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Concentration–Response Evidence for Antioxidant, α-Amylase Inhibitory, Anti-Denaturation and Antibacterial Activities of Four Himalayan Medicinal Plants: An Endpoint-Specific Comparison

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Abstract—

Comparative screening of medicinal plants often ranks species by a single potency value, although different assays measure different properties and half-maximal estimates depend on how they are derived. This study compares extracts of four Himalayan medicinal plants—*Bergenia ligulata* (underground organ), *Thalictrum foliolosum* (leaf), *Selinum tenuifolium* and *Wulfenia amherstiana* (underground organs)—across phenolic and flavonoid responses, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, α-amylase inhibition, inhibition of heat-induced protein denaturation and agar-diffusion antibacterial screening, each tested at 50–250 µg/ml. Each endpoint is interpreted separately, and each reported half-maximal concentration (IC_{50}) is checked against the concentration interval in which the measured curve crosses 50%. *S. tenuifolium* and *W. amherstiana* gave the strongest DPPH responses (90.6% and 91.2% at 250 µg/ml), whereas *B. ligulata* did not reach 50%. All four extracts inhibited α-amylase (67–71% at 250 µg/ml) and protein denaturation (72–83%) more strongly than the reference compounds tested in the same series. Of 15 reported IC_{50} values, 8 lay within the observed crossing interval, 4 were extrapolated beyond the tested range and 3 contradicted the measured data. Normalised phenolic and flavonoid totals rose 1.8- to 3.4-fold with assay concentration, indicating a calculation or working-range problem. The results identify preparations that merit confirmation but do not establish therapeutic efficacy, and they show that potency rankings should be reported with the evidence that supports them.

Index Terms—*Medicinal plants, Himalaya, DPPH, α-amylase, protein denaturation, antibacterial screening, IC50, concentration–response, phenolic content*

1. Introduction

Herbal medicines are widely used, and the safety and quality of many preparations remain poorly characterised [1]. Natural products also remain an important source of new drugs [2], which motivates laboratory screening of traditionally used plants. However, the value of such screening depends on how carefully the plant material, the extract and the assay are described. Guidelines for pharmacological research on extracts stress that results apply to a defined preparation, not to a species in general [3].

Many Himalayan medicinal plants are harvested from the wild, often for their underground organs, and several are under conservation pressure [4]. Four such plants were studied here. *Bergenia ligulata* is used for urinary and inflammatory complaints and is rich in phenolic constituents such as bergenin [5]. *Thalictrum* species contain isoquinoline alkaloids and have diverse traditional uses [6]. Root extracts of *Selinum tenuifolium* have shown antioxidant and antibacterial activity [7], and a flavone isolated from *Wulfenia amherstiana* has shown antimicrobial and antioxidant potential [8].

Screening studies commonly summarise activity as an IC_{50} and rank species accordingly. This has two weaknesses. First, assays measure different properties, so a ranking on one endpoint does not transfer to another. Second, an IC_{50} is only as reliable as the data and procedure behind it; a value that lies outside the tested range, or that disagrees with the measured curve, should not be used for ranking. This paper, derived from a doctoral thesis, makes three contributions:

1) An endpoint-by-endpoint comparison of four Himalayan medicinal plants under a common concentration series.
2) A simple consistency check that compares each reported IC_{50} with the interval in which the measured curve crosses 50%.
3) An evidence-graded interpretation that separates what each assay shows from what it cannot show, with the experiments needed to strengthen each claim.

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2. Related Work

2.1 Chemical and Biological Screening Assays

Total phenolic content is commonly estimated with the Folin–Ciocalteu reagent and expressed as gallic acid equivalents [9]; flavonoid content is estimated colorimetrically against quercetin [10]. Phenolics are linked to antioxidant activity, but the reagent responds to many reducing substances [11]. The DPPH assay measures the loss of a stable radical's absorbance [12]; its results depend on reaction time, solvent and the ratio of antioxidant to radical, which complicates comparison between studies [13]. α-Amylase inhibition is commonly measured with dinitrosalicylic acid detection of reducing sugars [14] and is used to screen for plants that may slow starch digestion [15]. Inhibition of heat-induced protein denaturation is a simple screen related to anti-inflammatory drug action [16].

2.2 Antibacterial Screening

Agar-diffusion methods are simple and widely used, but zone size depends on how well the extract diffuses through agar as well as on its activity, so diffusion results are qualitative [17]. Broth or microplate dilution methods that give a minimal inhibitory concentration are preferred for comparing potency [18].

