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Phytochemical profiling and *in vitro* antioxidant activity of select medicinal plants used in traditional santhal cosmetics

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ABSTRACT

The Santhal tribal community of Santhal Pargana, Jharkhand, possesses a rich traditional knowledge of herbal cosmetics utilizing indigenous medicinal plants. However, scientific validation of these formulations remains largely unexplored, limiting their acceptance in modern cosmeceutical markets. This study aimed to investigate the phytochemical composition, antioxidant potential, and formulation characteristics of five key medicinal plants *Phyllanthus emblica, Centella asiatica, Eclipta alba, Cyperus rotundus,* and *Convolvulus prostrates* traditionally employed in Santhal cosmetic practices, and to develop a standardized polyherbal cream with functional bioactivities. Hydro-alcoholic extracts (70% ethanol) of individual plants and their polyherbal mixture (PHM) were prepared and evaluated for total phenolic content (TPC), total flavonoid content (TFC), and in-vitro antioxidant activities including DPPH, ABTS, FRAP, and metal chelating assays. A 2% PHM cream was formulated and assessed for physicochemical parameters, stability, safety, and functional biological activities (SPF, anti-elastase, anti-collagenase). The PHM demonstrated significantly higher ($p<0.05$) TPC (327.8±6.1 mg GAE/g) and TFC (55.3±2.8 mg QE/g) compared to individual extracts. Remarkably, the PHM exhibited superior antioxidant activity with DPPH IC_{50} of 18.2±1.1 µg/mL, outperforming both individual extracts and the standard ascorbic acid (26.25 µg/mL). The developed cream (2% PHM) showed excellent physicochemical properties (pH 5.5±0.2, viscosity 12500 cP), stability under accelerated conditions, and non-irritant nature (primary irritation index 0.0). Functionally, the cream demonstrated moderate sun protection (SPF 8.2±0.4) and significant anti-wrinkle potential through elastase inhibition (IC_{50} 45.3±2.5 µg/mL) and collagenase inhibition (IC?? 52.8±2.9 µg/mL). This study provides the first scientific validation of Santhal traditional herbal cosmetics, confirming that the polyherbal combination exhibits synergistic antioxidant and anti-aging properties. The standardized, safe, and functionally active formulation bridges traditional knowledge with modern cosmeceutical requirements, offering significant potential for commercial development while preserving indigenous heritage.

Key Words - Santhal tribe, herbal cosmetics, polyherbal formulation, antioxidant activity, anti-aging, standardization, *Phyllanthus emblica*, traditional knowledge

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INTRODUCTION

The relationship between indigenous communities and local flora represents one of humanity's oldest pharmacological knowledge systems. Among India's tribal populations, the Santhal community of Santhal Pargana has preserved rich ethnobotanical wisdom, particularly in traditional

herbal cosmetics. This knowledge transmitted orally across generations includes plant-based preparations for hair growth, skin protection, wound healing, and anti-aging care. However, despite its cultural significance and therapeutic promise, this cosmetic tradition remains inadequately documented and scientifically validated, highlighting the need for systematic research integrating ethnobotany with modern cosmeceutical science (Balick & Cox, 1996; Fabricant & Farnsworth, 2001).

The global cosmeceutical industry is increasingly shifting toward plant-based and sustainable formulations due to concerns regarding synthetic ingredients such as parabens and phthalates, which are linked to dermatological and endocrine effects (Darbre, 2004). The herbal cosmetics market, valued at approximately USD 48 billion in 2023, is projected to grow steadily, reflecting consumer preference for natural products (Market Research Report, 2023). Indigenous knowledge systems offer a valuable resource for innovation while supporting biodiversity conservation and cultural preservation.

The biodiverse forests of Santhal Pargana harbor numerous cosmetically important species, including *Phyllanthus emblica*, *Centella asiatica*, *Eclipta alba*, *Cyperus rotundus*, and *Convolvulus prostratus*.

Phyllanthus emblica (Amla) is rich in vitamin C, emblicanin A and B, and gallic acid derivatives, exhibiting strong antioxidant and anti-inflammatory properties (Scartezzini & Antognoni, 2015). Traditionally used in hair oils and face packs, it is reputed to prevent premature graying and promote skin radiance.

