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Authentication, Methanolic Extraction and Preliminary Phytochemical Profiling of *Piper methysticum* G. Forst. Leaves

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Abstract

Piper methysticum G. Forst. (kava) is a medicinal species of the family Piperaceae whose pharmacological value depends on the identity, plant part, processing history and chemical profile of the material examined. This study was restricted to the pre-pharmacological stages of leaf authentication, crude methanolic extraction and qualitative phytochemical screening. Leaf samples were authenticated through macroscopic and taxonomical examination and were checked for foreign matter, fungal growth and visible substitution. The material was cleaned, shade-dried, pulverized and passed through a No. 40 sieve. Five hundred grams of dried leaf powder were extracted in a Soxhlet apparatus with 2.5 L of 95% methanol at 60-65 degrees C for a cumulative 24 h. The filtrate was concentrated under reduced pressure at 40 degrees C and 80 rpm, dried at 40 degrees C for 24 h and stored at 4 degrees C. A dark-brown semisolid extract weighing 68.50 g was obtained, corresponding to a yield of 13.70%. Standard qualitative tests, performed in triplicate, detected alkaloids, flavonoids, phenolic compounds, tannins, saponins, carbohydrates, reducing sugars, glycosides, steroids and terpenoids, whereas proteins and amino acids were not detected. Phenolic compounds and terpenoids gave the strongest reactions, while flavonoids, tannins, glycosides and steroids were moderately present. Overall positivity was 90.90%, with an intensity score of 18/33. The findings establish a reproducible botanical and phytochemical foundation for subsequent toxicological and neuropharmacological investigation. However, the screening remains qualitative and does not identify or quantify individual kavalactones; chromatographic standardization is therefore required before definitive chemical or therapeutic claims can be made.

1. Introduction

Medicinal-plant research begins long before a biological assay is performed. The reliability of any pharmacological claim depends on the identity of the plant, the part collected, the manner in which it was dried and stored, the solvent used for extraction and the chemical classes present in the final

preparation. Errors at any of these stages can change the composition of an extract sufficiently to produce conflicting efficacy or safety results. This is especially important for *Piper methysticum* G. Forst., commonly known as kava, because commercial and traditional discussions frequently emphasize roots and rhizomes, whereas the present investigation concerns an authenticated methanolic leaf extract. A leaf-based result cannot automatically be generalized to beverages, root preparations or extracts produced with different solvents.

Piper methysticum belongs to the Piperaceae and is culturally associated with Pacific Island traditions. Its central nervous system relevance has commonly been linked with a group of lipophilic lactones known as kavalactones, including kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin. Experimental literature has related these constituents to anxiolytic, calming and neurobehavioural effects through several possible targets rather than a single mechanism (Bian et al., 2020; Volgin et al., 2020). Kavain, for example, can modulate GABA-A receptor function, providing a plausible chemical basis for some inhibitory central effects (Chua et al., 2016). Nevertheless, the occurrence and relative abundance of these constituents depend on cultivar, plant organ, maturity, extraction technique and storage history.

Botanical authentication is therefore not a ceremonial step. A mislabeled, substituted or contaminated sample can invalidate all later findings. Macroscopic examination can provide a first layer of quality control by documenting leaf shape, margin, apex, base, venation, texture and colour and by detecting foreign material or fungal deterioration. Voucher documentation further supports traceability by linking the experimental material to a reference specimen that can be re-examined by future researchers. Current herbal quality-control literature emphasizes that authenticity and traceability are essential to product quality and safety (Wang et al., 2023).

Post-harvest handling is equally important. Direct sunlight and excessive heat can degrade light-sensitive or thermolabile compounds, while incomplete drying encourages microbial growth and enzymatic deterioration. Shade drying at room temperature reduces moisture while limiting harsh thermal exposure. Grinding then increases surface area and improves solvent contact, but very fine material can compact in an extraction thimble and obstruct solvent circulation. A standardized coarse powder passed through an appropriate sieve offers a practical balance between surface area, uniformity and permeability.