2.3 Research Gap

Earlier work on these plants has examined single species or single assays [7], [8], [19]. A comparison of all four under the same concentration series, with explicit checks on how potency estimates were derived, has not been reported.

3. Materials and Methods

3.1 Plant Material and Extracts

Plant material was collected in Himachal Pradesh, identified by comparison with floristic descriptions and herbarium material, shade-dried and powdered. Table I lists the preparations. The experimental names are retained; current accepted names are noted because botanical updates affect traceability. Because the *T. foliolosum* preparation was made from leaves while the others came from underground organs, differences between species are partly confounded with organ.

TABLE I PLANT MATERIALS AND PREPARATIONS COMPARED

Species (experimental name)	Family	Organ	Extracts used
Bergenia ligulata	Saxifragaceae	Underground organ	Methanolic; aqueous and ethanolic (antibacterial)
Thalictrum foliolosum	Ranunculaceae	Leaf	Methanolic; aqueous and ethanolic (antibacterial)
Selinum tenuifolium	Apiaceae	Underground organ	Methanolic; aqueous and ethanolic (antibacterial)
Wulfenia amherstiana (now *Wulfeniopsis amherstiana*)	Plantaginaceae	Underground organ	Methanolic; aqueous and ethanolic (antibacterial)

3.2 Chemical and Biological Assays

Phenolic responses were expressed as mg gallic acid equivalents (GAE) per gram and flavonoid responses as mg quercetin equivalents (QE) per gram [9], [10]. DPPH scavenging was measured with ascorbic acid as reference [12]; α-amylase inhibition with acarbose as reference [14]; and inhibition of heat-induced albumin denaturation with aspirin as reference [16]. Each assay used extract concentrations of 50, 100, 150, 200 and 250 µg/ml in three replicates. Antibacterial activity of aqueous and ethanolic extracts was screened by agar-well diffusion against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella abony*, with wells about 6 mm in diameter [17].

3.3 Consistency Check of Half-Maximal Estimates

For each concentration–response series, the crossing interval was defined as the pair of adjacent tested concentrations between which the mean inhibition first reaches 50%. If inhibition already exceeded 50% at 50 µg/ml, the interval was "below range"; if it remained under 50% at 250 µg/ml, it was "above range". Each reported IC_{50} was classed as consistent (inside the crossing interval), extrapolated (outside the tested range but on the correct side) or contradictory (on the wrong side of the measured data). No new IC_{50}

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values were calculated, because the raw absorbance data and fitting procedure were not available; the check uses only the reported means.

Procedure 1: Consistency check of a reported IC_{50}

Input: mean inhibition $y_1 \ldots y_5$ at c = 50, 100, 150, 200, 250 µg/ml; reported IC_{50}
Output: crossing interval and consistency class
1) **if** $y_1 \geq 50$ **then** interval ← (0, 50) — below range
2) **else if** $y_5 < 50$ **then** interval ← (250, ∞) — above range
3) **else** interval ← (c_{i-1}, c_i) for the first i with $y_i \geq 50$
4) **if** IC_{50} lies in a bracketed interval **then** class ← consistent
5) **else if** interval is open and IC_{50} lies on its open side **then** class ← extrapolated
6) **else** class ← contradictory; flag for recalculation from raw data

The overall analytical workflow is shown in Fig. 1.

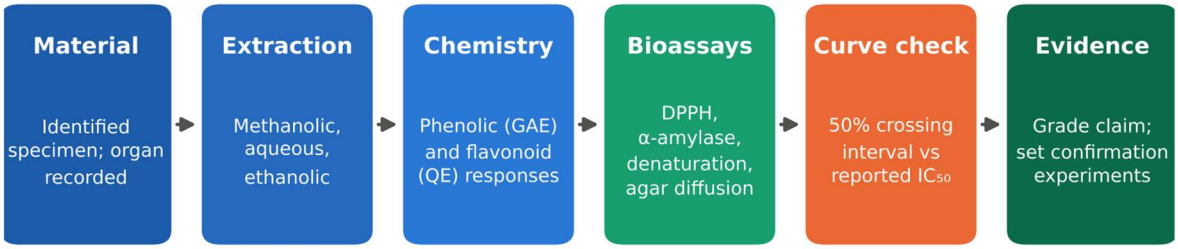


Fig. 1. Analytical workflow, from identified material to an evidence-graded interpretation. The curve-check step compares reported IC_{50} values with the measured concentration–response data.

