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Formulation and Evaluation of Herbal Based Immunomodulatory Gel Prepared from the Leaf Extract of *Hippophae rhamnoides L.*

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Abstract: ***Background:*** *Hippophae rhamnoides L. (sea buckthorn) is a medicinal plant containing phenolic acids, flavonoids and other bioactive constituents. The leaves are of particular interest because they can contain substantial phenolic and flavonoid fractions. Objective: The present work aimed to develop and evaluate a topical herbal gel containing ethanolic leaf extract of Hippophae L., with emphasis on formulation quality, antioxidant activity, preliminary anti-inflammatory activity, analytical characterization and in-vitro release. Methods: Leaves collected from Sissu, Lahaul and Spiti, Himachal Pradesh, were shade-dried and extracted with ethanol using Soxhlet extraction. Five formulations (F1–F5) were prepared using Carbopol 940 with increasing extract concentrations and evaluated for appearance, homogeneity, washability, pH, viscosity and spreadability. The optimized formulation was examined by UV-visible spectroscopy and HPLC. Antioxidant activity was assessed by DPPH radical-scavenging assay, and preliminary anti-inflammatory activity by egg albumin denaturation. In-vitro release was studied using a membrane diffusion setup. Results: The extract yield was 12.80%. F3 was selected as the optimized formulation, showing pH 6.0, viscosity 7725 cps and spreadability 4.0 cm. The extract produced 91.6% DPPH inhibition at 100 µg/mL, with an IC50 of 27.7 µg/mL. F3 produced 58.0% inhibition of protein denaturation. UV-visible scanning showed a major absorption maximum at 260 nm, while HPLC showed a major peak at approximately 5.6 min under the reported chromatographic conditions. Cumulative release reached 96.3% at 360 min. Conclusion: The findings support the feasibility of incorporating Hippophae rhamnoides L. leaf extract into a Carbopol-based topical gel with satisfactory physicochemical properties and preliminary antioxidant/anti-inflammatory activity. The work should be considered a formulation and screening study; validated cell-based immunomodulatory, permeation, safety and stability studies are needed before therapeutic claims are made.*

Keywords: Hippophae rhamnoidesL.; sea buckthorn; herbal gel; Carbopol 940; antioxidant; DPPH; protein denaturation; topical delivery; immunomodulatory potential

I. INTRODUCTION

Medicinal plants remain important sources of chemically diverse compounds for pharmaceutical and nutraceutical development. Their activity is often associated with combinations of phenolic acids, flavonoids, tannins, terpenoids, vitamins and other secondary metabolites rather than a single constituent. This complexity makes botanical standardization and dosage reproducibility important considerations during formulation development [1–4].

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Hippophae rhamnoides L., commonly known as sea buckthorn, is a hardy, deciduous shrub of the Elaeagnaceae family that occurs across parts of Europe and Asia and is well adapted to cold and high-altitude environments. In the Indian Himalaya it occurs in trans-Himalayan regions and is used traditionally as a food and medicinal resource. Reviews of the plant describe cytoprotective, antioxidant, antimicrobial, anti-inflammatory and immunomodulatory activities among the reported biological properties [1,5,6].

Fig. 1 *Hippophae rhamnoides L.*

The phytochemical profile of *Hippophae rhamnoides L.* is organ-dependent. Recent reviews and analytical studies indicate that leaves and branches may contain high levels of phenolic compounds and flavonoids, including quercetin-, isorhamnetin- and kaempferol-derived compounds [2,7–10]. Isolated several flavonoid glycosides from *Hippophae rhamnoides L.* leaves and demonstrated antioxidant activity of leaf-derived fractions [11]. Similarly reported antioxidant and antimicrobial properties of phenolic-rich leaf fractions and described RP-HPLC analysis of phenolic constituents [12].

Oxidative stress is closely related to inflammatory signalling and tissue injury. Antioxidant activity is therefore frequently used as an initial indicator of the biological potential of plant extracts. The DPPH assay is a rapid chemical screening method based on reduction of the stable DPPH radical by hydrogen- or electron-donating compounds [13,14]. Although a chemical antioxidant assay cannot establish cellular immunomodulatory activity, it can provide useful preliminary evidence supporting further biological investigation.

