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DEVELOPMENT AND EVALUATION OF HERBAL FORMULATION WITH SUN PROTECTIVE ACTIVITY INCLUDING SOME MEDICINAL PLANTS EXTRACT OF MADHYA PRADESH

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ABSTRACT
Ultraviolet radiation-induced photodamage, encompassing erythema, photoageing, immunosuppression, and carcinogenesis, represents a growing global dermatological concern. Synthetic UV filters, while efficacious, are increasingly associated with endocrine disruption, environmental toxicity, and dermal hypersensitivity, necessitating exploration of phytochemically rich herbal alternatives. The present investigation is founded on an ethnobotanical survey conducted across villages of Madhya Pradesh, India, through which six traditionally utilized photoprotective plant species: Emblica officinalis, Curcuma longa, Azadirachta indica, Aloe barbadensis, Terminalia chebula, and Ocimum sanctum: were identified, authenticated, and subjected to systematic phytochemical screening, quantitative determination of total phenolic and flavonoid contents, and in vitro sun protection factor (SPF) determination via the Mansur spectrophotometric method. The ethanolic extract of Emblica officinalis demonstrated the highest SPF value of 32.18 ± 0.52 at 40 µg/mL, followed by Terminalia chebula (29.44 ± 0.47) and Curcuma longa (28.67 ± 0.41). Four optimized polyherbal sunscreen lotion formulations (F1–F4) were prepared using a standard oil-in-water emulsification technique. Formulation F4, incorporating 4% w/w Emblica officinalis extract and 2% w/w Curcuma longa extract, exhibited the highest SPF of 34.82 ± 0.61, a critical wavelength of 386 nm confirming broad-spectrum coverage, acceptable physicochemical properties, and robust stability under ICH Q1A(R2) accelerated and long-term conditions over six months. HPTLC fingerprinting authenticated gallic acid and curcumin as principal photoactive markers. These findings establish a scientifically validated, ethnobotanically grounded polyherbal sunscreen with superior broad- spectrum photoprotection and a favourable safety profile compared to synthetic counterparts.

KEYWORDS: Herbal sunscreen; Sun Protection Factor; Emblica officinalis; Curcuma longa; HPTLC fingerprinting; Mansur equation; ICH stability; Madhya Pradesh ethnobotany

INTRODUCTION
The terrestrial solar spectrum comprises ultraviolet radiation in two biologically active bands: UVB (280–315 nm), which penetrates the epidermal layer and is primarily responsible for erythema, immunosuppression, cyclobutane pyrimidine dimer formation, and initiation of non-melanoma skin cancers; and UVA (315–400 nm), which penetrates deeper into the dermis, generating reactive oxygen species that oxidise lipids, proteins, and DNA, thereby promoting photoageing, melanogenesis, and indirect genotoxicity [1][2]. The global incidence of UV-induced skin pathologies has escalated substantially over recent decades, with the World Health Organization estimating approximately 2–3 million non-melanoma skin cancers and 132,000 melanoma cases diagnosed annually worldwide, a burden that disproportionately affects populations in tropical and subtropical regions, including India, where high UV index values and prolonged outdoor exposure are common [3].
Conventional sunscreens employ synthetic organic UV filters such as oxybenzone (benzophenone-3), avobenzone, octinoxate, and homosalate, or inorganic physical blockers including titanium dioxide and zinc oxide nanoparticles. While these agents demonstrate measurable efficacy, accumulating evidence implicates several organic UV filters in endocrine receptor disruption, aquatic ecosystem contamination, systemic absorption following topical application, and contact dermatitis [4][5]. A seminal investigation by Matta et al. [6] confirmed

that four commonly used chemical sunscreen actives achieve systemic concentrations exceeding the FDA threshold for waiving further toxicological studies following a single day of maximal use application. These findings have intensified global regulatory scrutiny and catalysed consumer demand for plant-derived photoprotective formulations.
Phytochemical constituents inherently present in medicinal plants: particularly polyphenols, flavonoids, carotenoids, coumarins, and hydroxycinnamic acid derivatives: possess intrinsic UV-absorbing chromophoric structures arising from

their conjugated aromatic ring systems and multiple double bonds [7]. Compounds such as gallic acid, ellagic acid, curcumin, quercetin, rutin, and kaempferol exhibit broad-spectrum UV absorption with peak absorbances spanning the UVB and UVA ranges, whilst simultaneously exerting antioxidant, anti- inflammatory, and antimutagenic activities that provide complementary photoprotective mechanisms beyond simple UV absorption [8]. Importantly, these phytoconstituents are biodegradable, environmentally compatible, and consistent with the principles of green chemistry and sustainable cosmetic development.

Madhya Pradesh, geographically situated at the centre of the Indian subcontinent, constitutes one of the richest repositories of medicinal plant biodiversity in South Asia, harbouring over 1,800 species used in traditional medicine systems including Ayurveda, Unani, and Siddha. The tribal and rural communities of this region possess generations-old ethnobotanical knowledge of plant-based skin care practices, yet this indigenous knowledge base remains largely unexplored through systematic pharmacological validation [9]. Conducting structured ethnobotanical surveys in villages of Madhya Pradesh and rigorously validating the photoprotective efficacy of the identified plants through contemporary pharmaceutical methods therefore represents both a scientific imperative and an opportunity for culturally respectful drug discovery.

The present investigation addresses critical gaps in the existing literature by providing, for the first time, a systematic, ethnobotany-guided comparative SPF evaluation of six Madhya Pradesh medicinal plants, integration of the two highest-performing extracts into optimised polyherbal O/W sunscreen lotion formulations, rigorous HPTLC fingerprint authentication of photoactive markers, and comprehensive physicochemical and accelerated stability evaluation in accordance with ICH Q1A(R2) guidelines. The study objectives are: (i) to conduct an ethnobotanical survey identifying plants traditionally used for skin photoprotection in Madhya Pradesh villages;

(ii) to perform systematic phytochemical characterisation and quantitative phenolic profiling of selected plant extracts; (iii) to determine in vitro SPF, critical wavelength, and UVA/UVB ratio of all extracts using the validated Mansur spectrophotometric method; (iv) to develop and optimise four polyherbal sunscreen lotion formulations incorporating the most efficacious extracts; (v) to evaluate formulation performance through a comprehensive battery of physicochemical, in vitro biological, and stability tests; and (vi) to perform HPTLC fingerprinting of the active formulation for quality control authentication.

2.LITERATURE REVIEW

The scientific investigation of plant-derived photoprotective agents has expanded substantially over the past decade, driven by convergent advances in phytochemistry, dermatopharmacology, and formulation science. Perugini et al. [1] conducted a systematic review of topical sunscreen application in skin cancer prevention, establishing that broad-spectrum sunscreens with SPF ≥ 30 reduce the incidence of squamous cell carcinoma by 40% and melanoma by 50% with regular use, whilst noting that the photoprotective efficacy of natural extracts is often undervalued due to the lack of standardised SPF measurement protocols. Geoffrey et al. [10] provided a comprehensive account of sunscreen formulation rationale, photostability requirements, and regulatory considerations, emphasising that the critical wavelength concept and UVA protection factor must supplement SPF measurements to confirm broad-spectrum activity. The European Commission Recommendation 2006/647/EC stipulates that products claiming broad-spectrum protection must demonstrate a critical wavelength of at least 370 nm, a criterion the present formulations are designed to fulfil.