Methanol is widely used in preliminary phytochemical studies because it can recover a broad spectrum of polar and moderately polar secondary metabolites. Soxhlet extraction repeatedly exposes the plant powder to freshly condensed solvent, improving mass transfer and producing an exhaustive crude extract under controlled conditions. However, the method also introduces heat and yields a complex mixture rather than a standardized single-compound preparation. Consequently, extraction yield should be interpreted as a measure of recovered methanol-soluble material, not as direct proof of pharmacological potency. A high-yield extract may contain abundant inactive constituents, while a lower-yield extract may contain smaller quantities of highly active compounds (Abubakar & Haque, 2020; Kumar et al., 2023).

Preliminary phytochemical screening provides a rapid chemical map of the extract. Colour and precipitation reactions can indicate broad groups such as alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, glycosides, steroids and terpenoids. Such tests are inexpensive and valuable for planning later fractionation, but they are presumptive. Reaction intensity may be influenced by concentration, solvent, interfering compounds and observer judgement. Qualitative screening cannot confirm molecular identity, purity or concentration and should therefore lead to, rather than replace, chromatographic and spectrometric analysis (Peiris et al., 2023).

The present paper isolates these foundational stages from the wider thesis and addresses one focused question: can authenticated *Piper methysticum* leaf material be processed reproducibly into a methanolic extract with a documented yield and a preliminary phytochemical profile? By limiting the paper to botanical quality, extraction and screening, the study avoids mixing early chemical characterization with toxicological or behavioural outcomes. The objectives were to authenticate the leaf material, standardize cleaning, shade drying, powdering and Soxhlet extraction, determine extractive yield, and qualitatively evaluate the major classes of phytoconstituents present in the crude extract.

A further reason for separating extraction and screening from the later biological phases is that chemical quality must be established independently of an attractive pharmacological result. When a behavioural effect is known in advance, there is a risk of interpreting every colour reaction as support for that effect. In the present paper, botanical identity, yield and phytochemical reactions are reported as primary observations in their own right. This structure permits later investigators

to repeat the preparation and test whether comparable chemical fingerprints are obtained before comparing biological outcomes.

The choice of leaf material also raises a useful scientific question. Most public familiarity with kava concerns preparations made from below-ground organs, yet other organs may contain a different balance of kavalactones, flavokavains, chlorophyll-derived compounds, phenolics and structural metabolites. A leaf extract could therefore show a distinct efficacy-to-toxicity relationship. Rather than assuming equivalence, each organ should be treated as a separate raw material with its own authentication, extraction and standardization requirements. Clear reporting of the plant part is consequently part of safety science as well as botanical description.

Quality assurance during extraction included use of analytical-grade reagents, cleaned and dried glassware, labeled containers, controlled temperatures and a calibrated analytical balance. These seemingly routine controls influence reproducibility. Solvent evaporation to different endpoints can change the apparent extract mass, and exposure of a hygroscopic semisolid to ambient humidity can alter yield calculations. Recording the final physical state and storing the preparation at 4 degrees C reduce, but do not eliminate, such variation. Replicate extraction batches would strengthen future estimates of yield precision.

The qualitative screening panel was intentionally broad. It was designed to determine whether the crude methanolic matrix contained chemical groups that could justify more selective analysis, not to establish a complete metabolome. Orthogonal confirmation is important because several traditional tests are vulnerable to cross-reactivity. For example, strongly coloured crude extracts can obscure subtle colour changes, and precipitating reagents may respond to more than one class of compound. Blanks, positive controls and instrument-based confirmation should therefore accompany future screening programmes.

For publication and inter-laboratory comparison, the extraction record should ultimately function like a manufacturing batch record. It should identify the source and voucher, dry mass, residual moisture, particle-size range, solvent grade, solid-to-solvent ratio, cycle rate, temperature, duration, concentration pressure, final drying endpoint, storage container and elapsed time before testing. Reporting these variables would permit another laboratory to distinguish a true biological difference from a preparation difference. The current procedure supplies most of this information and can therefore serve as a practical starting protocol for replication.

The study also illustrates the difference between screening and standardization. Screening asks which broad chemical classes may be present; standardization asks whether a defined preparation consistently contains specified markers within an acceptable range. A future specification for *Piper methysticum* leaf extract might include a chromatographic fingerprint, minimum and maximum concentrations for selected kavalactones, limits for flavokavains, residual methanol, microbial burden, heavy metals, pesticides and moisture. Only after these attributes are controlled can toxicological or behavioural results be meaningfully compared across batches and institutions.