Hippophae rhamnoides L. has also been investigated in relation to immune responses. Geetha et al. reported immunomodulatory effects of sea buckthorn leaf extract in a chromium-induced immunosuppression model, including effects on delayed-type hypersensitivity and cytokine-related endpoints [15]. This provides a rationale for exploring leaf-derived preparations; however, the present study did not directly reproduce a validated cell-based immunomodulatory assay.

Topical gels are useful dosage forms for localized delivery because they can be spread over the skin, are generally washable and can provide a hydrated polymeric matrix for controlled diffusion. Carbopol polymers are widely used as gelling agents because neutralization produces a structured gel with useful rheological properties [16–20]. The quality of

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a topical gel is influenced by pH, viscosity, spreadability, homogeneity and the concentration of the incorporated active material.

The objective of the present study was therefore to formulate a Carbopol-based topical gel containing ethanolic leaf extract of *Hippophae rhamnoides L.* and to evaluate its physicochemical characteristics, antioxidant activity, preliminary anti-inflammatory activity, UV-visible/HPLC profile and in-vitro release behaviour.

II. MATERIALS AND METHODS

2.1 Plant material and authentication

Fresh leaves of *Hippophae rhamnoides L.* were collected from healthy plants at Sissu, Lahaul and Spiti, Himachal Pradesh, during September 2025. The leaves were washed, separated, shade-dried for approximately 2–3 weeks and powdered. Botanical authentication was performed by Dr. Ved Prakash, Assistant Professor of Botany, Government College Bassa, Gohar, Mandi, Himachal Pradesh. These details are retained from the underlying thesis record.

Fig. 2 *Hippophae rhamnoides L.* Dried Leaves

2.2 Extraction

The powdered leaves were subjected to Soxhlet extraction using ethanol. Extraction was conducted at approximately 60–70°C. The solvent was removed after extraction and the concentrated extract was used for phytochemical and formulation studies. Extractive yield was calculated as: Extractive yield (%) = (weight of dried extract / weight of dried plant material) × 100.

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Fig. 3 Soxhlet extraction

2.3 Preliminary phytochemical screening

The ethanolic extract was subjected to qualitative tests for major classes of phytoconstituents, including alkaloids, flavonoids, phenolic compounds, tannins, saponins and glycosides. Standard colour- and precipitate-based reactions were interpreted as positive or negative according to the characteristic response.

2.4 Formulation development

Five formulations (F1–F5) were prepared by the dispersion method. Carbopol 940 was dispersed gradually in distilled water with continuous stirring and allowed to hydrate. Propylene glycol, methyl paraben and propyl paraben were incorporated, followed by addition of the plant extract. Triethanolamine was used to adjust pH and gel consistency. The extract concentration was varied from 0.5 to 2.5%, while Carbopol 940 was maintained at 0.5%. The formulation composition recorded in the source thesis is shown in Table 1.

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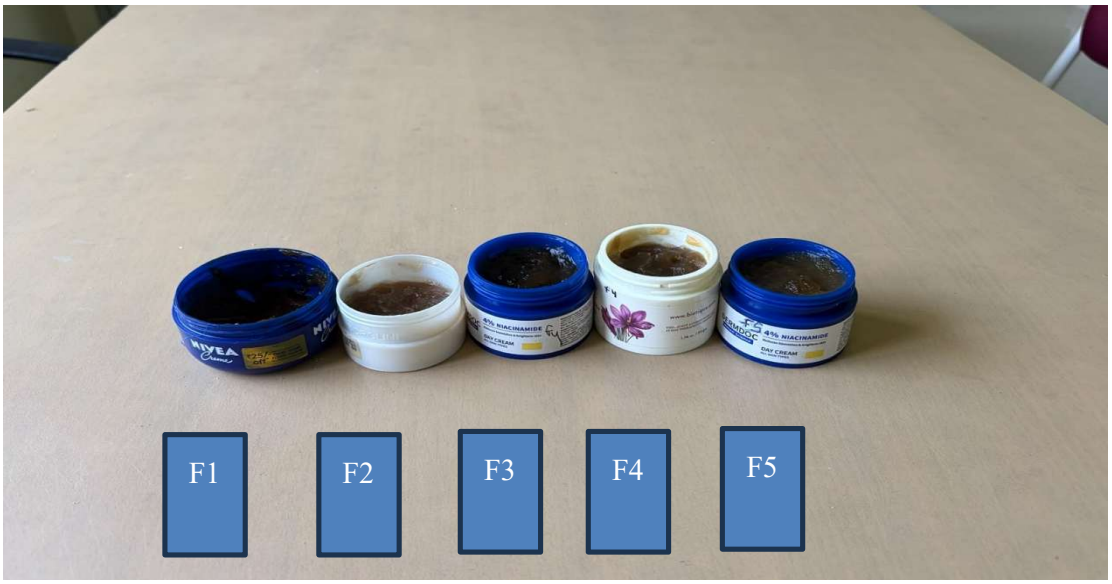