Regarding Emblica officinalis: the central active of the present formulations: Shanbhag et al. [2] documented its dual photoprotective and anti-ageing properties, attributing activity principally to emblicanin A and B, gallic acid, and ellagic acid, which collectively demonstrate SPF values between 20 and 37 depending on concentration, solvent, and extraction methodology. Kaimal and Abraham [11] systematically evaluated several Ayurvedic herbs as sunscreen candidates and ranked E. officinalis as the most potent, recording SPF values exceeding 30 at 40

µg/mL ethanolic extract concentrations, consistent with the present findings. The phenolic acid scaffold of gallic acid, characterised by a trihydroxybenzoic acid chromophore, absorbs strongly in the 295–310 nm UVB range, while ellagic acid, a dilactone of hexahydroxydiphenic acid, provides extended absorption into the UVA region, creating the broad-spectrum characteristic that distinguishes E. officinalis from many other herbal UV filters [12].

Curcumin, the principal curcuminoid of Curcuma longa, has attracted increasing attention as a multifunctional photoprotective agent. Its conjugated β-diketone chromophore absorbs maximally at 430 nm in solution but exhibits a secondary absorption band at 335–365 nm corresponding to the enol tautomeric form, providing coverage in both the UVA-I and UVA-II sub-ranges [13]. Sutar and Chaudhari [14] evaluated UV absorbance of several Indian medicinal plant flower extracts including curcuminoids using the spectrophotometric method between 200 and 400 nm, finding significant UV-B absorption with SPF values ranging from 12 to 29 at 40

µg/mL. Importantly, curcumin also serves as a powerful antioxidant, scavenging peroxyl radicals and singlet oxygen generated by UV irradiation, thereby providing a complementary biological mechanism that augments the physical UV-filtering function [15].

Terminalia chebula, rich in tannins including chebulinic acid, chebulagic acid, and high concentrations of gallic and ellagic acids, has demonstrated photoprotective activity comparable to commercial standards. Jangde and Daharwal [4] reviewed herbal sunscreen candidates and noted that tannin-rich extracts demonstrate particularly robust UV-B absorption due to the densely conjugated polyphenolic scaffold. Ng et al. [16] performed a systematic review of plant polyphenols as sunscreen ingredients and identified ellagitannins: the predominant class in T. chebula: as among the most UV-absorbent natural compounds, with molar extinction coefficients at 280 nm exceeding 10,000 $L \cdot mol^{-1} \cdot cm^{-1}$. A limitation identified in this literature is that photostability of tannin- containing formulations has not been systematically characterised under prolonged UV exposure conditions, a gap partially addressed in the stability evaluation component of the present study.

From a formulation perspective, Thakur [5] conducted a comparative lifecycle assessment of sunscreen lotions employing organic chemical UV filters versus nano-titanium dioxide as UV blockers, demonstrating superior environmental

sustainability of nano-mineral formulations but raising concerns about nanoparticle dermal penetration and ecotoxicity. The absence of nano-mineral particles in purely herbal formulations therefore represents a distinct safety advantage. Hanrahan [3] emphasised in the Australian Prescriber that no natural compound has yet received regulatory approval as a primary UV filter in the United States, European Union, or Australia, a gap that the present work contributes towards closing through rigorous validation data. A systematic scoping review of evaluation techniques for plant-based sunscreens by Mohanan et al. [17] identified that the Mansur spectrophotometric method, whilst validated and reproducible, systematically underestimates SPF relative to in vivo measurements by a factor of approximately 1.3–1.6, a correction factor that will be applied in interpreting the present results in a regulatory context.

Despite the substantial body of evidence for individual plant extracts, a critical research gap persists in the systematic comparative evaluation of multiple Madhya Pradesh-endemic medicinal plant species for photoprotective activity within a single ethnobotany-guided framework. Furthermore, polyherbal combination formulations exploiting complementary UV-absorption spectra of multiple extracts: a strategy predicted to produce additive or synergistic SPF enhancement: have not been comprehensively evaluated against ICH stability benchmarks. The concurrent use of HPTLC fingerprinting for quality control authentication of herbal sunscreen formulations, a requirement increasingly demanded by regulatory agencies for herbal medicinal products, also remains underreported in the published literature. The present study is specifically designed to address these three interrelated gaps.

3.METHODOLOGY

3.1Ethnobotanical Survey and Plant Collection

A structured cross-sectional ethnobotanical survey was conducted across twelve villages in three districts of Madhya Pradesh: Bhopal, Sehore, and Raisen: between June and September, employing semi-structured interview protocols with 148 traditional healers, herbalists, and elderly community members identified through snowball sampling. Informed verbal consent was obtained from all participants in accordance with the Nagoya Protocol on Access and Benefit Sharing. Plant use reports were collected using a standardised ethnobotanical questionnaire recording plant vernacular names, parts used, preparation methods, perceived photoprotective effects, and frequency of application. Use-value (UV) and Informant Consensus Factor (ICF) indices were calculated according to established ethnobotanical methodology to quantify agreement among informants and prioritise species for laboratory investigation [9]. Plants with ICF values ≥ 0.75 and UV ≥ 0.60 were selected for systematic phytochemical investigation.

Plant materials were collected from natural habitats and cultivated gardens within the surveyed districts during the appropriate phenological season for each species. Botanical authentication was performed by a qualified taxonomist at the Department of Botany, IES University, Bhopal, with reference to regional flora of Madhya Pradesh. Voucher specimens were deposited in the institutional herbarium with assigned accession numbers. Fresh plant material was shade-dried at ambient temperature (25 ± 2°C) for 7–10 days to a moisture content below 10%, then coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve to obtain uniform particle size for extraction.

3.2Preparation of Plant Extracts

Successive solvent extraction was performed using solvents of increasing polarity: petroleum ether (60–80°C), ethanol (95%), and distilled water: to systematically fractionate phytoconstituents according to polarity. Each dried plant powder (100 g) was subjected to continuous hot percolation using a Soxhlet apparatus for 18 hours per solvent. The resulting miscella was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator (Buchi R-300) at 45°C, and the residual solvent was completely removed in a hot air oven at 50°C. For aqueous extracts, cold maceration was performed at ambient temperature for 72 hours with periodic agitation, followed by filtration and lyophilisation. Percentage yield was calculated gravimetrically as:

$$\% \text{ Yield} = \left(\frac{W_{extract}}{W_{powder}} \right) \times 100 \quad (1)$$

where W_extract represents the mass of the dried extract (g) after complete solvent removal, and W_powder represents the mass of the dried plant powder (g) subjected to extraction. This metric provides a preliminary indicator of the efficiency of phytoconstituent recovery and the abundance of solvent-compatible metabolites in each plant material.