Finally, chemical data should be integrated cautiously with ethnopharmacological knowledge. Traditional use can guide plant selection, but it does not validate every organ, solvent or concentrated extract. Organic-solvent extraction may enrich constituents that are present at lower levels in customary aqueous preparations. The scientifically responsible position is therefore neither to dismiss traditional knowledge nor to assume automatic equivalence. Instead, traditional context should generate hypotheses, while authenticated material, transparent extraction and modern analytical chemistry determine the properties of the exact preparation tested.

2. Materials and Methods

2.1 Study design

A laboratory-based descriptive experimental design was used. The investigation covered the sequence from raw leaf material to a stored crude methanolic extract and its qualitative chemical profile. All observations and measurements were documented on prepared data sheets. The procedures were organized to improve traceability and reproducibility in accordance with accepted principles of medicinal-plant preparation and phytochemical screening.

2.2 Plant material and scope

The plant part examined was the leaf of *Piper methysticum* G. Forst. The use of leaves was stated explicitly because chemical composition differs among leaves, stems, roots and rhizomes. The study did not attempt to characterize traditional aqueous kava beverages or commercial root products. Samples were labeled with the botanical name, common name, plant part, collection or procurement details and laboratory sample code.

2.3 Authentication

The specimen was authenticated by ACME Research Solutions through macroscopic and organoleptic examination under reference number ACME/PA/1301 dated 25 February 2024. The sample was compared with accepted taxonomical descriptions and available herbarium information. Diagnostic observations included leaf type, shape, margin, apex, base, venation, texture and colour before and after drying. The material was also inspected for foreign matter, fungal growth, physical deterioration and visible substitution or adulteration. Authentication was completed before extraction.

2.4 Cleaning and drying

Damaged portions, insects, dust, soil particles and other foreign materials were removed. Washing was used only where required, after which surface moisture was allowed to dissipate. Leaves were spread in a clean, ventilated, shaded area at room temperature. They were protected from direct sunlight, excessive heat, moisture and contamination. Drying continued until the material was sufficiently brittle for grinding and showed no visible fungal growth. Shade drying was selected to reduce moisture while limiting degradation of light- and heat-sensitive constituents.

2.5 Powder preparation and storage

Dried leaves were cut into smaller pieces and pulverized with a mechanical grinder fitted with stainless-steel blades. The powder was passed through a No. 40 mesh sieve to obtain a relatively uniform particle size. Oversized material was reground where necessary. The powder was transferred to clean, dry, airtight and properly labeled containers and was protected from direct light, excessive heat and humidity until extraction.

2.6 Soxhlet extraction

Five hundred grams of dried leaf powder were placed in a cellulose extraction thimble. The material was extracted with 2.5 L of 95% methanol in a Soxhlet apparatus. The heating mantle was adjusted to maintain an extraction temperature of 60-65 degrees C. Extraction was carried out for 8 h per day over three consecutive days, giving a cumulative extraction time of 24 h. The apparatus was checked for secure joints, appropriate solvent cycling and avoidance of excessive boiling or solvent loss.

2.7 Concentration and final drying

The methanolic extract was filtered and concentrated under reduced pressure using a rotary evaporator. The water-bath temperature was maintained at 40 degrees C and the rotation speed at approximately 80 rpm. Reduced-pressure concentration was used to remove solvent at a lower thermal load than open evaporation. The concentrated material was then placed in a hot-air oven at 40 degrees C for 24 h to obtain a stable semisolid crude extract. The dried extract was weighed on an analytical balance with 0.001 g sensitivity.

2.8 Yield calculation and storage

Percentage yield was calculated from the weight of the final dried crude extract relative to the initial dry plant powder: percentage yield = (weight of dried extract / weight of dried powder) x 100. The extract was transferred to a labeled airtight container and stored at 4 degrees C. Its colour, physical nature, mass and yield were recorded.

2.9 Preparation for phytochemical tests

For each qualitative assay, 50 mg of crude extract were dissolved in 5 mL of methanol or distilled water according to the chemical requirement of the test. Each solution was filtered through Whatman No. 1 filter paper. Tests were performed in clean test tubes, generally at room temperature except where heating was specified. Each assay was conducted in triplicate. Results were scored as absent (-), weakly present (+), moderately present (++), or strongly present (+++).

