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Dr. Kiriti Attri
 Lecturer in Botany,
 Department of Higher
 Education, Government
 Degree College, Bani, Kathua,
 Jammu and Kashmir, India

Anjali Garg
 Assistant Professor,
 Department of Zoology
 KVA DAV College for Women,
 Karnal, Haryana, India

***In vitro* phytochemical profiling and antioxidant evaluation of twelve traditionally used Ayurvedic medicinal plants from Jammu division, Jammu & Kashmir, India, with reference to Gynecological disorders**

Kiriti Attri and Anjali Garg

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Abstract

In vitro phytochemical characterization offers a scientific route for evaluating ethnobotanical claims and for guiding the development of standardized phytopharmaceuticals. This study examined the phytochemical composition, solvent-dependent extractability and interspecies variation of twelve Ayurvedic medicinal plants traditionally used across the Jammu Division (Jammu & Kashmir, India) for the management of gynecological complaints, including *Cissampelos pareira*, *Punica granatum*, *Berberis lyceum*, *Artemisia vulgaris*, *Vitex negundo*, *Cymbopogon citratus*, *Mentha longifolia*, *Asparagus racemosus*, *Ageratum conyzoides*, *Trachyspermum ammi*, *Cinnamomum zeylanicum* and *Foeniculum vulgare*. Authenticated plant material was sequentially extracted with hexane, ethyl acetate and methanol, followed by qualitative screening for major secondary metabolite classes and spectrophotometric quantification of total phenolic (TPC), flavonoid (TFC), alkaloid (TAC) and saponin (TSC) content. Antioxidant capacity was assessed using DPPH radical-scavenging and ferric reducing antioxidant power (FRAP) assays, and interrelationships among variables were examined using correlation, regression, principal component and hierarchical cluster analyses.

Methanol consistently produced the greatest extraction yield (mean 10.7%), followed by aqueous, ethyl acetate and hexane fractions, confirming a polarity-dependent recovery of phytoconstituents. *Vitex negundo*, *Cinnamomum zeylanicum* and *Punica granatum* showed the highest phenolic and flavonoid content and the strongest antioxidant activity (DPPH IC₅₀ 45.2, 49.6 and 51.8 µg/mL, respectively; FRAP 489.6, 469.2 and 457.9 µmol Fe²⁺/g, respectively), while *Berberis lyceum* and *Cissampelos pareira* were distinguished by high alkaloid content (4.28% and 3.92% w/w) and *Asparagus racemosus* and *Foeniculum vulgare* by high saponin content (31.4 and 26.5 mg diosgenin equivalents/g). Total phenolic and flavonoid contents correlated strongly with antioxidant activity ($r = 0.91-0.94$, $p < 0.01$), and principal component analysis resolved the species into three chemotypic clusters – polyphenolic-antioxidant, saponin-adaptogenic and alkaloid-uterotonic – that align closely with their respective traditional indications.

These findings provide a comparative phytochemical and antioxidant profile for regionally important Ayurvedic herbs used in women's reproductive healthcare and offer a scientific basis for their future standardization, quality control and pharmacological validation.

Keywords: Phytochemical screening, Ayurvedic medicinal plants, gynecological disorders, solvent extraction, antioxidant activity, Jammu Division

Introduction

Plant-based remedies remain central to primary healthcare for a large proportion of the world's population, and traditional systems such as Ayurveda continue to inform the management of women's reproductive disorders in South Asia. In the Jammu Division of Jammu & Kashmir, India, an ecologically diverse Himalayan transition zone spanning subtropical plains to temperate and subalpine belts, local healers, Vaidyas, Hakims and rural communities have long used regionally available herbs to treat dysmenorrhea, menorrhagia, leucorrhea, infertility and related gynecological conditions. Despite this rich ethnomedicinal record, systematic phytochemical validation of the plants concerned has been limited, and much of the existing regional literature remains descriptive rather than quantitative.

Corresponding Author:
Dr. Kiriti Attri
 Lecturer in Botany,
 Department of Higher
 Education, Government
 Degree College, Bani, Kathua,
 Jammu and Kashmir, India

Phytochemical profiling bridges this gap by identifying and quantifying the secondary metabolites—alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides—that underlie the pharmacological activity of medicinal plants. These compound classes are differentially soluble according to polarity, so the choice of extraction solvent strongly influences both the yield and the chemical composition of the resulting extract. A polarity-graded, sequential extraction approach (non-polar to polar solvents) is therefore widely used to obtain a comprehensive metabolite fingerprint of a given species.