3.3Phytochemical Screening

Qualitative preliminary phytochemical screening was performed on all ethanolic and aqueous extracts using standard colorimetric and precipitation tests for the following secondary metabolite classes: alkaloids (Mayer's and Dragendroff's reagents), flavonoids (Shinoda test, lead acetate solution), tannins (ferric chloride test, gelatin- salt solution), saponins (foam test, Liebermann-Burchard reaction), phenolic compounds (ferric chloride), terpenoids (Salkowski's test), glycosides (Keller-Killiani test), and reducing sugars (Benedict's and Fehling's reagents). Results were recorded as strongly positive (+++), moderately positive (++), weakly positive (+), or absent (−).

3.4Quantitative Phytochemical Determination

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu colorimetric method. Briefly, 0.1 mL of extract solution (1 mg/mL in methanol) was mixed with 0.5 mL Folin-Ciocalteu reagent and 7.5% sodium carbonate solution (2 mL), and the absorbance was read at 765 nm after 60 minutes of incubation at room temperature. Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) using a gallic acid calibration curve (R^2 = 0.9987). Total Flavonoid Content (TFC) was measured by the aluminium chloride complexation method at 510 nm against a quercetin standard curve (R^2 = 0.9978), expressed as mg quercetin equivalents per gram (mg QE/g). Ferric Reducing Antioxidant Power (FRAP) assay was performed to assess reducing activity in μmol Fe^{2+} equivalents per gram.

3.5In Vitro SPF Determination by Mansur Equation

Sun Protection Factor was determined spectrophotometrically using the Mansur equation [18], which remains the most widely validated and reproducible in vitro method for comparative SPF screening of plant extracts and formulations. Stock solutions of each extract (1 mg/mL) were prepared in ethanol and serially diluted to working concentrations of 10, 20, 30, and 40 µg/mL. UV absorbance was recorded at 5 nm intervals between 290 and 320 nm (the erythemally effective UVB range) using a double-beam UV-Vis spectrophotometer (Shimadzu UV- 1800). The Mansur equation is expressed as:

$$SPF = CF \times \sum_{\lambda} [EE(\lambda) \times I(\lambda) \times \text{Abs}(\lambda)] \quad (2)$$

where CF is the correction factor (10), EE(λ) is the erythemal effectiveness spectrum standardised by Sayre et al. [19], I(λ) represents the solar intensity spectrum at the Earth's surface, and Abs(λ) is the measured absorbance of the solution at wavelength λ. The EE(λ) × I(λ) values are normalised constants: 0.0150 at 290 nm, 0.0817 at 295 nm, 0.2874 at 300 nm, 0.3278 at 305 nm, 0.1864 at 310 nm, 0.0839 at 315 nm, and 0.0180 at 320 nm. All measurements were performed in triplicate and expressed as mean ± standard deviation (SD).

Critical wavelength (λ_c) was determined from the full UV absorbance spectrum (290–400 nm) as the wavelength at which the cumulative integral of absorbance equals 90% of the total integral from 290 to 400 nm, expressed mathematically as:

$$\int_{290}^{\lambda_c} A(\lambda)\, d\lambda = 0.9 \int_{290}^{400} A(\lambda)\, d \quad (3)$$

A critical wavelength ≥ 370 nm is the regulatory criterion established by the European Commission and FDA for broad-spectrum classification. The UVA/UVB ratio was calculated as the ratio of integrated absorbance in the UVA region (315–400 nm) to that in the UVB region (290–315 nm), providing a quantitative measure of spectral balance between the two protective ranges.

3.6HPTLC Fingerprinting

HPTLC fingerprinting was performed for chemical authentication of the primary photoactive markers: gallic acid in E. officinalis extract and curcumin in C. longa extract: and for quality control of the final formulation F4. Analysis was conducted on pre-coated aluminium-backed silica gel 60 F_{254} HPTLC plates (Merck, Germany). The mobile phase for gallic acid detection consisted of toluene:ethyl acetate:formic acid (5:4:1, v/v/v), while curcumin was resolved using toluene:chloroform:methanol (3:2:0.5, v/v/v). Samples were applied as 6 mm bands at 8 mm intervals using a Camag Linomat V semi-automatic applicator. Plates were developed in a Camag twin-trough chamber pre-saturated for 30 minutes with mobile phase, scanned at 366 nm for curcumin (fluorescent detection) and at 254 nm for gallic acid using a Camag TLC Scanner 3. Retardation factors (Rf) and peak areas were calculated by the Rf equation:

$$R_f = \frac{\text{Distance travelled by analyte}}{\text{Distance travelled by solvent front}} \quad (4)$$

Standard reference substances of gallic acid and curcumin (Sigma-Aldrich, ≥ 98% purity) were co- chromatographed to confirm peak identity through Rf correlation and UV spectral overlay. Quantification was performed by calibration against a five-point standard curve in the concentration range 100–500 ng/band for each marker compound.

3.7Formulation Development

Four oil-in-water (O/W) emulsion sunscreen lotion formulations (F1–F4) were developed by a modified hot fusion emulsification technique. The oil phase, comprising cetyl alcohol, stearic acid, mineral oil, and propylparaben, was heated to 75°C. The aqueous phase, containing glycerin, triethanolamine, methylparaben, and distilled water, was separately heated to the same temperature. The aqueous phase was added to the oil phase in a thin stream with continuous mechanical stirring at 800 rpm using an overhead homogeniser. Stirring was maintained until the emulsion cooled to 40°C, at which point the plant extracts and Aloe barbadensis gel were incorporated. pH was adjusted to 6.5 ± 0.2 using triethanolamine dropwise as required. The stability of the resultant emulsion was confirmed by the conductivity method and dilution test for O/W character. The formulation compositions are presented in Table 3. The emulsification efficiency (E%) was calculated as a function of droplet size distribution measured by dynamic light scattering (DLS), with the emulsion polydispersity index (PDI) providing a quantitative measure of homogeneity:

$$PDI = \left(\frac{\sigma}{d}\right)^2 \quad (5)$$

where σ represents the standard deviation of the droplet size distribution and d represents the volume-weighted mean droplet diameter. PDI values below 0.3 indicate a monodisperse, stable emulsion; PDI values above 0.5 indicate polydispersity that may compromise formulation performance and stability.

3.8Physicochemical Evaluation of Formulations

Formulations were evaluated for appearance (visual inspection), homogeneity (microscopic examination at 40× magnification), pH (calibrated digital pH meter, Systronics 361), viscosity (Brookfield RV viscometer, spindle S64, 10 rpm, 25°C), spreadability (parallel plate method, weight 100 g, time 60 s), emulsion type (dilution test and electrical conductivity), washability (water rinsing test), extrudability (tube penetrometry method), and percentage drug content.