- Alkaloids were examined by Mayer's and Dragendorff's reactions. Extract was dissolved in 1% hydrochloric acid, warmed at approximately 60 degrees C for 5 min and filtered. A cream or pale-yellow precipitate after Mayer's reagent or an orange to reddish-brown precipitate after Dragendorff's reagent was regarded as a positive reaction. Flavonoids were evaluated by the alkaline reagent test: a yellow colour following 10% sodium hydroxide that disappeared after addition of dilute hydrochloric acid indicated a positive response.
- Phenolic compounds were tested by adding 2-3 drops of 5% ferric chloride to the extract solution; blue, green or black coloration indicated a positive reaction. Tannins were assessed with 10% lead acetate, with a white or yellowish precipitate considered positive. Saponins were evaluated by the foam test after vigorous shaking with distilled water and standing for 15 min; persistent foam indicated their presence.

- Carbohydrates were assessed with Molisch's reagent followed by concentrated sulphuric acid, producing a violet ring at the interface when positive. Reducing sugars were examined with Benedict's reagent after heating at 80 degrees C for 5 min; green, yellow, orange or brick-red precipitate was considered positive.
- Glycosides were screened using the Keller-Killiani reaction with glacial acetic acid, ferric chloride and concentrated sulphuric acid; a brown ring at the interface indicated a positive result.
- Steroids were assessed by the Liebermann-Burchard test using chloroform, acetic anhydride and concentrated sulphuric acid; green or bluish-green coloration was considered positive.
- Terpenoids were examined by the Salkowski test, in which a reddish-brown interface after chloroform and concentrated sulphuric acid indicated a positive reaction.
- Proteins and amino acids were screened by the ninhydrin test after heating at 80 degrees C for 5 min; purple or bluish coloration indicated a positive response.

Because the phytochemical procedures were qualitative, results were summarized descriptively. For an exploratory intensity comparison, scores of 0, 1, 2 and 3 were assigned to -, +, ++ and +++, respectively. The positivity percentage was calculated as the number of chemical groups yielding any positive reaction divided by the total number tested, multiplied by 100. The total intensity score was expressed relative to the theoretical maximum score of 33 for 11 tests.

3. Results

3.1 Authentication and raw-material quality

The examined sample displayed diagnostic characteristics consistent with *Piper methysticum* leaf material. It was a simple, broad, cordate or heart-shaped leaf with an entire margin, an acute to acuminate apex, a cordate base and prominent reticulate to palmate venation. The texture was smooth to slightly leathery. Leaves were green to dark green before drying and brownish-green after drying. Foreign matter, fungal growth and visible evidence of adulteration or substitution were not observed. The material therefore met the study's macroscopic acceptance criteria and was used for extraction.

Table 1: Characteristics of *Piper methysticum* leaf

Characteristic	Observation for *Piper methysticum* leaf
Plant part / type	Leaf; simple
Shape / margin	Broad cordate or heart-shaped; entire margin
Apex / base	Acute to acuminate apex; cordate base
Venation / texture	Prominent reticulate-palmate venation; smooth to slightly leathery
Colour	Green to dark green before drying; brownish-green after drying
Quality observations	No foreign matter, fungal growth or visible substitution/adulteration
Authentication	Matched taxonomical description; ACME/PA/1301, 25 February 2024

3.2 Extraction outcome

Soxhlet processing of 500 g dried leaf powder with 2.5 L of 95% methanol produced a dark-brown semisolid crude extract. The final dry-equivalent mass was 68.50 g. The calculated extractive yield was 13.70%. The physical consistency was suitable for transfer into an airtight container, and the extract was stored at 4 degrees C until screening.