4. Results

4.1 Concentration–Response Profiles

Fig. 2 shows the three concentration–response series, and Table II gives the responses at the lowest and highest concentrations. In the DPPH assay, *S. tenuifolium* and *W. amherstiana* rose from 46.5% and 56.1% at 50 µg/ml to 90.6% and 91.2% at 250 µg/ml, and the *T. foliolosum* leaf extract rose from 64.35% to 88.20%. *B. ligulata* responded weakly, reaching only 47.24%. In the α-amylase assay, the four extracts converged at high concentration (67.1–70.7% at 250 µg/ml) but differed at 50 µg/ml, where *B. ligulata* (36.42%) and *T. foliolosum* (32.14%) exceeded *S. tenuifolium* (20.14%) and *W. amherstiana* (16.24%). In the denaturation assay, all extracts showed graded responses, with *B. ligulata* and *W. amherstiana* reaching 82.5% and 82.6%.

TABLE II MEAN INHIBITION (%) AT THE LOWEST AND HIGHEST TESTED CONCENTRATION

Preparation	DPPH 50	DPPH 250	α-Amylase 50	α-Amylase 250	Denaturation 50	Denaturation 250
B. ligulata	35.79	47.24	36.42	67.14	30.10	82.50
T. foliolosum	64.35	88.20	32.14	70.74	25.30	71.60
S. tenuifolium	46.50	90.60	20.14	67.79	33.10	75.60
W. amherstiana	56.10	91.20	16.24	69.45	32.30	82.60
Reference	17.38	41.63	20.54	61.42	14.90	47.90

Ascorbic acid (DPPH), acarbose (α-amylase) and aspirin (denaturation). Concentrations in µg/ml.

The reference compounds responded weakly in this series. Ascorbic acid reached only 41.63% DPPH scavenging at 250 µg/ml, although it is normally a rapid and potent scavenger in this assay [12], [13]. The apparent superiority of every extract over the references therefore cannot be accepted at face value; it may reflect reaction conditions, reference preparation or background correction, and needs to be re-examined before extract-to-reference comparisons are used.

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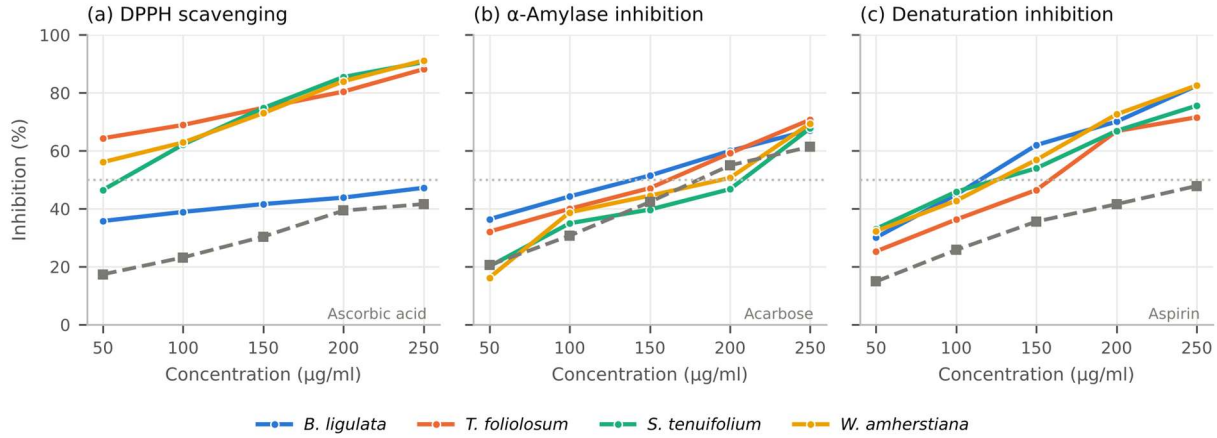


Fig. 2. Concentration–response profiles of the four extracts (solid) and the reference compound (dashed) for (a) DPPH radical scavenging, (b) α-amylase inhibition and (c) inhibition of heat-induced protein denaturation. The dotted line marks 50% inhibition. Means of three replicates as reported.

4.2 Consistency of Reported Half-Maximal Estimates

Fig. 3 and Table III compare each reported IC_{50} with the crossing interval. Eight values were consistent: all four denaturation estimates, the α-amylase estimates for *B. ligulata*, *T. foliolosum* and acarbose, and the DPPH estimate for *S. tenuifolium*. Four were extrapolated beyond the tested range: *B. ligulata* (302.6 µg/ml) and ascorbic acid (301.5 µg/ml) for DPPH, aspirin (252.9 µg/ml), and *W. amherstiana* DPPH (21.64 µg/ml). These estimates point in the right direction but are less secure because no measurement brackets them.