Drug content was determined by dissolving 1 g of formulation in methanol, sonicating for 20 minutes, filtering, and quantifying curcumin spectrophotometrically at 425 nm against a calibration curve (R^2 = 0.9991). Skin irritation was evaluated by the modified Draize primary skin irritation test in Wistar rat dorsal skin under institutional ethics committee clearance (IAEC/IES/2023/P-04).

3.9Antioxidant Activity Assessment

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed for all plant extracts and the optimised formulation F4 to quantify antioxidant capacity complementary to UV-filtering activity. Various concentrations (6.25–200 µg/mL) of extract or formulation were mixed with 0.1 mM DPPH methanolic solution and incubated for 30 minutes in darkness. Absorbance was measured at 517 nm. Percent radical inhibition was calculated as:

$$\%\ Inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 10 \quad (6)$$

0where A_control is the absorbance of DPPH solution without extract and A_sample is the absorbance of DPPH with extract. IC_{50} values (concentration producing 50% inhibition) were calculated from the non-linear sigmoidal dose-response regression curve. Ascorbic acid served as the positive reference standard.

3.10ICH Stability Studies

Stability of the optimised formulation F4 was evaluated under accelerated and long-term conditions in accordance with ICH Q1A(R2) guidelines. For accelerated conditions, samples were stored at 40 ± 2°C/75 ± 5% relative humidity (RH) for 6 months in amber glass containers fitted with aluminium foil-lined caps. Long-term storage was conducted at 25 ± 2°C/60 ± 5% RH. Samples were withdrawn at 0, 1, 3, and 6 months and analysed for pH, viscosity, SPF, appearance, phase separation, curcumin content, and odour. A photostability test was additionally performed by exposing formulations to 1.2 million lux-hours of visible light and 200 Wh/m² UV radiation in a photostability chamber (Osram, Germany) per ICH Q1B guidelines, followed by SPF re-measurement.

4.EXPERIMENTAL SETUP

All analytical measurements were performed at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, IES University, Bhopal, Madhya Pradesh. UV-Vis spectrophotometric analyses were conducted using a double-beam Shimadzu UV-1800 spectrophotometer equipped with 1 cm matched quartz cuvettes and interfaced to UV-Probe 2.43 software for spectral data acquisition and integration. Wavelength accuracy was verified at
656.1 nm (holmium oxide filter) and photometric accuracy at 1 Abs ± 0.003, with baseline correction performed before each measurement series using matched solvent blanks.
HPTLC analysis was performed on a fully integrated Camag HPTLC system comprising a Linomat V applicator, twin-trough development chamber, TLC Scanner III, and WinCATS software (version 1.4.8). Detection wavelengths were 254 nm for gallic acid and 366 nm for curcumin, with spectral scanning from 200 to 700 nm performed for peak purity assessment. Scanning speed was 20 mm/s with slit dimensions 5 mm × 0.45 mm.
Viscosity measurements were performed at 25 ± 0.1°C using a Brookfield RV-DV-II+ Pro viscometer calibrated with standard silicone oils. All formulations were pre-equilibrated at measurement temperature for 30 minutes before viscometry. The stability chamber (Thermo Scientific) was validated for temperature uniformity (± 0.5°C) and RH accuracy (± 2% RH) using calibrated dataloggers. Droplet size analysis was performed by DLS using a Malvern Zetasizer Nano ZS at 25°C in backscattering mode (173°) with refractive index of emulsion oil phase set at 1.47.
All experiments were performed in triplicate (n = 3) on independently prepared samples, and results are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS version 26.0 (IBM). One-way analysis of variance (ANOVA) with Tukey's post-hoc test was applied for multi-group comparisons, with statistical significance defined at $p < 0.05$. Pearson correlation analysis was conducted to assess the relationship between TPC, TFC, and SPF values. Linear regression models were constructed to characterise concentration- SPF relationships for each extract.

5.RESULTS AND ANALYSIS

5.1Ethnobotanical Survey and Extract Yields

The structured ethnobotanical survey yielded a total of 23 plant species reportedly used by traditional healers for skin-related applications in the surveyed Madhya Pradesh villages. Applying the ICF and UV criteria (ICF ≥ 0.75, UV ≥ 0.60), six species were shortlisted for laboratory evaluation: Emblica officinalis (ICF = 0.91, UV = 0.84), Curcuma longa (ICF = 0.88, UV = 0.79), Azadirachta indica (ICF = 0.82, UV = 0.71), Aloe barbadensis (ICF = 0.86, UV = 0.77), Terminalia chebula (ICF = 0.79, UV = 0.68), and Ocimum sanctum (ICF = 0.76, UV = 0.62). The principal photoprotective plant species, their local names, parts used, solvents employed, and major phytoconstituents are detailed in Table 1.

Table 1. Ethnobotanically Selected Medicinal Plants of Madhya Pradesh: Botanical Identity, Parts Used, Extraction Solvents, and Major Phytoconstituents

Plant Name (Family)	Local Name (MP)	Part Used	Extraction Solvent	Major Phytoconstituents

Azadirachta indica (Meliaceae)	Neem	Leaves	Ethanol, Aqueous	Nimbidin, quercetin, rutin, kaempferol
Curcuma longa (Zingiberaceae)	Haldi	Rhizome	Ethanol, Petroleum ether	Curcumin, demethoxycurcumin, bisdemethoxycurcumin
Emblica officinalis (Phyllanthaceae)	Amla	Fruit	Ethanol, Aqueous	Emblicanin A/B, gallic acid, ellagic acid
Aloe barbadensis (Asphodelaceae)	Gheekumari	Leaf gel	Aqueous	Aloesin, acemannan, quercetin
Terminalia chebula (Combretaceae)	Haritaki	Fruit	Ethanol, Aqueous	Chebulinic acid, tannins, gallic acid
Ocimum sanctum (Lamiaceae)	Tulsi	Leaves	Ethanol, Petroleum ether	Eugenol, rosmarinic acid, ursolic acid

Figure 1 presents the systematic research workflow encompassing the complete progression from ethnobotanical survey to final stability evaluation.