The present study was undertaken to scientifically evaluate twelve Ayurvedic medicinal herbs traditionally used for gynecological disorders in the Jammu Division, selected on the basis of ethnobotanical prevalence, taxonomic authenticity and literature support. The specific objectives were to (i) document and authenticate the selected medicinal plants; (ii) perform qualitative and quantitative phytochemical screening of sequential hexane, ethyl acetate and methanol extracts; (iii) evaluate the antioxidant potential of the extracts using DPPH and FRAP assays; (iv) statistically assess interspecies and solvent-related variation; and (v) correlate the resulting phytochemical profiles with the traditional Ayurvedic indications of each species. In addressing these objectives, the study aims to provide a reproducible, comparative phytochemical and antioxidant atlas that can support the standardization, quality control and future pharmacological validation of these regionally important herbs.

Materials and Methods

Study area and plant collection

Plant material was collected from multiple districts of the Jammu Division (approximately 32°17'–33°13' N, 74°19'–75°20' E), a region encompassing subtropical plains, mid-hill zones and temperate forest belts (mean temperature 18–31 °C; relative humidity 45–80%; annual rainfall 1100–1400 mm; soil pH 6.5–7.5). Ethnobotanical surveys, comprising semi-structured interviews and informal discussions with traditional healers, elderly villagers and herbal practitioners conducted in Dogri, Gojri, Pahari and Hindi, were used to document local names, plant parts used, preparation methods, dosage and the specific gynecological conditions treated. Cross-verification among multiple informants was performed to ensure the reliability of ethnomedicinal claims. From an initial pool of approximately fifty candidate species, twelve were selected for phytochemical evaluation on the basis of frequency of traditional use, regional availability, literature support and taxonomic authenticity (Table 1). Voucher specimens were authenticated using standard regional floras, taxonomic keys and herbarium comparison, with expert verification from the Department of Ayurveda and the Department of Botany, University of Jammu, and cross-checking against herbarium material held at the Botanical Survey of India (Northern Regional Centre, Dehradun). Collected material was cleaned, shade-dried at 25–30 °C for 10–15 days, powdered using a stainless-steel grinder, passed through a 60-mesh sieve and stored in airtight amber containers under moisture-free, low-light conditions prior to extraction.

Chemicals, reagents and instrumentation

Analytical- and HPLC-grade solvents and reagents (hexane, ethyl acetate, methanol, chloroform, petroleum ether,

acetone, DMSO, Folin–Ciocalteu reagent, aluminium chloride, sodium carbonate, DPPH, gallic acid, quercetin, ascorbic acid) were procured from Sigma-Aldrich, HiMedia Laboratories and Merck India. Extraction and analysis were performed using a Soxhlet apparatus, rotary evaporator (Buchi R-300), UV–Vis spectrophotometer (Shimadzu UV-1800), analytical balance, pH meter, water bath, magnetic stirrer, centrifuge and refrigerated storage, with all experimental work conducted at DNA Labs – CRIS (Centre for Research and Innovative Studies), Dehradun, under controlled laboratory conditions (24 ± 2 °C, 50 ± 5% relative humidity).

Sequential solvent extraction

Fifty grams of powdered plant material from each species was extracted sequentially in a Soxhlet apparatus using solvents of increasing polarity – hexane, ethyl acetate, methanol and, subsequently, distilled water – for 8–10 h per solvent until the eluate became visibly pale.

Extracts were filtered, concentrated under reduced pressure at 40 °C using a rotary evaporator, and stored at 4 °C in amber vials. Extraction yield was calculated as the percentage ratio of the weight of dried extract to the weight of the initial powdered sample. Concentrated extracts were reconstituted in methanol or DMSO to prepare 10 mg/mL stock solutions for subsequent qualitative and quantitative assays.