Three values contradicted the measured data. The *T. foliolosum* DPPH extract already exceeded 50% at 50 µg/ml, yet its IC_{50} was reported as 109.5 µg/ml. For α-amylase, *S. tenuifolium* crossed 50% only between 200 and 250 µg/ml and *W. amherstiana* between 150 and 200 µg/ml, but their reported values were 144.8 and 130.8 µg/ml. These three values should not be used for ranking until they are recalculated from the raw absorbance data. If they were accepted, *W. amherstiana* would appear to be the most potent α-amylase inhibitor, whereas its measured response at 50 µg/ml was the weakest of the four.

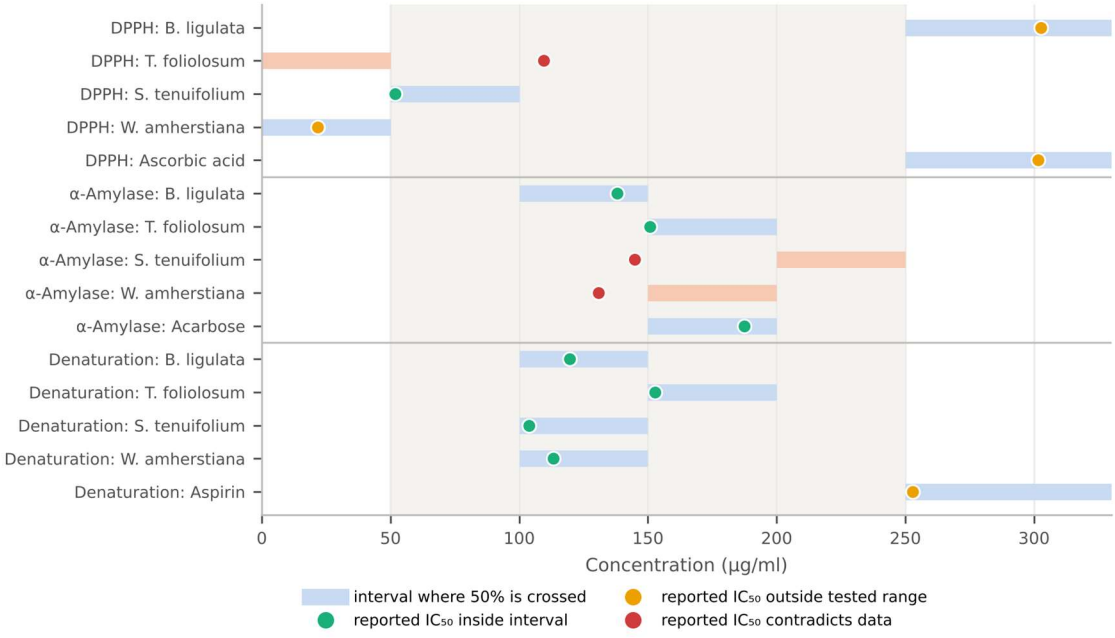


Fig. 3. Reported IC_{50} values (points) compared with the concentration interval in which the measured curve crosses 50% (bars). The shaded band is the tested range (50–250 µg/ml); open-ended bars indicate crossings below or above that range.

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TABLE III REPORTED IC_{50} VALUES AND OBSERVED CROSSING INTERVALS

Assay	Preparation	Reported IC_{50} (µg/ml)	Crossing interval (µg/ml)	Class
DPPH	*B. ligulata*	302.6	> 250	Extrapolated
DPPH	*T. foliolosum*	109.5	< 50	Contradictory
DPPH	*S. tenuifolium*	51.89	50–100	Consistent
DPPH	*W. amherstiana*	21.64	< 50	Extrapolated
DPPH	Ascorbic acid	301.5	> 250	Extrapolated
α-Amylase	*B. ligulata*	138.04	100–150	Consistent
α-Amylase	*T. foliolosum*	150.8	150–200	Consistent
α-Amylase	*S. tenuifolium*	144.8	200–250	Contradictory
α-Amylase	*W. amherstiana*	130.8	150–200	Contradictory
α-Amylase	Acarbose	187.4	150–200	Consistent
Denaturation	*B. ligulata*	119.5	100–150	Consistent
Denaturation	*T. foliolosum*	152.8	150–200	Consistent
Denaturation	*S. tenuifolium*	103.8	100–150	Consistent
Denaturation	*W. amherstiana*	113.2	100–150	Consistent
Denaturation	Aspirin	252.9	> 250	Extrapolated

