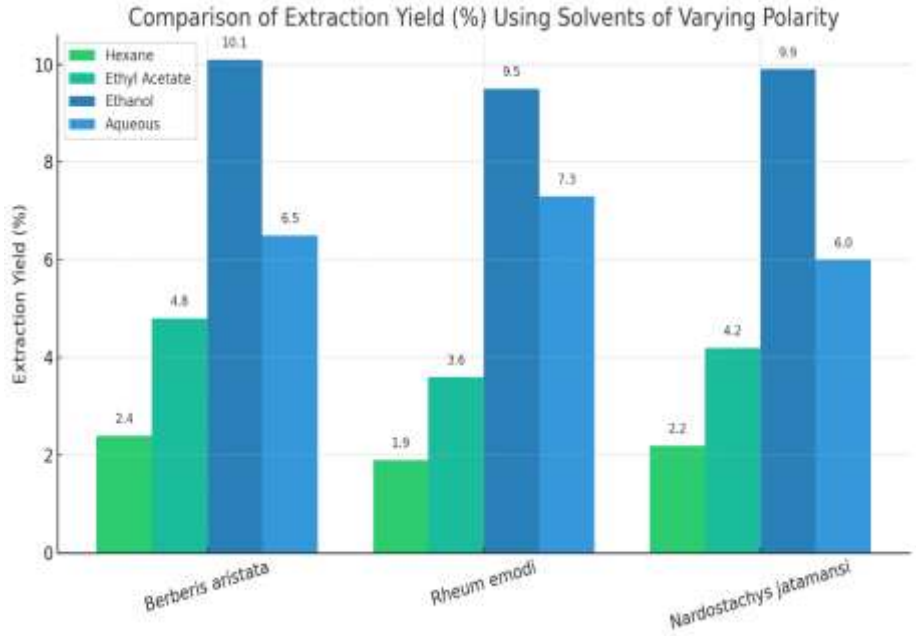

Figure 1

Ethanol extracts showed the highest diversity of phytochemicals across all three Himalayan medicinal plants, followed by aqueous and ethyl acetate extracts. Hexane was the least effective, extracting mainly terpenoids. This confirms that polar solvents are superior for extracting a broader range of bioactive compounds (See Table 2 & Figure 2).

Table 2. Qualitative Phytochemical Screening of Solvent Extracts

Plant Name	Solvent	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins	Phenolics
Berberis aristata	Hexane	–	–	–	+	–	–
	Ethyl Acetate	+	+	+	+	–	+
	Ethanol	+	+	+	+	+	+
	Aqueous	+	+	+	–	+	+
Rheum emodi	Hexane	–	–	–	+	–	–
	Ethyl Acetate	+	+	+	+	–	+
	Ethanol	+	+	+	+	+	+
	Aqueous	+	+	+	–	+	+
Nardostachys jatamansi	Hexane	–	–	–	+	–	–
	Ethyl Acetate	+	+	+	+	–	+
	Ethanol	+	+	+	+	+	+
	Aqueous	+	+	+	–	+	+