Table 2: Extraction outcome

Parameter	Recorded value
Dried powder	500 g
Methanol	95%; 2.5 L
Method	Soxhlet extraction
Temperature / duration	60-65 degrees C; 24 h total
Final concentration	Rotary evaporation at 40 degrees C and 80 rpm
Final drying	40 degrees C for 24 h
Extract appearance	Dark-brown semisolid
Dried extract mass	68.50 g
Percentage yield	13.70%
Storage	4 degrees C in airtight labeled container

Figure 1: A) *Piper methysticum* leaf B) *Piper methysticum* leaf extract

3.3 Phytochemical profile

Ten of the eleven chemical groups tested gave a positive reaction, corresponding to 90.90% positivity. Phenolic compounds and terpenoids were strongly present (+++). Flavonoids, tannins, glycosides and steroids were moderately present (++). Alkaloids, saponins, carbohydrates and reducing sugars were weakly present (+). Proteins and amino acids were not detected (-). The total qualitative intensity score was 18 of a possible 33, corresponding to a relative intensity of 54.54%.

Table 3.3: Phytochemical profile

Phytochemical group	Test	Result	Score
Alkaloids	Mayer's / Dragendorff's	+	1
Flavonoids	Alkaline reagent	++	2
Phenolic compounds	Ferric chloride	+++	3
Tannins	Lead acetate	++	2
Saponins	Foam test	+	1
Carbohydrates	Molisch's	+	1
Reducing sugars	Benedict's	+	1
Glycosides	Keller-Killiani	++	2
Steroids	Liebermann-Burchard	++	2
Terpenoids	Salkowski	+++	3
Proteins / amino acids	Ninhydrin	-	0

Key: - absent; + weakly present; ++ moderately present; +++ strongly present. Tests were performed in triplicate.

4. Discussion

The study established a traceable sequence from authenticated *Piper methysticum* leaves to a chemically characterized crude extract. This sequence matters because the reproducibility of herbal experiments is often weakened by incomplete reporting of plant identity, organ used, drying history, extraction ratio and storage conditions. By recording authentication details, diagnostic macroscopic characters, extraction temperature and duration, solvent volume, concentration conditions and final yield, the present work defines the preparation more precisely than the broad term 'kava extract.'

The macroscopic observations were consistent with the expected broad, cordate morphology of *Piper methysticum* leaves. The absence of visible fungal growth and foreign matter supported the suitability of the material for laboratory processing. Macroscopic authentication cannot, by itself, exclude all closely related species or establish chemotype, but it provides an essential first barrier against obvious substitution and degradation. For higher-level botanical quality assurance, future studies could supplement voucher documentation with microscopy, DNA barcoding or chemical fingerprinting. Advanced microscopy has been used to distinguish Piper species related to kava, illustrating the value of combining morphology with instrumental approaches.

Shade drying was appropriate for preserving a broad spectrum of constituents while lowering moisture. Direct sunlight can produce local heating and photodegradation, and prolonged damp storage can promote hydrolysis, oxidation and microbial growth. The brownish-green colour after drying was compatible with moisture loss and pigment alteration without visible decomposition. Uniform powdering through a No. 40 sieve likely improved solvent penetration and reduced between-batch variation. Nevertheless, particle size can influence extraction kinetics, so future reports should retain sieve information and, ideally, measure residual moisture content before weighing the powder.

The 13.70% yield indicates that a substantial fraction of the dried leaf mass was soluble in hot 95% methanol under the selected Soxhlet conditions. The extraction ratio of 500 g powder to 2.5 L solvent, the 60-65 degrees C temperature range and the cumulative 24-h exposure are important because changing any of them may alter both yield and composition. The dark-brown semisolid nature of the product reflects concentration of multiple pigment, phenolic, lipid-soluble and moderately polar constituents rather than isolation of one active principle. The yield should

therefore be treated as a manufacturing and comparison parameter, not a measure of therapeutic strength.

Soxhlet extraction offers repeated solvent renewal and can provide efficient recovery, but it also exposes the plant material to prolonged heat. Some constituents may degrade, isomerize or oxidize under these conditions. Conversely, insufficient solvent cycling or incomplete drying can leave an extract with residual methanol or variable water content. Concentration at 40 degrees C under reduced pressure and final drying at 40 degrees C were intended to limit thermal damage and solvent retention. Future work should directly quantify residual solvent, determine moisture or loss on drying, and compare Soxhlet extraction with cold maceration, ultrasound-assisted extraction or other lower-temperature techniques.