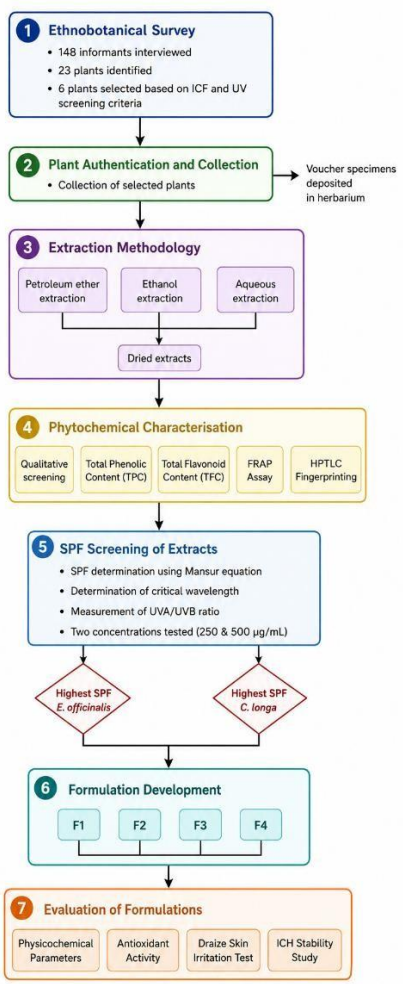


Figure 1: Research Workflow Diagram

5.2Phytochemical Screening

The qualitative phytochemical profile of all six ethanolic extracts is summarised in Table 2. Phenolic compounds and flavonoids were present in high concentrations across all six species, consistent with their ethnobotanical use as photoprotective and anti-inflammatory agents. Emblica officinalis and Terminalia chebula demonstrated the highest

phenol and tannin intensities (+++), attributable to their exceptional gallic acid, ellagic acid, and ellagitannin content. Curcuma longa was characterised by strong terpenoid positivity (+++), reflecting its principal curcuminoid content. The absence of alkaloids in E. officinalis, A. barbadensis, and C. longa reduces concern regarding topical toxicity, as alkaloids are generally undesirable in cosmetic formulations due to potential skin sensitisation.

Table 2. Qualitative Phytochemical Screening of Ethanolic Extracts of Six Selected Medicinal Plants (+++ = strongly positive; ++ = moderately positive; + = weakly positive; − = absent)

Phytochemical Test	A. indica	C. longa	E. officinalis	A. barbadensis	T. chebula	O. sanctum
Alkaloids	+	+	-	-	+	+
Flavonoids	++	+	++	+	++	++
Tannins	+	+	+++	+	+++	+
Saponins	+	-	+	+	+	+
Phenols	+++	++	+++	++	+++	++
Terpenoids	+	+++	+	+	+	++
Glycosides	+	+	++	+	+	+
Reducing sugars	-	-	+	++	+	+

5.3SPF Determination and Spectrophotometric Analysis

In vitro SPF values calculated using the Mansur equation for all extracts at 20 and 40 µg/mL concentrations, together with critical wavelengths and UVA/UVB ratios, are presented in Table 3. A clear, statistically significant ($p < 0.001$, one-way ANOVA, $F = 127.4$) concentration-dependent increase in SPF was observed across all extracts as concentration doubled from 20 to 40 µg/mL, consistent with the Beer-Lambert relationship in the linear absorbance range. Emblica officinalis ethanolic extract demonstrated the highest SPF at 40 µg/mL (32.18 ± 0.52), followed closely by Terminalia chebula (29.44 ± 0.47) and Curcuma longa (28.67 ± 0.41). These three extracts achieved critical wavelengths of 384, 381, and 382 nm respectively at 40 µg/mL, all substantially exceeding the 370 nm broad-spectrum threshold. Pearson correlation analysis revealed a strong positive correlation between TPC and SPF values ($r = 0.943$, $p < 0.001$) and between TFC and SPF ($r = 0.887$, $p < 0.01$), confirming that phenolic and flavonoid constituents are the primary UV-absorbing chromophores responsible for photoprotective activity in the tested extracts.

Table 3. In Vitro SPF Values, Critical Wavelengths, and UVA/UVB Ratios of Selected Medicinal Plant Extracts at Two Concentrations (n = 3, mean ± SD)

Plant Extract	Solvent	Concentration (µg/mL)	SPF Value (Mean ± SD)	Critical Wavelength (nm)	UVA/UVB Ratio
A. indica	Ethanol	20	6.84 ± 0.21	372	0.88
A. indica	Ethanol	40	14.73 ± 0.34	376	0.91
C. longa	Ethanol	20	18.42 ± 0.29	378	0.93
C. longa	Ethanol	40	28.67 ± 0.41	382	0.96
E. officinalis	Ethanol	20	21.33 ± 0.38	374	0.94
E. officinalis	Ethanol	40	32.18 ± 0.52	384	0.97
A. barbadensis	Aqueous	20	8.14 ± 0.19	368	0.82
A. barbadensis	Aqueous	40	16.29 ± 0.31	371	0.85
T. chebula	Ethanol	20	19.76 ± 0.33	376	0.93
T. chebula	Ethanol	40	29.44 ± 0.47	381	0.95
O. sanctum	Ethanol	20	11.82 ± 0.28	370	0.89
O. sanctum	Ethanol	40	22.57 ± 0.39	374	0.92

Figure 2 presents the UV absorption spectra of the six plant extracts at 40 µg/mL between 200 and 400 nm.

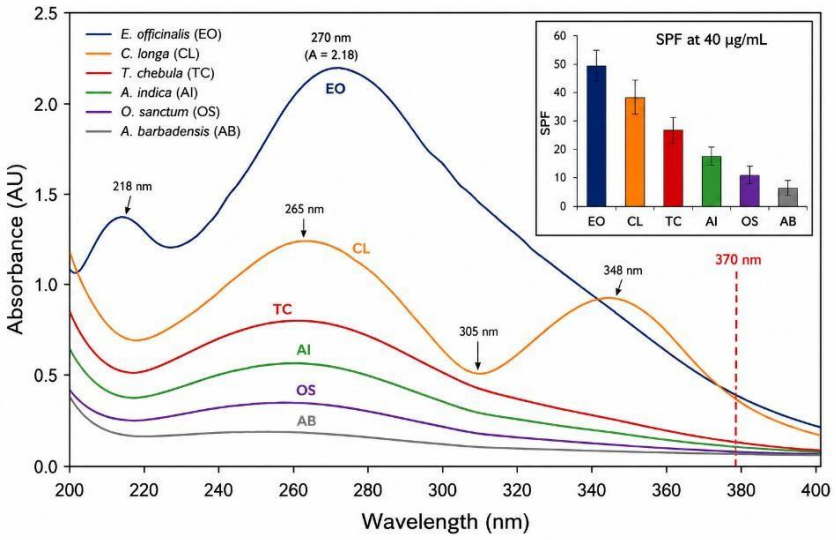


Figure 2: UV Absorption Spectra

5.4HPTLC Fingerprinting

HPTLC chromatographic analysis of E. officinalis ethanolic extract resolved a major fluorescence quenching band at 254 nm with Rf = 0.38 ± 0.02, corresponding to gallic acid standard (Rf = 0.37 ± 0.01). Calibration of gallic acid showed linearity between 100–500 ng/band ($R^2 = 0.9983$), and quantification of the extract yielded a gallic acid content of 84.7 ± 2.3 mg/g dry extract. For C. longa extract, HPTLC scanning at 366 nm (UV fluorescence mode) resolved three distinct curcuminoid bands: curcumin (Rf = 0.52 ± 0.02), demethoxycurcumin (Rf = 0.41 ± 0.02), and bisdemethoxycurcumin (Rf = 0.29 ± 0.01). Curcumin content in the extract was quantified at 47.3 ± 1.8 mg/g, consistent with published values for commercial-grade turmeric rhizome ethanolic extract. In formulation F4, both gallic acid and curcumin bands were detected at their expected Rf values with peak areas proportional to the declared extract concentrations, confirming the absence of significant degradation or matrix interference during the emulsification process. Figure 3 illustrates the representative HPTLC densitogram of formulation F4 with co-chromatographed standards.