Qualitative phytochemical screening

Standard colorimetric and precipitation tests were performed in triplicate on each extract to detect the major phytochemical classes: alkaloids (Mayer's, Dragendorff's and Wagner's tests), flavonoids (alkaline reagent and Shinoda tests), saponins (froth test), tannins/phenolics (ferric chloride and lead acetate tests), terpenoids/steroids (Liebermann–Burchard reaction) and glycosides (Keller–Killiani test), following established protocols (Harborne, 1998; Sofowora, 2008; Trease & Evans, 2009) [16, 22, 25]. Reaction intensities were scored semi-quantitatively (absent, mild, moderate or strong presence) and cross-checked using thin-layer chromatography.

Quantitative phytochemical assays

Total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method (absorbance at 765 nm, gallic acid equivalents, mg GAE/g dry extract; Singleton & Rossi, 1965, modified) [21]. Total flavonoid content (TFC) was determined by the aluminium chloride colorimetric assay (absorbance at 415 nm, quercetin equivalents, mg QE/g extract; Chang *et al.*, 2002) [15]. Total alkaloid content (TAC) was estimated by acid–base titrimetry of chloroform: ethanol: ammonia-extracted alkaloids using 0.02 N H₂SO₄ and methyl red indicator, expressed as% w/w (Harborne, 1998; Obadoni & Ochuco, 2001) [16, 19]. Total saponin content (TSC) was determined by the vanillin–sulfuric acid colorimetric method (absorbance at 544 nm, diosgenin equivalents, mg/g extract; Hiai, Oura, & Nakajima, 1976) [17]. All assays were performed in triplicate with five-point calibration curves (5–100 µg/mL; R₂ ≥ 0.98).

Antioxidant activity assays: DPPH radical-scavenging activity was assessed by combining extract (10–100 µg/mL) with 0.1 mM DPPH in methanol, incubating in the dark for 30 min, and measuring absorbance at 517 nm against a methanolic DPPH control; ascorbic acid served as the reference standard, and results were expressed as percentage

inhibition and IC₅₀ (Blois, 1958; Brand-Williams *et al.*, 1995) [13].

Ferric reducing antioxidant power (FRAP) was determined by reaction of the extract with a Fe³⁺–TPTZ-based FRAP reagent, incubation at 37 °C for 30 min, and measurement of absorbance at 593 nm, expressed as $\mu\text{mol Fe}^{2+}$ equivalents/g extract (Benzie & Strain, 1996) [12]. Both assays were performed on the methanolic extracts, which showed the highest phytochemical yield in preliminary screening.

Statistical analysis: All quantitative data are presented as mean $\pm$ standard deviation of triplicate determinations. One-

way ANOVA was used to test interspecies and inter-solvent differences, Pearson's correlation coefficient (*r*) was used to examine relationships among TPC, TFC, TAC, TSC and antioxidant indices, and multiple linear regression was used to model antioxidant activity as a function of phytochemical composition. Principal component analysis (PCA) and hierarchical cluster analysis (Ward's method, Euclidean distance) were applied to classify species according to overall chemotypic similarity. Analyses were performed in Microsoft Excel 2021, IBM SPSS Statistics 28 and OriginPro 2023, with significance set at $p < 0.05$.

Table 1: Selected medicinal plant species, family, part used and traditional relevance to gynecological disorders.

Botanical name	Family	Common name	Part used	Traditional relevance
<i>Cissampelos pareira</i> L.	Menispermaceae	Patha	Root	Uterine tonic, menstrual regulator
<i>Punica granatum</i> L.	Lythraceae	Pomegranate	Peel / fruit rind	Menorrhagia, uterine inflammation
<i>Berberis lyceum</i> Royle	Berberidaceae	Indian barberry	Root bark	Leucorrhea, infections
<i>Artemisia vulgaris</i> L.	Asteraceae	Mugwort	Aerial parts	Emmenagogue
<i>Vitex negundo</i> L.	Lamiaceae	Nirgundi	Leaves	PMS relief, hormonal balance
<i>Cymbopogon citratus</i> (DC.) Stapf	Poaceae	Lemongrass	Leaves	Antispasmodic
<i>Mentha longifolia</i> L.	Lamiaceae	Wild mint	Leaves	Dysmenorrhea
<i>Asparagus racemosus</i> Willd.	Liliaceae	Shatavari	Roots	Fertility enhancer
<i>Ageratum conyzoides</i> L.	Asteraceae	Goat weed	Whole plant	Vaginal infections
<i>Trachyspermum ammi</i> (L.) Sprague	Apiaceae	Ajwain	Seeds	Menstrual cramps
<i>Cinnamomum zeylanicum</i> Blume	Lauraceae	Cinnamon	Bark	Uterine stimulant
<i>Foeniculum vulgare</i> Mill.	Apiaceae	Fennel	Seeds	Menstrual regulator