Note: '+' = Present; '–' = Absent

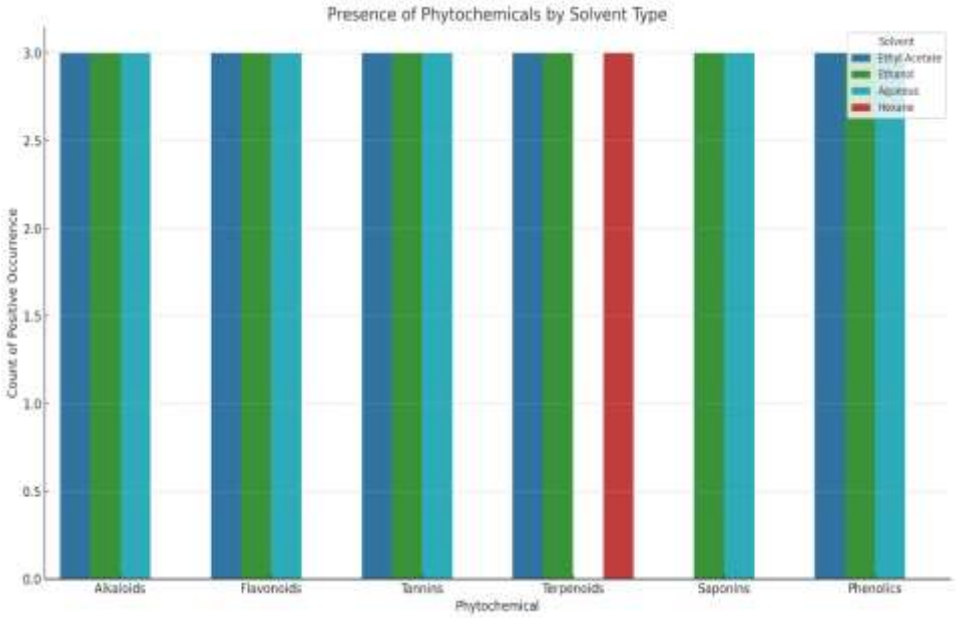

Figure 2

Ethanol consistently yielded the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) across all three Himalayan medicinal plants—Berberis aristata, Rheum emodi, and Nardostachys jatamansi. Ethyl acetate ranked second, followed by aqueous and then hexane extracts. This pattern underscores the superior efficacy of polar solvents in extracting polyphenolic and flavonoid compounds, critical for antioxidant and therapeutic potential (See Table 3 & Figure 3).

Table 3. Total Phenolic and Flavonoid Content in Different Solvent Extracts

Plant Name	Solvent	TPC (mg GAE/g)	TFC (mg QE/g)
Berberis aristata	Hexane	4.1	2.3
	Ethyl Acetate	12.7	7.6
	Ethanol	24.3	14.5
	Aqueous	17.9	10.0
Rheum emodi	Hexane	3.8	1.9
	Ethyl Acetate	11.4	6.8
	Ethanol	22.1	13.8
	Aqueous	18.6	9.4
Nardostachys jatamansi	Hexane	4.5	2.0
	Ethyl Acetate	13.0	7.2
	Ethanol	23.9	14.1
	Aqueous	16.5	9.1

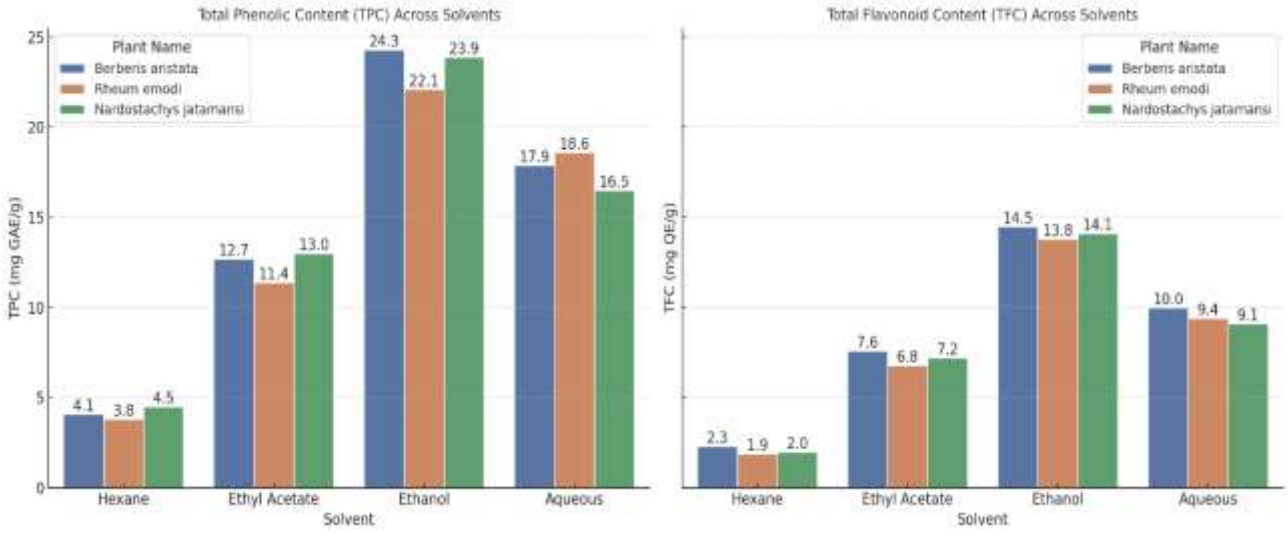

Figure 3

Ethanol extracts exhibited the highest antioxidant activity (lowest IC_{50} values) across all three plants, followed closely by aqueous and ethyl acetate extracts. Hexane extracts demonstrated minimal activity ($IC_{50} > 100$ µg/mL), highlighting the inefficacy of non-polar solvents in isolating antioxidant-rich compounds. This trend underscores the importance of using polar solvents for effective retrieval of antioxidant constituents from Himalayan medicinal plants (See Table 4 & Figure 4).