4.3 Phenolic and Flavonoid Responses

S. tenuifolium and *W. amherstiana* gave the highest phenolic and flavonoid equivalents at every concentration (Fig. 4), which agrees with their strong DPPH responses. *B. ligulata* gave low phenolic values despite the known phenolic richness of the genus [5], which is consistent with its weak DPPH response in this series. However, the values are expressed per gram of material and should therefore be constant across the dilution series. Instead, phenolic totals rose 2.3-, 2.6-, 1.8- and 2.5-fold from 50 to 250 µg/ml for *B. ligulata*, *T. foliolosum*, *S. tenuifolium* and *W. amherstiana*, and flavonoid totals rose 3.4-, 2.7-, 1.7- and 2.3-fold. The composition of an extract does not change with dilution, so this pattern points to a problem with the calibration range, dilution factors or denominator. The values are useful for ranking within a concentration, not as absolute compositional estimates.

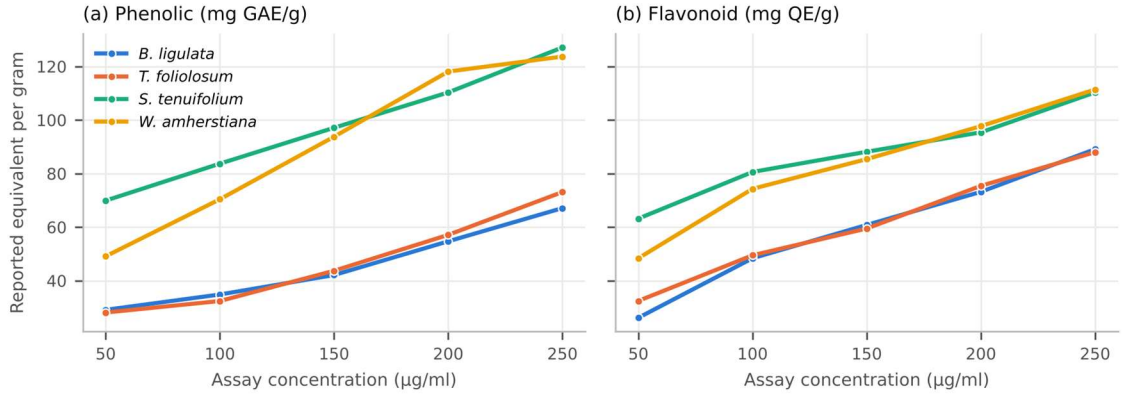


Fig. 4. Reported (a) phenolic and (b) flavonoid equivalents per gram at each assay concentration. Values expressed per gram should be independent of assay concentration; the rising trends indicate a calculation or working-range issue.

4.4 Antibacterial Screening

Aqueous extracts produced larger zones than ethanolic extracts against all four organisms (Fig. 5). *B. ligulata* aqueous extract gave zones of 9.0–12.07 mm against all organisms, and *E. coli* zones of 10.64–12.10 mm were recorded for all four aqueous extracts. Ethanolic extracts were active mainly against *B. subtilis* (7.4–7.88 mm). Two features limit interpretation. Some reported zones (2.0 and 2.75 mm) are smaller than the 6-mm well, so it is unclear whether values describe the total diameter or the margin beyond the well. In addition, the aqueous results for *T. foliolosum* and *S. tenuifolium* are identical for all four organisms, and their ethanolic results nearly so, which suggests a transcription error that should be checked against the original plates.