The phytochemical results demonstrate chemical diversity. Strong phenolic and terpenoid reactions are particularly relevant because phenolic compounds can contribute antioxidant and neuroprotective properties, while terpenoid or lactone-related constituents may contribute to central nervous system activity. Kava's best-known constituents are kavalactones, and the strong terpenoid-type reaction is compatible with the presence of non-polar or moderately polar constituents; however, a Salkowski reaction cannot specifically identify kavalactones. It would be scientifically incorrect to equate a positive terpenoid test with quantitative proof of kavain, methysticin or any other named molecule.

Moderate flavonoid and tannin reactions indicate additional polyphenolic complexity. Flavonoids may influence oxidative stress, inflammation and neuronal signalling, while tannins have protein-binding and antioxidant properties. Moderate glycoside and steroid reactions and weak alkaloid, saponin, carbohydrate and reducing-sugar reactions further show that the methanolic extract is a multicomponent matrix. Such diversity creates the possibility of additive, synergistic or antagonistic interactions, but qualitative tests cannot establish which group dominates a later pharmacological response.

The non-detection of proteins and amino acids may reflect low abundance, limited extraction into 95% methanol, degradation during processing or the sensitivity of the ninhydrin method. A negative qualitative result does not prove absolute absence. Similarly, a weak reaction may result from low concentration or chemical interference. Triplicate testing improves consistency but does

not eliminate subjectivity in colour grading. The intensity score of 18/33 is therefore best viewed as a semi-quantitative descriptive index rather than an analytical concentration measurement.

The overall positivity of 90.90% suggests that methanol extracted a wide range of chemical classes from the leaf material. This supports the use of methanol for exploratory screening but also highlights a translational limitation. Traditional kava preparations are commonly aqueous and often use roots or rhizomes, while the present preparation is an organic-solvent leaf extract. Its chemical profile and safety cannot be assumed to mirror traditional use. The paper intentionally confines its conclusion to the prepared methanolic leaf extract.

For further standardization, thin-layer chromatography could first provide a rapid fingerprint and confirm batch similarity. High-performance thin-layer chromatography or high-performance liquid chromatography could then separate and quantify marker compounds. Gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry would support identification of volatile and non-volatile constituents, while validated reference standards could quantify the six major kavalactones. Such work should also examine flavokavains and other constituents relevant to safety. Botanical identity, extraction yield and preliminary screening provide a necessary foundation, but they are not sufficient for a standardized medicinal product.

Several limitations should be acknowledged. Only one extraction solvent and one extraction method were evaluated, so extraction efficiency cannot be compared across techniques. The study used qualitative chemical reactions rather than validated quantitative assays. No chromatographic fingerprint, residual-solvent test, total phenolic content, total flavonoid content or individual kavalactone assay was performed. The authentication relied primarily on macroscopic and taxonomical examination, without molecular confirmation. These limitations do not invalidate the descriptive results; rather, they define the appropriate level of inference.

The practical contribution of the work is a documented, reproducible preparation pathway. Future pharmacological or toxicological experiments can refer to a specific authenticated plant part and a defined extraction protocol rather than an unspecified kava product. This is critical because variations in plant part, chemotype, solvent and thermal history may contribute to inconsistent findings in the kava literature. A strong research programme should preserve this chain of identity and processing as it advances from crude screening to fractionation, mechanism studies and safety assessment.

5. Conclusion

Authenticated *Piper methysticum* leaves were successfully cleaned, shade-dried, powdered and extracted with 95% methanol by Soxhlet processing. The procedure yielded 68.50 g of dark-brown semisolid extract from 500 g dry powder, corresponding to 13.70%. Preliminary screening detected ten of eleven tested phytochemical groups, with strong phenolic and terpenoid reactions and moderate flavonoid, tannin, glycoside and steroid reactions. These findings establish botanical traceability, extraction reproducibility and a preliminary chemical basis for subsequent studies. The results apply specifically to the methanolic leaf extract and should not be generalized to root, rhizome or aqueous kava preparations. Chromatographic fingerprinting and quantitative kavalactone analysis are required before the extract can be considered chemically standardized.

6. Declarations

Conflict of interest: The author declares no conflict of interest.

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Author Contribution: Poonam Yadav contributed to conceptualization, methodology, investigation, data collection, formal analysis, and preparation of the original manuscript. Sushila Kaura provided supervision, validation, project administration, and critical review and editing. Both authors reviewed and approved the final manuscript.

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