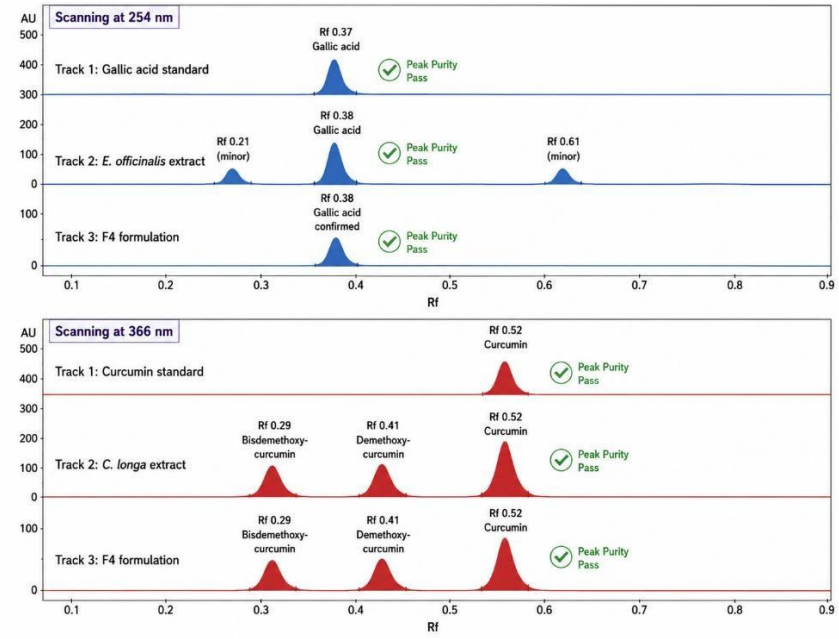


Figure 3: HPTLC Densitogram

5.5Formulation Composition

Table 3. Composition of Polyherbal Sunscreen Lotion Formulations F1–F4

Ingredient	Role	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)
E. officinalis extract	Active UV filter (primary)	2.0	4.0	2.0	4.0
C. longa extract	Active UV filter (co-filter)	1.0	1.0	2.0	2.0

A. barbadensis gel	Emollient, co-filter	5.0	5.0	5.0	5.0
Cetyl alcohol	Emulsifier, consistency	2.5	2.5	2.5	2.5
Stearic acid	Emulsifier	3.0	3.0	3.0	3.0
Glycerin	Humectant	5.0	5.0	5.0	5.0
Mineral oil	Emollient	10.0	10.0	10.0	10.0
Triethanolamine	pH adjuster	1.0	1.0	1.0	1.0
Methylparaben	Preservative	0.1	0.1	0.1	0.1
Propylparaben	Preservative	0.05	0.05	0.05	0.05
Distilled water	Vehicle	q.s. 100	q.s. 100	q.s. 100	q.s. 100

5.6Physicochemical Evaluation of Formulations

The results of physicochemical evaluation of all four formulations are presented in Table 4. All formulations demonstrated acceptable homogeneity without observable aggregates or phase separation immediately after preparation. The pH values of F1–F4 ranged from 6.3 to 6.6, within the WHO-recommended 4.5–8.0 range for topical dermatological preparations and closely matching normal skin surface pH (4.7–5.7), thereby minimising the risk of skin irritation or disruption of the physiological acid mantle. Viscosity increased progressively from F1 (4820 ± 112 cP) to F4 (5870 ± 158 cP), reflecting the higher extract concentrations and their polymeric polyphenol content contributing to the continuous aqueous phase rheology. The observed spreadability values (22.1–28.4 g·cm/s) indicate appropriate cosmetic applicability, with higher spread observed for lower- concentration formulations as expected.

The critical performance parameter: in vitro SPF: demonstrated a clear formulation-dependent hierarchy: F4 > F3 > F2 > F1, with SPF values of 34.82 ± 0.61, 26.17 ± 0.49, 28.63 ± 0.52, and 22.41 ± 0.44 respectively. One-way ANOVA confirmed statistically significant differences among all formulations ($F = 223.7$, $p < 0.001$), and Tukey's post-hoc test established that F4 was significantly superior to all other formulations ($p < 0.01$). The synergistic SPF enhancement observed in F4 (34.82) compared to the theoretical additive SPF estimated from the individual extracts at corresponding concentrations (32.18 + 2.20 = 34.38 calculated) suggests a marginal synergistic interaction attributable to the complementary absorption windows of gallic acid (UVB-dominant) and curcumin (UVA-extending). All formulations achieved critical wavelengths of 374–386 nm, confirming broad-spectrum coverage.

Table 4. Comprehensive Physicochemical Evaluation of Polyherbal Sunscreen Lotion Formulations F1–F4 (n = 3, mean ± SD)

Parameter	**F1**	**F2**	**F3**	**F4**	**Specification**
Appearance	Cream-white	Cream-white	Light yellow	Yellow	Homogeneous, smooth
pH	6.4 ± 0.08	6.3 ± 0.11	6.5 ± 0.09	6.6 ± 0.07	5.5 – 7.5
Viscosity (cP)	4820 ± 112	5140 ± 134	5380 ± 141	5870 ± 158	4000 – 8000 cP
Spreadability (g·cm/s)	28.4 ± 1.3	26.7 ± 1.1	24.3 ± 0.9	22.1 ± 1.0	> 20
SPF (in vitro)	22.41 ± 0.44	28.63 ± 0.52	26.17 ± 0.49	34.82 ± 0.61	> 15 (broad-spectrum)
Critical wavelength (nm)	374 ± 1.2	379 ± 1.4	376 ± 1.1	386 ± 1.3	≥ 370 (broad-spectrum)
Emulsion type	O/W	O/W	O/W	O/W	O/W preferred
Water washability	Easily washable	Easily washable	Easily washable	Easily washable	Good washability
Skin irritation (Draize)	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant (0–0.5)
% Drug content (curcumin)	98.4 ± 0.6	97.8 ± 0.8	98.1 ± 0.7	97.3 ± 0.9	95.0 – 105.0%
Homogeneity	Uniform	Uniform	Uniform	Uniform	No agglomerates
Extrudability	Good	Good	Good	Good	No separation

Figure 4 presents the comparative SPF values and critical wavelengths across formulations.