Fig. 4 *Hippophae rhamnoides L.* leaf-extract gel formulations

Table 1. Composition of *Hippophae rhamnoides L.* leaf-extract gel formulations. Water was used q.s. to the final formulation volume/weight as recorded in the thesis.

Ingredient	F1	F2	F3	F4	F5
H. rhamnoides extract (%)	0.5	1.0	1.5	2.0	2.5
Carbopol 940 (%)	0.5	0.5	0.5	0.5	0.5
Propylene glycol	5 mL	5 mL	5 mL	5 mL	5 mL
Methyl paraben (%)	0.10	0.10	0.10	0.10	0.10
Propyl paraben (%)	0.05	0.05	0.05	0.05	0.05
Triethanolamine	0.2	0.2	0.2	0.2	0.2

2.5 Evaluation of gel formulations

Appearance, colour, homogeneity and washability were evaluated visually. Surface pH was measured using a calibrated digital pH meter. Viscosity was measured using a Brookfield viscometer at room temperature. Spreadability was assessed by the slip-and-drag method, using the relationship S = M×L/T, where S is spreadability, M is the applied mass, L is the length of the slide and T is the time required for separation [16–20].

2.6 UV-visible analysis

The optimized gel was scanned from 200 to 800 nm using a UV-visible spectrophotometer. The major absorption maximum was recorded as an analytical fingerprint of the formulation. Because the study did not include a validated reference standard for each individual phytoconstituent, the UV peak was interpreted as evidence of extract-derived UV-absorbing constituents rather than as proof of a specific compound.

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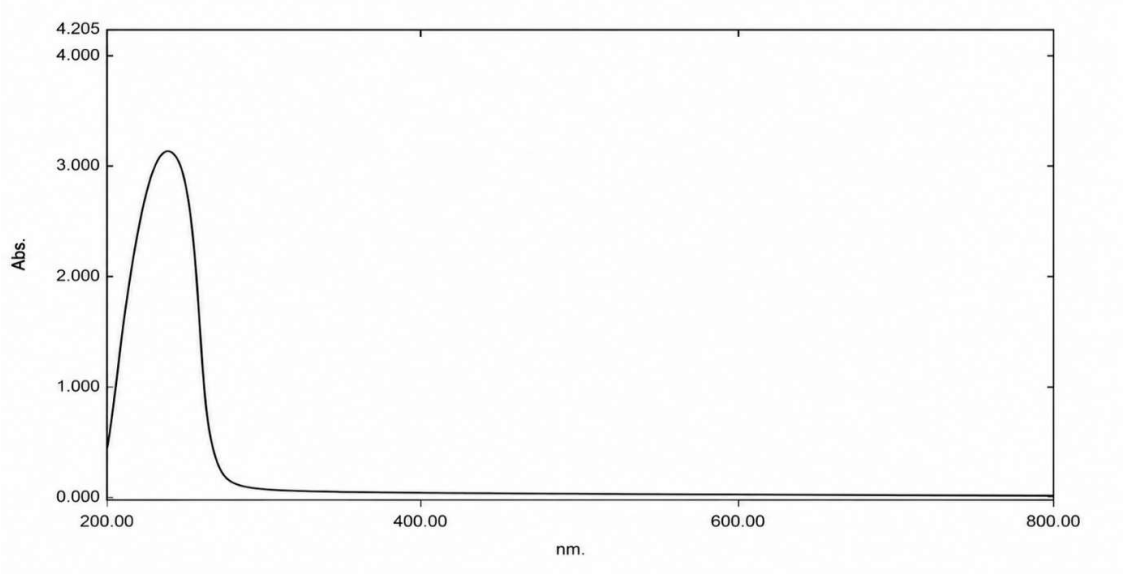