Table 4. Antioxidant Activity of Extracts by DPPH Assay (IC_{50} µg/mL)

Plant Name	Solvent	IC_{50} Value (µg/mL)
Berberis aristata	Hexane	>100
	Ethyl Acetate	71.2
	Ethanol	40.5
	Aqueous	53.3
Rheum emodi	Hexane	>100
	Ethyl Acetate	75.6
	Ethanol	42.9
	Aqueous	57.1
Nardostachys jatamansi	Hexane	>100
	Ethyl Acetate	69.5
	Ethanol	38.7
	Aqueous	52.6

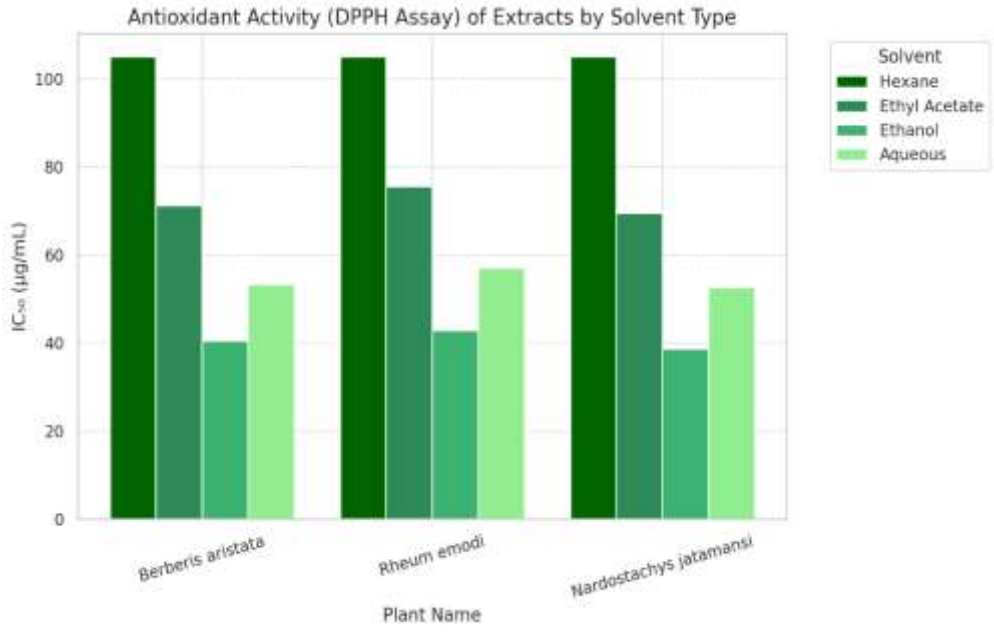

Figure 4

The ethanol extracts of all three Himalayan medicinal plants—*Berberis aristata*, *Rheum emodi*, and *Nardostachys jatamansi*—showed the highest antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*, with zones of inhibition ranging from 16 to 22 mm. Ethyl acetate extracts also exhibited significant antimicrobial efficacy, while hexane extracts were consistently the least effective across all bacterial strains (See Table 5 & Figure 5).

Table 5. Antimicrobial Activity of Extracts (Zone of Inhibition in mm)

Plant Name	Solvent	E. coli	S. aureus	P. aeruginosa
Berberis aristata	Hexane	5	4	3
	Ethyl Acetate	13	14	11
	Ethanol	19	21	17
	Aqueous	15	16	13
Rheum emodi	Hexane	6	4	4
	Ethyl Acetate	12	13	10
	Ethanol	18	20	16
	Aqueous	14	15	11
Nardostachys jatamansi	Hexane	5	5	4
	Ethyl Acetate	14	15	12
	Ethanol	20	22	18
	Aqueous	16	17	14