Results

Extraction yield

Sequential extraction yielded a consistent solvent-dependent pattern across all twelve species, with mean yields following the order methanol ($10.73 \pm 0.74\%$) > aqueous ($6.37 \pm 0.79\%$) > ethyl acetate ($3.70 \pm 0.48\%$) > hexane ($2.12 \pm 0.54\%$) (one-way ANOVA, $p < 0.01$). The highest total yields (>23%) were obtained for *Vitex negundo*,

Cissampelos pareira and *Cinnamomum zeylanicum*, while *Cymbopogon citratus* gave the lowest overall recovery (20.7%). The methanolic fraction was consistently the richest across species, indicating a predominance of moderately polar to polar constituents such as flavonoids, tannins and saponins, consistent with the traditional preparation of these herbs as aqueous or hydroalcoholic decoctions.

Table 2: Extraction yield (%) of the studied plant species in hexane, ethyl acetate, methanol and aqueous solvents (mean of triplicate determinations).

Plant species	Hexane (%)	Ethyl acetate (%)	Methanol (%)	Aqueous (%)	Total (%)
<i>Cissampelos pareira</i>	2.8	4.2	10.4	5.6	23.0
<i>Punica granatum</i>	1.5	3.1	11.2	6.8	22.6
<i>Berberis lyceum</i>	2.2	3.9	9.7	7.1	22.9
<i>Artemisia vulgaris</i>	1.9	4.4	10.9	5.4	22.6
<i>Vitex negundo</i>	3.0	4.0	11.6	6.2	24.8
<i>Cymbopogon citratus</i>	1.8	3.6	9.5	5.8	20.7
<i>Mentha longifolia</i>	2.6	3.8	10.7	6.5	23.6
<i>Asparagus racemosus</i>	1.4	3.5	10.9	6.7	22.5
<i>Ageratum conyzoides</i>	2.1	4.1	9.8	5.9	21.9
<i>Trachyspermum ammi</i>	2.9	3.3	10.5	5.6	22.3
<i>Cinnamomum zeylanicum</i>	1.5	3.0	11.9	7.4	23.8
<i>Foeniculum vulgare</i>	1.7	2.8	10.4	7.6	22.5

Qualitative phytochemical screening

All twelve species tested positive for at least four of the six major phytochemical classes screened. Alkaloids were most strongly represented in *Cissampelos pareira* and *Berberis lyceum*; flavonoids in *Vitex negundo*, *Punica granatum* and *Mentha longifolia*; saponins in *Asparagus racemosus* and *Foeniculum vulgare*; and tannins/phenolics in *Punica granatum* and *Cinnamomum zeylanicum*. A phytochemical richness index (PRI, the summed intensity of positive reactions across compound classes) ranged from 7.8 (*Ageratum conyzoides*) to 9.4 (*Vitex negundo*). Across

solvents, methanol most effectively recovered alkaloids, flavonoids and phenolics, aqueous extracts were most effective for saponins and tannins, and hexane/ ethyl acetate fractions were enriched in terpenoids and steroids – a pattern consistent with the polarity of each metabolite class. Hierarchical clustering of the qualitative data grouped the species into three chemotypic clusters: a polyphenol-rich cluster (*Punica granatum*, *Cinnamomum zeylanicum*, *Vitex negundo*), an alkaloid/terpenoid-dominant cluster (*Cissampelos pareira*, *Berberis lyceum*, *Artemisia vulgaris*)

and a saponin-dominant cluster (*Asparagus racemosus*, *Foeniculum vulgare*, *Mentha longifolia*).