Fig. 5 UV-visible analysis

2.7 HPLC analysis

The optimized gel was examined by RP-HPLC using a C18 column. The mobile phase reported in the thesis was methanol: acetonitrile: water containing 1% acetic acid in a 40:15:45 ratio. The chromatographic peak profile was recorded and the major peak was observed at approximately 5.6 min. In the absence of a co-injected authentic standard in the supplied results, the peak is reported here as a major chromatographic peak rather than being assigned definitively to quercetin.

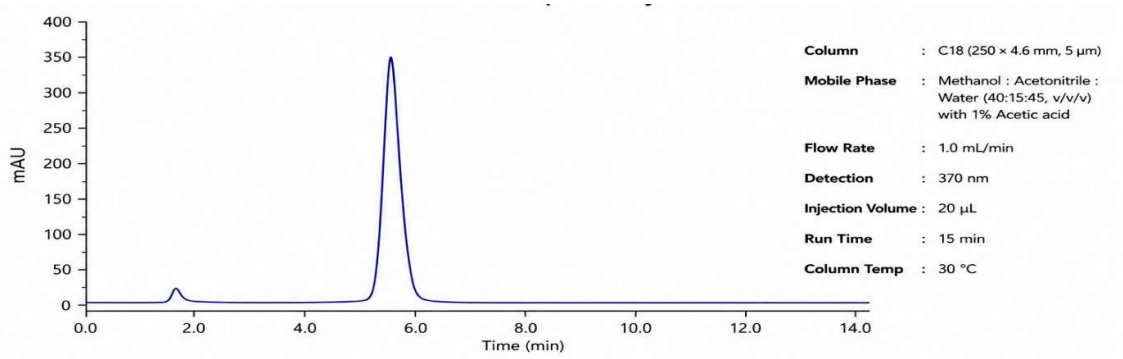

Fig. 6 HPLC analysis

2.8 DPPH radical-scavenging assay

The antioxidant activity of the leaf extract was determined at 20–100 µg/mL, with ascorbic acid as the reference antioxidant. DPPH solution was reacted with the test samples and absorbance was measured after incubation in the dark. Percentage inhibition was calculated as: % inhibition = [(A0−A1)/A0]×100. IC50 was obtained from the concentration-response relationship. The approach is consistent with established DPPH methodology [13,14].

2.9 Egg albumin denaturation assay

Preliminary anti-inflammatory activity was assessed by inhibition of heat-induced egg albumin denaturation. The test formulation was compared with a reference preparation and absorbance was measured at 660 nm. Percentage inhibition was calculated as [(Ac−At)/Ac]×100, where Ac is the control absorbance and at is the test absorbance. Protein-denaturation methods have long been used as simple in-vitro screens for anti-inflammatory potential [21,22].

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2.10 In-vitro release study

The optimized F3 gel was evaluated using a membrane diffusion setup with phosphate buffer pH 6.8 as receptor medium. An egg membrane pre-soaked in buffer was used as the diffusion barrier. The receptor medium was maintained at approximately 35±5°C and stirred at 50 rpm. Samples were withdrawn at predetermined intervals, replaced with fresh buffer, diluted as required and measured spectrophotometrically at 270 nm. Cumulative release was calculated from the calibration relationship. Membrane/dialysis approaches are widely used for preliminary release assessment of topical gels, although they should not be equated with true skin permeation [23].

2.11 Statistical considerations

The article reports the numerical observations available in the thesis. Where the source record did not provide replicate-level observations, inferential statistics and p-values were not introduced. This avoids presenting unsupported statistical significance. Future experiments should use independent replicates and report mean±SD with appropriate statistical tests.