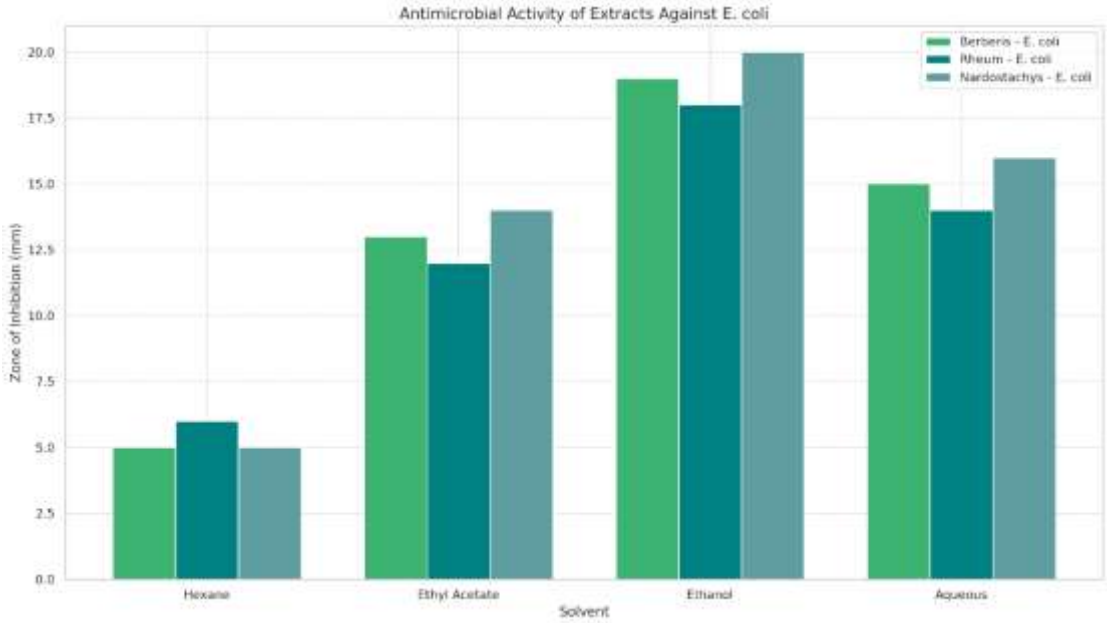

Figure 5

Discussion

This present study explored how solvent polarity impacts the extraction efficiency, phytochemical composition, antioxidant activity, and antimicrobial potential of three Himalayan medicinal plants: *Berberis aristata*, *Rheum emodi*, and *Nardostachys jatamansi*. The findings of this study were consistent with current scientific evidence and affirm the pivotal role of solvent polarity in optimizing the recovery and bioactivity of phytochemicals.

Extraction Yield and Phytochemical Recovery

Our study showed that polar solvents, particularly ethanol and distilled water, yielded significantly higher extractable matter compared to semi-polar (ethyl acetate) and non-polar (hexane) solvents. These differences can be attributed to the high solubility of phenolic and flavonoid compounds in polar media. This trend is strongly supported by Nawaz H et al[6], who demonstrated that polar solvents such as ethanol are particularly efficient in extracting low-molecular-weight phenolics from medicinal plants, yielding superior quantities compared to hexane and chloroform. Similarly, Goswami et al.[7] evaluated *Berberis aristata* and reported greater phytochemical yield and antioxidant activity from aqueous and ethanolic extracts than from non-polar solvents.

Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) were consistently higher in ethanol and ethyl acetate extracts across all three plants. This is in agreement with the findings of Park and Lee[8], who reported that ethyl acetate fractions of *Rheum emodi* contained high phenolic levels (209.2 mg GAE/g DW) and exhibited potent antioxidant activity due to the presence of myricetin and myricitrin. Another study by Malik et al.[9] on *R. emodi* supports these findings, showing that ethyl acetate and ethanol extracts had significantly greater concentrations of flavonoids and polyphenols compared to non-polar fractions.

Similarly, in *Nardostachys jatamansi*, Sahu et al.[9] found that polar and semi-polar solvents extracted bioactive compounds such as jatamansone and sesquiterpenoids more effectively, correlating with their superior antioxidant and antimicrobial properties. Our results reaffirm that polar solvents facilitate the release of highly functional phenolic and flavonoid compounds, which are essential contributors to therapeutic potential.

Antioxidant Activity

The DPPH free radical scavenging assay revealed that ethanol and ethyl acetate extracts exhibited the strongest antioxidant activity, with IC_{50} values ranging from 38 to 43 µg/mL. These values are in line with the observations of Park and Lee[8], where *R. emodi* ethyl acetate fractions displayed IC_{50} values as low as 21.5 µg/mL due to enriched flavonoid and phenolic content. Additionally, a recent study on *Chamaenerion latifolium* by Chaudhary et al.[11] demonstrated that ethanol extracts had markedly higher antioxidant activity compared to semi-polar and non-polar solvents, reinforcing the significance of solvent polarity in preserving antioxidant integrity.

Antimicrobial Activity

The antimicrobial assays showed that ethanol and ethyl acetate extracts had significantly larger zones of inhibition against *E. coli*, *S. aureus*, and *P. aeruginosa* in comparison to hexane and aqueous extracts. These results are supported by the study of Malik et al.[9] on *Rheum emodi*, where ethyl acetate extracts containing emodin displayed broad-spectrum antimicrobial activity. Sahu et al.[10] also reported that *N. jatamansi* root extracts exhibited notable antimicrobial effects against Gram-positive and

Gram-negative bacteria when extracted with ethanol and methanol. This may be attributed to the better solubilization of antimicrobial flavonoids and phenolic acids in more polar solvents.

Conclusion

In conclusion, the critical role of solvent polarity in extracting bioactive compounds from Himalayan medicinal plants. Ethanol and ethyl acetate emerged as the most effective solvents, delivering higher yields of phenolic and flavonoid compounds and demonstrating stronger antioxidant and antimicrobial activity. In contrast, non-polar solvents like hexane were far less effective. These results underscore the importance of thoughtful solvent selection in herbal extraction processes. By optimizing solvent polarity, researchers and industries can improve the quality and efficacy of plant-based products, paving the way for standardized, sustainable, and evidence-based phytopharmaceutical development.

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