Quantitative phytochemical content

Total phenolic content averaged 72.8 ± 3.1 mg GAE/g across species, with the highest values recorded for *Vitex negundo* (91.7 mg GAE/g), *Cinnamomum zeylanicum* (89.4 mg GAE/g) and *Punica granatum* (87.5 mg GAE/g), and the lowest for *Ageratum conyzoides* (58.7 mg GAE/g). Total flavonoid content followed a closely parallel trend (mean 63.4 ± 2.4 mg QE/g), again led by *Vitex negundo* (79.1 mg QE/g) and *Cinnamomum zeylanicum* (74.2 mg QE/g), with a

strong TPC–TFC correlation ($r = 0.94$, $p < 0.01$) indicating overlapping biosynthetic origins. Total alkaloid content was highest in *Berberis lyceum* (4.28% w/w) and *Cissampelos pareira* (3.92% w/w), consistent with their classification as alkaloid-bearing uterotonic species, and lowest in *Asparagus racemosus* (0.85%) and *Foeniculum vulgare* (0.98%). Conversely, total saponin content was greatest in *Asparagus racemosus* (31.4 mg diosgenin eq./g) and *Foeniculum vulgare* (26.5 mg/g), consistent with their traditional use as fertility-enhancing and galactagogue agents (Table 3).

Table 3: Comparative quantitative phytochemical parameters of the studied plant species (mean $\pm$ SD, $n = 3$).

Plant species	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (% w/w)	TSC (mg/g)	Dominant group
<i>Cissampelos pareira</i>	62.4 ± 1.8	49.3 ± 1.4	3.92 ± 0.15	22.3 ± 0.9	Alkaloids
<i>Punica granatum</i>	87.5 ± 2.5	71.4 ± 2.2	1.64 ± 0.10	18.5 ± 0.8	Phenolics
<i>Berberis lyceum</i>	71.2 ± 1.9	59.9 ± 1.7	4.28 ± 0.19	15.6 ± 0.7	Alkaloids
<i>Artemisia vulgaris</i>	64.1 ± 1.7	62.4 ± 1.9	2.31 ± 0.12	14.8 ± 0.6	Terpenoids
<i>Vitex negundo</i>	91.7 ± 2.4	79.1 ± 2.1	1.95 ± 0.11	25.7 ± 1.0	Flavonoids
<i>Cymbopogon citratus</i>	60.9 ± 1.8	56.3 ± 1.8	1.34 ± 0.09	16.4 ± 0.7	Terpenoids
<i>Mentha longifolia</i>	73.6 ± 2.1	65.7 ± 2.0	1.72 ± 0.10	19.1 ± 0.8	Flavonoids
<i>Asparagus racemosus</i>	68.5 ± 2.3	58.2 ± 1.6	0.85 ± 0.07	31.4 ± 1.2	Saponins
<i>Ageratum conyzoides</i>	58.7 ± 1.5	46.5 ± 1.3	2.85 ± 0.14	17.2 ± 0.7	Alkaloids
<i>Trachyspermum ammi</i>	65.9 ± 2.0	63.5 ± 1.9	1.43 ± 0.09	20.6 ± 0.8	Flavonoids
<i>Cinnamomum zeylanicum</i>	89.4 ± 2.7	74.2 ± 2.3	1.27 ± 0.08	18.9 ± 0.9	Phenolics
<i>Foeniculum vulgare</i>	69.2 ± 1.8	67.1 ± 2.0	0.98 ± 0.06	26.5 ± 1.0	Saponins

Antioxidant activity

DPPH radical-scavenging activity and FRAP values both ranked *Vitex negundo* as the most potent antioxidant species (IC₅₀ 45.2 μ g/mL; FRAP 489.6 μ mol Fe²⁺/g), followed closely by *Cinnamomum zeylanicum* (IC₅₀ 49.6 μ g/mL; FRAP 469.2 μ mol Fe²⁺/g) and *Punica granatum* (IC₅₀ 51.8 μ g/mL; FRAP 457.9 μ mol Fe²⁺/g); all three exceeded 84% DPPH inhibition at 100 μ g/mL. *Ageratum conyzoides* and *Cymbopogon citratus* showed the weakest activity in both assays (IC₅₀ > 80 μ g/mL; FRA $P < 275$ μ mol Fe²⁺/g). Mean

IC₅₀ and FRAP values across all species were 63.4 ± 3.9 μ g/mL and 382.8 ± 9.0 μ mol Fe²⁺/g, respectively. A composite antioxidant efficiency index (AEI), normalizing both assays to a 0–1 scale, reproduced the same species ranking (Table 4), and both TPC and TFC correlated strongly with antioxidant activity (TPC vs. DPPH IC₅₀, $r = -0.91$; TPC vs. FRAP, $r = 0.93$; $p < 0.01$ for both), confirming phenolic and flavonoid content as the principal determinants of radical-scavenging and reducing capacity in this plant set.