III. RESULTS

3.1 Organoleptic and extraction characteristics

The dried leaves were dry, brittle and slightly rough, with olive-green to brownish-green colour, characteristic herbal odour and bitter/astringent taste. Loss on drying was 6.0%. Soxhlet extraction with ethanol produced an extractive yield of 12.80%.

3.2 Preliminary phytochemical screening

Qualitative screening indicated the presence of several secondary-metabolite classes, including flavonoids, phenolic compounds, tannins, alkaloids, saponins and glycosides. The presence of phenolic and flavonoid constituents is consistent with published compositional studies of sea buckthorn leaves [7–12].

3.3 Physicochemical evaluation and optimization

Table 2. Physicochemical evaluation of F1–F5. F3 was selected as the optimized formulation because it provided a balanced pH, viscosity and spreadability profile.

Formulation	Appearance	Homogeneity	Washability	pH	Viscosity (cps)	Spreadability (cm)
F1	Greenish	Smooth/even	Easy	5.0	7666	3.5
F2	Transparent	Smooth/even	Easy	5.5	7686	3.8
F3	Transparent	Smooth/even	Easy	6.0	7725	4.0
F4	Transparent	Smooth/even	Easy	6.5	7753	4.2
F5	Transparent	Smooth/even	Easy	7.0	7782	4.5

3.4 UV-visible and HPLC characterization

The optimized gel showed a prominent UV-visible absorption maximum at 260 nm. The HPLC chromatogram showed a major peak at approximately 5.6 min under the reported RP-HPLC conditions. These findings demonstrate that extract-derived chromatographic/UV-absorbing constituents remained detectable after incorporation into the gel matrix.

3.5 DPPH antioxidant activity

Table 3. DPPH radical-scavenging activity of *Hippophae rhamnoides L.* leaf extract and ascorbic acid. The extract showed concentration-dependent activity and an IC50 of 27.7 µg/mL; the corresponding value for ascorbic acid was 14.8 µg/ml.

Concentration (µg/mL)	H. rhamnoides extract (% inhibition)	Ascorbic acid (% inhibition)
20	42.8	55.6
40	61.4	72.8
60	76.5	85.9
80	85.2	92.7
100	91.6	97.8

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3.6 Protein-denaturation inhibition

The reference preparation showed an absorbance of 0.800, while the optimized herbal gel showed an absorbance of 0.336 at 660 nm. Using the reported equation, the formulation produced 58.00% inhibition of protein denaturation.

3.7 In-vitro release

Table 4. In-vitro release profile of optimized F3 formulation.

Time (min)	Absorbance	Drug released (mg)	Cumulative release (%)
0	0.000	0.00	0.0
15	0.128	0.62	8.4
30	0.196	1.15	15.7
60	0.291	2.05	27.6
90	0.366	2.90	39.3
120	0.441	3.82	51.6
180	0.534	5.02	67.8
240	0.612	6.08	82.1
300	0.669	6.75	91.2
360	0.714	7.12	96.3

IV. DISCUSSION

The present study demonstrates a complete preliminary development pathway from plant material to a topical herbal dosage form. The 12.80% ethanolic extractive yield indicates that the selected extraction process recovered a measurable fraction of constituents from the dried leaves. Because extraction yield is influenced by plant origin, drying, particle size, solvent polarity and extraction temperature, the value should be treated as specific to the present batch rather than as a universal characteristic of *Hippophae rhamnoides L.*.

The phytochemical findings are biologically plausible in view of published analytical work. Sea buckthorn leaves have been reported to contain phenolic acids and flavonols such as quercetin, isorhamnetin and kaempferol derivatives, and leaves can contain greater phenolic concentrations than edible fruit tissues [7–12]. These compounds provide a reasonable chemical basis for the antioxidant activity observed in the present study, although qualitative phytochemical tests alone cannot establish individual compound concentrations.

F3 was selected because it provided the best overall balance of formulation properties. Its pH of 6.0 is within the range commonly encountered in topical semisolid formulations, while its viscosity of 7725 cps provided sufficient structure without preventing spreadability. The progression in viscosity and spreadability across F1–F5 also illustrates how formulation composition can alter handling and application characteristics. Similar studies of Carbopol-based gels emphasize the importance of balancing viscosity and spreadability during optimization [16–20,24,25].