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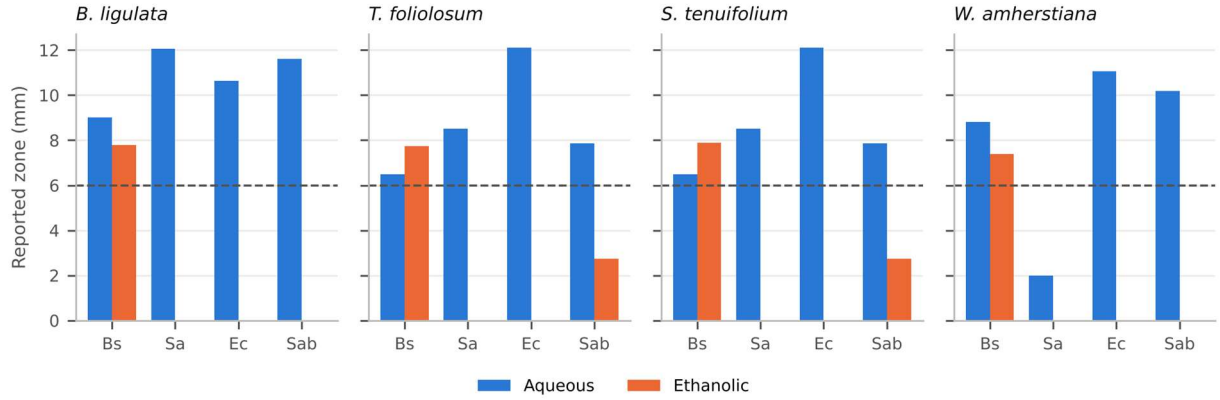


Fig. 5. Reported inhibition zones of aqueous and ethanolic extracts against *B. subtilis* (Bs), *S. aureus* (Sa), *E. coli* (Ec) and *S. abony* (Sab). The dashed line marks the 6-mm well diameter; values below it are ambiguous.

5. Discussion

5.1 Endpoint-Specific Ranking

No single extract ranked first on every endpoint. *S. tenuifolium* and *W. amherstiana* were the strongest radical scavengers and had the highest phenolic responses; *B. ligulata* and *T. foliolosum* were the strongest α-amylase inhibitors at low concentration; denaturation responses were similar across species; and aqueous extracts were the more active antibacterial preparations. Ranking by a single IC_{50} would hide these differences. The leaf-versus-underground difference for *T. foliolosum* also means that its results cannot be attributed to species alone.

5.2 What the Assays Do and Do Not Show

Table IV summarises the evidence. Each assay identifies a preparation that changes a defined chemical or biochemical signal. None shows that an extract is safe, absorbed or effective in people. DPPH and the phenolic assay respond to many reducing compounds [11]; α-amylase inhibition in crude extracts can reflect non-specific protein binding or interference with colour detection [15]; and diffusion zones depend on how far extract constituents travel through agar [17].

TABLE IV EVIDENCE SUPPORTED BY EACH ENDPOINT AND THE NEXT CONFIRMATION STEP

Endpoint	What the result supports	What it does not show	Next step
Phenolic and flavonoid	Relative chemical response at a given concentration	Absolute content or identity of compounds	Recalculate from raw data; chromatographic quantification
DPPH	Radical-scavenging capacity of the extract	Antioxidant protection in cells or tissues	Second assay with a different principle; cell model
α-Amylase	Reduction of the enzyme assay signal	Glucose control or antidiabetic effect	Extract-only controls; independent detection; selectivity
Denaturation	Reduced heat-induced turbidity of protein	Anti-inflammatory activity in tissue	Mediator-based cellular assays with toxicity testing
Agar diffusion	Qualitative growth inhibition	Potency or clinical usefulness	Broth microdilution to obtain MIC values

5.3 Reporting Recommendations

Three recommendations follow. First, IC_{50} values should be reported only when the tested range brackets the 50% response, together with the fitting method; otherwise the result should be stated as "> highest" or "< lowest concentration". Second, every assay should include extract-only blanks to correct for colour and turbidity, and the reference compound should behave as expected before comparisons are made [13]. Third, antibacterial screening should state the measurement convention and be followed by minimal inhibitory concentration tests [18]. These steps are inexpensive, and they would make comparisons between laboratories more reliable [3].

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5.4 Limitations

The analysis uses reported means; raw absorbance data, replicate values and fitting procedures were not available, so no new statistical tests or IC_{50} values were derived. Single collections were studied, organ differs between species, and extraction details limit replication. The findings therefore describe these preparations under these conditions.

6. Conclusion and Future Work

Extracts of four Himalayan medicinal plants showed concentration-dependent responses in DPPH, α-amylase and protein-denaturation assays and qualitative antibacterial activity, with different species leading on different endpoints. A simple consistency check showed that 8 of 15 reported IC_{50} values were supported by the measured data, 4 were extrapolated and 3 contradicted it, and that normalised phenolic and flavonoid totals varied with assay concentration. Future work should recalculate the flagged estimates from raw data, repeat the most promising responses—*S. tenuifolium* and *W. amherstiana* for radical scavenging and *B. ligulata* for α-amylase inhibition—with independent extraction batches and appropriate controls, determine minimal inhibitory concentrations and identify the active constituents.

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