Table 4: Comparative DPPH and FRAP antioxidant activity and composite antioxidant efficiency index (AEI) of the studied plant species, ranked from highest to lowest activity.

Plant species	DPPH IC ₅₀ (μ g/mL)	FRAP (μ mol Fe ²⁺ /g)	AEI	Rank
<i>Vitex negundo</i>	45.2	489.6	1.00	1
<i>Cinnamomum zeylanicum</i>	49.6	469.2	0.96	2
<i>Punica granatum</i>	51.8	457.9	0.92	3
<i>Mentha longifolia</i>	59.2	402.7	0.84	4
<i>Foeniculum vulgare</i>	60.3	383.9	0.80	5
<i>Trachyspermum ammi</i>	65.0	377.6	0.78	6
<i>Berberis lyceum</i>	67.4	374.5	0.77	7
<i>Asparagus racemosus</i>	70.8	353.5	0.74	8
<i>Cissampelos pareira</i>	72.5	321.4	0.71	9
<i>Artemisia vulgaris</i>	78.2	298.7	0.66	10
<i>Cymbopogon citratus</i>	81.3	272.8	0.62	11
<i>Ageratum conyzoides</i>	85.1	260.4	0.60	12

Correlation and multivariate analysis

Pearson correlation analysis across all species confirmed strong positive relationships between TPC and TFC ($r = 0.94$), and between both parameters and antioxidant capacity (TPC–DPPH $r = 0.91$; TPC–FRAP $r = 0.93$; TFC–DPPH $r = 0.89$; TFC–FRAP $r = 0.86$; all $p < 0.01$), whereas TAC correlated only weakly with these variables ($r < 0.5$), suggesting a limited direct contribution of alkaloids to antioxidant capacity. Multiple linear regression using TPC, TFC, TAC and TSC as predictors explained 94% of the

variance in the composite antioxidant index ($R^2 = 0.94$, $p < 0.01$), with TPC and TFC contributing the largest coefficients.

Principal component analysis reduced the six measured variables (TPC, TFC, TAC, TSC, DPPH, FRAP) to two dominant components explaining 81.6% of total variance (PC1 = 63.0%, PC2 = 18.6%). PC1 was defined by high loadings of TPC, TFC, DPPH and FRAP and represented overall polyphenolic-antioxidant strength, while PC2 was associated with TAC and TSC and reflected saponin–

alkaloid variation. Hierarchical cluster analysis (Ward's method) corroborated the PCA groupings, resolving the twelve species into a polyphenolic-antioxidant cluster (*Vitex negundo*, *Cinnamomum zeylanicum*, *Punica granatum*, *Mentha longifolia*), a saponin-adaptogenic cluster

(*Asparagus racemosus*, *Foeniculum vulgare*), an alkaloid/terpenoid-dominant cluster (*Berberis lyceum*, *Cissampelos pareira*, *Artemisia vulgaris*) and a lower-diversity cluster (*Cymbopogon citratus*, *Ageratum conyzoides*, *Trachyspermum ammi*).

Table 5: Pearson correlation matrix among phytochemical and antioxidant parameters

Parameter	TPC	TFC	TAC	TSC	DPPH (–IC50)	FRAP
TPC	1.000	0.937	0.481	0.633	0.910	0.934
TFC	0.937	1.000	0.512	0.598	0.892	0.861
TAC	0.481	0.512	1.000	0.382	0.435	0.421
TSC	0.633	0.598	0.382	1.000	0.689	0.702
DPPH (–IC50)	0.910	0.892	0.435	0.689	1.000	0.927
FRAP	0.934	0.861	0.421	0.702	0.927	1.000

Discussion: The extraction and phytochemical data obtained here reinforce the established principle that solvent polarity governs both the yield and the compound-class selectivity of plant extracts. The consistently higher methanolic yields across all twelve species, together with methanol's superior recovery of alkaloids, flavonoids and phenolics, support the continued use of hydroalcoholic media in Ayurvedic preparation while indicating that aqueous decoctions preferentially capture saponins and tannins – a pattern that maps closely onto traditional preparation methods for these herbs.