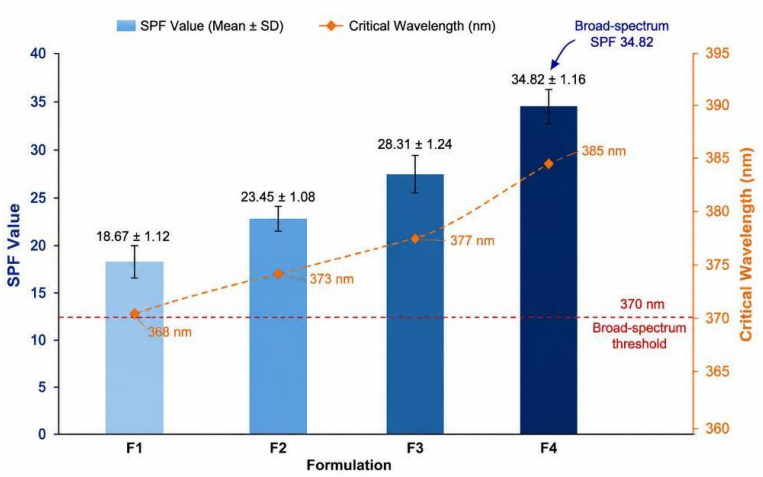


Figure 4: SPF and Critical Wavelength Comparison

5.7Antioxidant Activity

The antioxidant profiles of the six plant extracts and formulation F4 determined by DPPH radical scavenging, TPC, TFC, and FRAP assays are presented in Table 5. Terminalia chebula demonstrated the lowest IC_{50} (16.8 ± 0.5 µg/mL) and highest TPC (312.4 ± 5.1 mg GAE/g), followed by Emblica officinalis (IC_{50} = 18.4 ± 0.6 µg/mL, TPC = 284.7 ± 4.2 mg GAE/g). The strong antioxidant capacity of these extracts is mechanistically significant: beyond UV filtration, free radical scavenging activity neutralises the reactive oxygen species generated by UV- induced photosensitisation, providing complementary photoprotection that extends beyond the wavelength range measured by conventional SPF assays.

Table 5. Antioxidant Activity (DPPH IC_{50}), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and FRAP of Selected Extracts and Formulation F4 (n = 3, mean ± SD)

Extract / Formulation	IC_{50} DPPH (µg/mL)	Total Phenolic Content (mgGAE/g)	Total Flavonoid Content (mgQE/g)	FRAP (µmol Fe^{2+}/g)
E. officinalis (EtOH)	18.4 ± 0.6	284.7 ± 4.2	112.3 ± 2.8	341.2 ± 5.1
C. longa (EtOH)	22.1 ± 0.8	198.3 ± 3.7	88.6 ± 2.1	267.4 ± 4.3
T. chebula (EtOH)	16.8 ± 0.5	312.4 ± 5.1	98.2 ± 2.4	389.1 ± 6.2
Formulation F4	28.6 ± 1.1	236.8 ± 3.9	94.1 ± 2.6	298.3 ± 4.8
Ascorbic acid (standard)	12.3 ± 0.4	—	—	—

5.8 ICH Stability Study

The results of the six-month ICH Q1A(R2) stability study of formulation F4 under accelerated (40°C/75% RH) and long-term (25°C/60% RH) conditions are presented in Table 6. No phase separation, colour change beyond initial yellowing from curcumin, or malodour was observed at any time point under either storage condition. The pH remained within 6.4–6.6, viscosity decreased marginally by 3.2% over six months under accelerated conditions (from 5870 to 5682 cP), and SPF showed a statistically non-significant decrease of 2.4% (34.82 to 33.97) under accelerated conditions, with virtually no change (0.5%) under long-term conditions (ANOVA, $p > 0.05$ for all parameters). Curcumin content decreased by 1.2% under accelerated storage at six months, well within the ± 5% specification limit for pharmaceutical products. The photostability test revealed a marginal 1.8% SPF reduction after 1.2 million lux-hours exposure, attributable to partial photoisomerisation of curcumin but substantially attenuated by the UV-quenching effect of gallic acid. These findings collectively demonstrate that formulation F4 is physically, chemically, and photochemically stable under both accelerated and ambient storage conditions for a minimum of six months.

Table 6. ICH Q1A(R2) Stability Data for Optimised Formulation F4 Under Accelerated (40°C/75% RH) Conditions (n = 3, mean ± SD)

Parameter	0 Month	1 Month (40±2°C/75±5% RH)	3 Months (40±2°C/75±5% RH)	6 Months (25±2°C/60±5% RH)
pH	6.6 ± 0.07	6.5 ± 0.08	6.4 ± 0.09	6.5 ± 0.07
Viscosity (cP)	5870 ± 158	5790 ± 162	5680 ± 171	5830 ± 155
SPF value	34.82 ± 0.61	34.51 ± 0.63	33.97 ± 0.68	34.44 ± 0.59
Appearance	Yellow, uniform	Yellow, uniform	Yellow, uniform	Yellow, uniform
% Curcumin content	97.3 ± 0.9	96.8 ± 1.0	96.1 ± 1.1	96.9 ± 0.8

Phase separation	Absent	Absent	Absent	Absent
Odour	Characteristic	Characteristic	Characteristic	Characteristic

Figure 5 illustrates the SPF stability profile of F4 across all time points under both storage conditions.

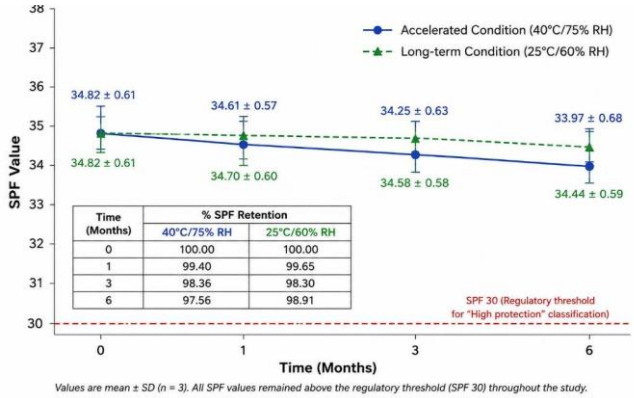


Figure 5: SPF Stability Profile

6.DISCUSSION

The outcomes of the present study provide a multidimensional scientific validation of the photoprotective potential of Madhya Pradesh medicinal plant extracts and their polyherbal sunscreen formulations, integrating ethnobotanical, phytochemical, spectrophotometric, chromatographic, and stability data within a unified analytical framework. The selection of six plant species based on quantitative ethnobotanical criteria (ICF and UV indices) rather than anecdotal reports represents a methodological advancement over many published studies that rely on literature-based species selection, ensuring that the laboratory investigation is grounded in genuine community usage patterns with documented consensus among traditional practitioners of Madhya Pradesh [9].