The DPPH assay showed a clear concentration-response relationship. The extract reached 91.6% inhibition at 100 µg/mL, while ascorbic acid reached 97.8%. The lower IC50 of ascorbic acid (14.8 µg/mL) compared with the extract (27.7 µg/mL) indicates greater potency of the purified reference antioxidant under these assay conditions. Nevertheless, the extract's high maximal inhibition suggests appreciable radical-scavenging capacity. Published studies likewise report substantial antioxidant activity in sea buckthorn leaves and associate this activity with phenolic and flavonoid composition [7,12,26].

The egg albumin denaturation result of 58.0% provides preliminary evidence of protein-stabilizing activity. Protein denaturation assays are useful screening tools, but they are not substitutes for mechanistic cellular assays. Therefore, the result should be described as preliminary anti-inflammatory potential rather than proof of anti-inflammatory efficacy in humans [21,22].

The UV-visible peak at 260 nm and the major HPLC peak at approximately 5.6 min support the analytical detectability of extract-derived constituents after formulation. Importantly, a retention time alone does not establish compound

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identity. Published sea buckthorn HPLC studies have demonstrated the usefulness of chromatographic methods for flavonoids and phenolics [9,11,12,27]. A future definitive assay should include authenticated reference standards, system suitability, linearity, precision, accuracy, specificity and recovery according to current analytical-validation guidance.

The optimized gel released 96.3% of the measured extract-related signal by 360 min. The gradual increase over six hours suggests that the Carbopol matrix did not cause immediate loss of the measured constituents and permitted progressive diffusion. However, the present membrane method measures release into a receptor medium rather than actual skin permeation or retention. More physiologically relevant Franz diffusion-cell experiments using validated synthetic or biological membranes would therefore be needed before claims about dermal penetration can be made [23,28].

The study is particularly relevant to the proposed immunomodulatory application because sea buckthorn leaf extract has previously been investigated for immune effects. Geetha et al. reported modulation of immune endpoints in an experimental immunosuppression model [15]. Nevertheless, the present work did not directly quantify cytokines, mast-cell mediators, lymphocyte proliferation or other validated cellular immunomodulatory endpoints. Thus, the appropriate conclusion is that the formulation shows preliminary antioxidant and anti-inflammatory properties and warrants further immunomodulatory investigation.

Several limitations should be acknowledged. First, the biological assays were preliminary and largely chemical or protein-based. Second, the supplied results do not provide replicate-level raw data for all formulation tests, preventing robust statistical inference. Third, the HPLC peak was not definitively assigned to a reference standard. Fourth, long-term stability, microbial quality, skin irritation, cytotoxicity and skin permeation were not comprehensively established. These limitations do not invalidate the formulation work, but they define the level of evidence supported by the study.

V. CONCLUSION

An ethanolic leaf extract of *Hippophae rhamnoides L.* was successfully incorporated into a Carbopol 940 topical gel. Among five formulations, F3 was selected as the optimized formulation based on its balanced physicochemical characteristics (pH 6.0, viscosity 7725 cps and spreadability 4.0 cm). The leaf extract exhibited concentration-dependent DPPH radical-scavenging activity with an IC50 of 27.7 µg/mL, while the optimized gel showed 58.0% inhibition of egg albumin denaturation. UV-visible and HPLC analysis demonstrated detectable extract-derived analytical signals, and the optimized gel showed progressive in-vitro release reaching 96.3% at 360 min. Collectively, these results support further investigation of *Hippophae rhamnoides L.* leaf extract as a topical herbal formulation with potential antioxidant, anti-inflammatory and immunomodulatory relevance. Definitive therapeutic or immunomodulatory claims require validated cell-based assays, standardized marker quantification, skin permeation studies, safety testing and stability studies.

VI. DECLARATIONS

Ethics approval: Not applicable to the reported in-vitro formulation and screening work; no animal or human intervention data are reported in this manuscript.

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests.

Funding: No specific external funding was reported.

Data availability: Data presented in the manuscript are derived from the underlying thesis records. Additional raw analytical files should be made available by the authors where required by the target journal.

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