The species-level phytochemical hierarchy identified in this study aligns closely with documented traditional indications. *Vitex negundo*, *Cinnamomum zeylanicum* and *Punica granatum*, the three species with the highest phenolic and flavonoid content, also showed the strongest antioxidant activity in both DPPH and FRAP assays, consistent with reports describing polyphenol-driven antioxidant and anti-inflammatory activity of *Vitex negundo* and *Cinnamomum zeylanicum* in other regional studies (Anwar *et al.*, 2020; Ajeet *et al.*, 2022; Abu-Darwish *et al.*, 2020) [7, 3], and with prior phenolic quantification of *Punica granatum* (Tavakkoli *et al.*, 2021; Ahmad *et al.*, 2021) [23, 2]. The dominance of alkaloids in *Berberis lyceum* and *Cissampelos pareira* corresponds to their traditional classification as uterotonic and antimicrobial agents, in agreement with previous work implicating berberine-type alkaloids in the modulation of uterine smooth muscle and reproductive hormone pathways (Arif *et al.*, 2021) [8]. Similarly, the high saponin content of *Asparagus racemosus* and *Foeniculum vulgare* is consistent with their long-standing use as fertility-enhancing and lactogenic agents (Alok *et al.*, 2013; Babu *et al.*, 2022) [5, 11], and with earlier saponin quantification of *Asparagus racemosus* (Tiwari *et al.*, 2023) [24].

The strong statistical correlation between total phenolic/flavonoid content and antioxidant capacity ($r = 0.91–0.94$) indicates that hydroxylated aromatic structures are the principal biochemical drivers of free-radical scavenging and reducing activity in this plant set, most plausibly through hydrogen-atom transfer and single-electron transfer mechanisms. Given that oxidative stress is implicated in the pathophysiology of several gynecological conditions, including dysmenorrhea and endometrial inflammation, the antioxidant profiles reported here provide a plausible biochemical rationale – though not direct mechanistic proof – for the traditional use of these polyphenol-rich species in managing oxidative reproductive disorders. The clustering of species by PCA and hierarchical analysis into polyphenolic-antioxidant, saponin-adaptogenic

and alkaloid-uterotonic chemotypes further suggests that distinct biochemical strategies may underlie the different traditional applications documented for these plants, offering a testable framework for prioritizing candidates for future pharmacological work.

Several limitations should be considered when interpreting these findings. Colorimetric assays for TPC, TFC, TAC and TSC are semi-quantitative and can be confounded by non-specific reducing agents; more specific techniques such as HPLC-DAD or LC-MS/MS would be needed to identify and quantify individual marker compounds. Antioxidant activity was assessed using two chemical assays (DPPH, FRAP) that serve as proxies for, but do not establish, *in vivo* pharmacological efficacy, and no cytotoxicity, receptor-binding or animal/clinical data were generated in this study. Sampling was also restricted to a limited number of sites per species, so intraspecific chemotypic variation across the full altitudinal and climatic gradient of the Jammu Division may not be fully captured. Future work incorporating chromatographic marker-compound identification, receptor-based or enzyme-inhibition bioassays, multi-site and multi-seasonal sampling, and *in vivo* toxicological and efficacy testing would be valuable next steps toward pharmacopoeial standardization of these species.

Conclusion: This study provides a comparative *in vitro* phytochemical and antioxidant profile of twelve Ayurvedic medicinal plants traditionally used for gynecological disorders in the Jammu Division. Methanol was the most effective extraction solvent across all species, and distinct chemotypic groupings – polyphenolic, alkaloid-rich and saponin-rich – were identified that correspond closely with the traditional therapeutic indications of the respective species. *Vitex negundo*, *Cinnamomum zeylanicum* and *Punica granatum* emerged as the most promising candidates for further antioxidant-related pharmacological investigation, while *Berberis lyceum*, *Cissampelos pareira*, *Asparagus racemosus* and *Foeniculum vulgare* showed chemical profiles consistent with their traditional uterotonic and fertility-related applications. These findings offer a scientific basis for the standardization, quality control and continued pharmacological evaluation of regionally important Ayurvedic herbs used in women's reproductive healthcare, and lay a foundation for future chromatographic, mechanistic and *in vivo* studies.

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Conflict of Interest

The authors declare no conflict of interest.

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