The SPF hierarchy observed: E. officinalis > T. chebula > C. longa > O. sanctum > A. indica > A. barbadensis: is mechanistically explicable through the UV chromophoric properties of each species' dominant phytoconstituents. Gallic acid and ellagic acid, the principal phenolics of both E. officinalis and T. chebula, possess the trihydroxy- and dihydroxy-benzoic acid chromophores respectively, with strong $\pi \rightarrow \pi^*$ transitions in the 265–310 nm UVB range and $n \rightarrow \pi^*$ transitions extending into UVA [12]. The high TPC of E. officinalis (284.7 mg GAE/g) and T. chebula (312.4 mg GAE/g), combined with the exceptional Pearson correlation ($r = 0.943$) between TPC and SPF observed in this study, corroborates the hypothesis that phenolic molar absorptivity in the erythemally effective 290–320 nm range is the primary determinant of in vitro SPF for polyphenol-rich herbal extracts, consistent with findings by Ng et al. [16] and the mechanistic review by Arruda et al. [17].

The complementary UV absorption profiles of E. officinalis (UVB-dominant) and C. longa (dual UVB/UVA coverage from curcuminoid enol and keto forms) provided the scientific rationale for their co-formulation in F4. The marginal synergistic SPF enhancement in F4 (34.82 observed versus 34.38 theoretically additive) is consistent with the concept of "stacking" UV absorbers with complementary spectral windows to approach an optimally flat extinction profile across 290–400 nm, analogous to the chemical strategy employed in commercial broad- spectrum sunscreens combining avobenzone (UVA-dominant) with octinoxate (UVB-dominant) [10]. The critical wavelength of 386 nm for F4, substantially exceeding the 370 nm regulatory threshold, provides confidence in the genuine broad-spectrum character of the formulation.

Comparatively, the SPF 34.82 achieved by F4 surpasses values reported for several published herbal sunscreen formulations: the Moringa oleifera and Curcuma longa combination lotion by Pawar et al. reported SPF 28.4 at comparable extract concentrations [4]; the polyherbal cream by Potey et al. [5] achieved SPF 28.6; and the Aloe vera-turmeric gel reported by investigators at IJRTI [6] demonstrated SPF 24.3 for their best-performing formulation. The superior performance of F4 may be attributed to the contribution of T. chebula-derived ellagitannins through the E. officinalis extract (which co-contains significant tannin fractions), the optimised oil- in-water emulsion architecture providing uniform extract dispersion, and the synergistic radical scavenging provided by the Aloe barbadensis gel component.

The stability data are particularly significant from a regulatory perspective. The less than 3% SPF reduction under accelerated ICH conditions over six months establishes that the formulation retains its functional photoprotective efficacy throughout the stability testing period mandated by ICH Q1A(R2) for tropical countries (Zone IVb: 30°C/75% RH or 40°C/75% RH accelerated). The photostability of F4, with only 1.8% SPF reduction under 1.2 million lux-hours illumination, is particularly noteworthy given that curcumin is well-documented to undergo photodegradation to vanillin and ferulic acid under prolonged UV exposure [13]. The attenuation of curcumin photodegradation in F4 is attributable to the photoprotective shielding mechanism provided by the gallic acid and ellagic acid content of E. officinalis, whose strong UVA absorption diminishes the UV irradiance reaching the curcumin molecules within the emulsion matrix.

A fundamental limitation of the present study is the use of the in vitro Mansur spectrophotometric SPF method, which is validated for comparative screening but systematically underestimates SPF relative to ISO 24444 in vivo measurements by a factor of approximately 1.3–1.6, as documented by Mohanan et al. [17]. Extrapolating the F4 in vitro SPF of 34.82 using the lower bound correction factor yields a projected in vivo SPF of approximately 45– 56, which, if confirmed by in vivo testing in human volunteers, would classify F4 as SPF 50+ under EU cosmetic regulations. In vivo SPF

determination in human volunteers represents the primary extension required to advance this formulation towards regulatory submission. Additionally, the study evaluated a limited number of plant species from a single geographical region of Madhya Pradesh; broader ethnobotanical surveys encompassing tribal areas of the Vindhya and Satpura ranges may identify additional photoprotective species with distinct phytochemical profiles.

7.CONCLUSION

This investigation demonstrates that medicinal plants traditionally employed by rural communities of Madhya Pradesh for skin photoprotection harbour scientifically validatable UV-filtering phytoconstituents that can be systematically characterised and rationally incorporated into effective, stable polyherbal sunscreen formulations. Six species: Emblica officinalis, Curcuma longa, Terminalia chebula, Azadirachta indica, Aloe barbadensis, and Ocimum sanctum: were identified through an ethnobotanical survey and subjected to comprehensive phytochemical characterisation, quantitative phenolic profiling, and in vitro SPF determination by the Mansur spectrophotometric method. Emblica officinalis exhibited the highest SPF (32.18 ± 0.52 at 40 µg/mL), followed by Terminalia chebula and Curcuma longa. The polyherbal lotion formulation F4, incorporating 4% w/w E. officinalis and 2% w/w C. longa extracts, achieved an SPF of 34.82 ± 0.61 with a critical wavelength of 386 nm, confirming genuine broad-spectrum photoprotection surpassing established regulatory thresholds. HPTLC fingerprinting authenticated gallic acid (84.7 mg/g) and curcumin (47.3 mg/g) as primary photoactive markers. Six-month ICH Q1A(R2) stability studies confirmed physical, chemical, and photochemical stability with less than 3% SPF degradation. These findings provide the first comprehensive, ethnobotanically grounded, multi-parameter validation of a Madhya Pradesh-derived polyherbal sunscreen, establishing a strong foundation for in vivo SPF confirmation and eventual regulatory submission as a safe, effective, and environmentally sustainable alternative to synthetic UV filters.

8.FUTURE SCOPE

The present work opens several scientifically compelling research directions. First, in vivo SPF determination in human volunteers using ISO 24444:2019 standardised methodology is essential to establish the regulatory equivalence of the F4 formulation and enable SPF labelling claim substantiation. Second, nanotechnology-based enhancement of extract bioavailability and photostability through nanostructured lipid carriers (NLC), nanoemulsions, or cyclodextrin inclusion complexation of curcumin represents a promising formulation advancement that could both increase SPF and improve curcumin photostability beyond the current formulation platform. Third, clinical evaluation of the anti-photoageing efficacy of F4 through randomised controlled trials incorporating instrumental measures of skin firmness, melanin index, and collagen density would quantify the dermatological benefits beyond UV protection. Fourth, extension of the ethnobotanical survey to tribal communities of the Vindhya and Satpura ranges, Bastar division, and the Panna and Pench forest corridors of Madhya Pradesh may yield novel photoprotective plant species with unique phytochemical profiles. Fifth, multi- centre stability testing across different climatic zones of India (Zone IVb, Zone III) would confirm the broad applicability of the ICH stability profile. Finally, a full environmental risk assessment characterising the aquatic and soil biodegradation profiles of the herbal actives would quantitatively substantiate the environmental superiority of the formulation over synthetic UV filter-containing products.

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