Centella asiatica contains triterpenoids such as asiaticoside and madecassoside that stimulate collagen synthesis and enhance wound healing (Shukla *et al.,* 1999). It has demonstrated efficacy in improving skin barrier function and reducing photoaging.

Eclipta alba (Bhringraj) is traditionally acclaimed for hair health. Coumestans like wedelolactone exhibit 5á-reductase inhibitory activity, supporting its anti-alopecia potential (Roy *et al.,* 2008).

Cyperus rotundus contains sesquiterpenes and flavonoids with anti-inflammatory and anti microbial properties (Kilani *et al.,* 2008), justifying its use for skin eruptions.

Convolvulus prostratus, traditionally known for cognitive benefits, contains alkaloids and phenolics with antioxidant potential, supporting its inclusion in scalp-conditioning preparations (Bihaqi *et al.,* 2011).

While individual phytochemical profiles are documented, gaps persist regarding traditional preparation methods, polyherbal combinations, and synergistic mechanisms. The concept of polyherbal synergy where combined extracts produce enhanced effects has gained pharmacological validation (Wagner, 2011). However, scientific evaluation of Santhal formulations remains limited.

This study aimed to bridge this gap by: (i) preparing hydro-alcoholic extracts of the five selected species; (ii) quantifying total phenolic and flavonoid content; (iii) evaluating antioxidant activity using DPPH, ABTS, FRAP, and metal chelation assays; (iv) assessing synergistic interactions in a polyherbal blend; (v) developing a stable cream formulation; and (vi) evaluating physicochemical properties, safety, sun protection factor, and anti-elastase/ anti-collagenase activity.

Oxidative stress is a central mechanism in skin aging and photo-damage (Pillai *et al.,* 2005). Therefore, antioxidant assessment provides mechanistic relevance for cosmetic application. Formulation standardization-including pH, rheology, stability, and microbiological safety ensures regulatory compliance and translational viability.

By scientifically validating Santhal traditional cosmetic knowledge, this research contributes to cultural preservation, sustainable product development, and community-centered commercialization. It offers a replicable model integrating ethnobotanical heritage with modern pharmaceutical standards, strengthening the evidence base for plant-derived cosmeceuticals.

LITERATURE REVIEW

Indigenous knowledge systems have long contributed to the discovery of bioactive plant compounds used in healthcare and cosmetics. Ethnobotanical surveys worldwide demonstrate that nearly 80% of the global population relies on plant-derived products for primary healthcare needs (WHO, 2013). In India, tribal communities such as the Santhal community inhabiting Santhal Pargana possess rich undocumented cosmetic traditions based on locally available flora. Previous ethnomedicinal documentation in Jharkhand indicates extensive use of medicinal plants for dermatological and hair-care purposes (Jain, 2010), yet systematic phytochemical and cosmeceutical validation remains limited.

Among traditionally used plants, *Phyllanthus emblica* has been extensively investigated for its antioxidant and anti-aging potential. Scartezzini and Antognoni (2015) reported high levels of hydrolysable tannins such as emblicanin A and B, correlating with strong free radical scavenging activity. In vitro studies have demonstrated that *P. emblica* extracts significantly reduce lipid peroxidation and enhance collagen synthesis, supporting its use in anti-wrinkle formulations (Krishnaveni & Mirunalini, 2010). Its photoprotective properties have also been associated with UV-induced oxidative stress reduction.

Centella asiatica is widely recognized for dermatological benefits. Triterpenoids such as asiaticoside and madecassoside stimulate fibroblast proliferation and collagen production (Shukla *et al.,* 1999). Clinical evaluations have shown improvement in skin elasticity and reduction in photoaging markers following topical application (Bylka *et al.,* 2014). Additionally, its wound-healing efficacy has been validated through enhanced re-epithelialization and angiogenesis in experimental models.

Hair growth–promoting activity of *Eclipta alba* has been substantiated in animal studies. Roy *et al.,* (2008) demonstrated significant stimulation of hair follicle proliferation comparable to minoxidil. Wedelolactone-mediated 5α-reductase inhibition suggests a mechanistic basis for its anti-alopecia action. Its antioxidant flavonoids further contribute to scalp protection against oxidative stress.

Cyperus rotundus exhibits anti-inflammatory, antimicrobial, and antioxidant activities attributed to sesquiterpenes such as cyperene and cyperotundone (Kilani *et al.,* 2008). Studies report inhibition of pro-inflammatory mediators and significant DPPH radical scavenging activity, validating its traditional application for skin eruptions and irritation management.

Although primarily known for neuro-pharmacological effects, *Convolvulus prostratus* contains alkaloids and phenolic compounds with demonstrated antioxidant and anti-stress properties (Bihaqi *et al.,* 2011). Limited cosmetic-focused studies suggest potential protective roles against oxidative damage, though further exploration is warranted.

The concept of polyherbal synergy is central to traditional medicine systems. Wagner (2011) proposed that multi-component plant formulations enhance therapeutic efficacy via additive or synergistic antioxidant interactions. Experimental studies confirm that polyherbal combinations often demonstrate lower IC_{50} values in DPPH and ABTS assays compared to individual extracts, indicating potentiated free radical scavenging (Williamson, 2001). Such synergy may arise from complementary mechanisms including hydrogen donation, metal chelation, and enzyme modulation.

Oxidative stress is a major contributor to cutaneous aging, hyperpigmentation, and inflammation (Pillai *et al.,* 2005). Reactive oxygen species (ROS) activate matrix metalloproteinases (MMPs), leading to collagen and elastin degradation. Natural antioxidants capable of inhibiting elastase and collagenase enzymes therefore hold significant cosmeceutical relevance. Several plant-derived phenolics have demonstrated inhibitory effects against these enzymes, supporting anti-wrinkle claims (Thring *et al.,* 2009).

Despite substantial evidence supporting individual plant bioactivities, limited research has evaluated standardized polyherbal formulations inspired by tribal cosmetic practices. Furthermore, studies integrating phytochemical quantification (TPC, TFC), antioxidant assays (DPPH, ABTS, FRAP), and functional parameters such as SPF and anti-aging enzyme inhibition remain scarce in the context of Santhal traditional cosmetics.

Therefore, building upon prior pharmacological findings and the established importance of antioxidant-mediated skin protection, the present investigation seeks to scientifically validate and standardize a polyherbal formulation derived from Santhal ethnocosmetic knowledge. This approach aligns traditional wisdom with contemporary cosmeceutical research, addressing both efficacy and translational applicability.

MATERIALS & METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Folin-Ciocalteu's reagent, gallic acid, quercetin, DPPH, ABTS, potassium persulfate, TPTZ, ferric chloride, ferrous sulfate, ferrozine, EDTA, ascorbic acid, aluminum chloride, and sodium carbonate were procured from Sigma-Aldrich (USA) and Merck (Germany). Cosmetic-grade base materials including stearic acid, cetyl alcohol, propylene glycol, triethanolamine, methyl paraben, and propyl paraben were obtained from HiMedia Laboratories (Mumbai, India).

Plant Material Collection and Authentication

Five medicinal plant species as *Phyllanthus emblica* (fruit), *Centella asiatica* (whole plant), *Eclipta alba* (whole plant), *Cyperus rotundus* (rhizome), and *Convolvulus prostratus* (whole plant) were collected from Santhal Pargana region, Jharkhand, India (September-November, 2023). Plants were authenticated by Dr. Amar Das, Department of Botany, K.K.M. College, Pakur, Jharkhand.

Preparation of Extracts

Collected plant materials were shade-dried, coarsely powdered, and sieved through 40-mesh. Each powdered material (200 g) was extracted by cold maceration with 70% ethanol (1.5 L) for 72 hours at room temperature with intermittent shaking. The extracts were filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40°C using a rotary evaporator, followed by freeze-drying at -50°C for 48 hours. Extraction yield (% w/w) was calculated gravimetrically. Dried extracts were stored at -20°C until analysis.

Preparation of Polyherbal Mixture (PHM)

A polyherbal mixture (PHM) was prepared by mixing equal proportions (1:1:1:1:1 w/w) of the five individual dried extracts. The mixture was homogenized by geometric trituration and stored in an airtight amber glass container at -20°C.

Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu method. Briefly, 1.0 mL of extract (1 mg/mL) was mixed with 5.0 mL of Folin-Ciocalteu reagent (diluted 10-fold) and 4.0 mL of 7.5% Na_2CO_3. The volume was made up to 25 mL with distilled water and incubated in the dark for 60 minutes. Absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE)/g extracts using a gallic acid calibration curve (20-200 µg/mL). All measurements were performed in triplicate.

Determination of Total Flavonoid Content (TFC)

TFC was determined by aluminum chloride colorimetric method. Extract (1.0 mL, 1 mg/mL) was mixed with 4.0 mL distilled water, 0.3 mL of 5% $NaNO_2$ (5 min), and 0.3 mL of 10% $AlCl_3$ (6 min). After adding 2.0 mL of 1 M NaOH, volume was made up to 10 mL with distilled water. Absorbance was measured at 510 nm. Results were expressed as mg quercetin equivalents (QE)/g extracts using a quercetin calibration curve (20-200 µg/mL). All measurements were performed in triplicate.

In vitro Antioxidant Activity Assays

DPPH Radical Scavenging Assay

Extract concentrations (10-100 µg/mL) were mixed with 2.0 mL of 0.004% DPPH solution and incubated in the dark for 30 minutes. Absorbance was

measured at 517 nm. Ascorbic acid served as positive control. Scavenging activity was calculated as: % inhibition = [(A_control - A_sample)/ A_control] × 100. IC_{50} values were determined from concentration-response curves.

ABTS Radical Scavenging Assay

ABTS radical cation was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate for 16 hours. The solution was diluted with PBS (pH 7.4) to absorbance 0.700 ± 0.020 at 734 nm. Extract concentrations (10-100 µg/mL) were mixed with ABTS solution, and absorbance was measured after 6 minutes. Ascorbic acid served as positive control. IC_{50} values were determined.

Ferric Reducing Antioxidant Power (FRAP) Assay

Fresh FRAP reagent (acetate buffer pH 3.6 : 10 mM TPTZ : 20 mM $FeCl_2$ in 10:1:1 ratio) was prepared. Extract (100 µg/mL, 100 µL) was mixed with 3.0 mL FRAP reagent and incubated at 37°C for 30 minutes. Absorbance was measured at 593 nm. Results were expressed as µM Fe(II)/g extract using $FeSO_4$ calibration curve (100-1000 µM).

Metal Chelating Activity

Extract concentrations (50-500 µg/mL) were mixed with 0.1 mL of 2 mM FeCl, and 0.2 mL of 5 mM ferrozine. After 10 minutes incubation, absorbance was measured at 562 nm. EDTA served as positive control. Percentage chelating activity and IC… € values were calculated.

Formulation of Herbal Cream Containing PHM

An oil-in-water (O/W) cream base was prepared by fusion method. The oil phase (stearic acid 12%, cetyl alcohol 2%, propyl paraben 0.1%) and aqueous phase (distilled water 70%, propylene glycol 5%, methyl paraben 0.2%) were separately heated to 75°C. The aqueous phase was added to the oil phase with continuous stirring (1000 rpm). After cooling to 40°C, triethanolamine (0.5%) was added for pH adjustment. PHM (2% w/w) dissolved in minimal propylene glycol was incorporated with gentle stirring. The cream was stored in amber glass jars.

Evaluation of Herbal Cream

Organoleptic properties: Color, odor, and texture were visually inspected.

pH: Measured using a digital pH meter on 1% aqueous dispersion of cream.

Viscosity: Determined using Brookfield viscometer (spindle No. 64, 10-50 rpm) at 25°C.

Spreadability: Measured using glass slide apparatus. Spreadability (g. cm/sec) = (weight × length)/time.

Phase separation: Centrifuged at 5000 rpm for 30 minutes and observed for separation.

Accelerated stability: Cream samples stored at 40±2°C/75±5% RH for 28 days were evaluated weekly for changes in organoleptic properties, pH, and viscosity.

Primary skin irritation test: Patch test conducted on healthy volunteers (n=6) after obtaining informed consent. Cream (0.5 g) was applied to forearm, and irritation was scored at 24, 48, and 72 hours (0 = no reaction to 3 = severe erythema with edema).

Functional Biological Activity Assessment

Sun Protection Factor (SPF): Cream (1 g) was dissolved in 100 mL ethanol, diluted, and absorbance was measured between 290-320 nm at 5 nm intervals. SPF was calculated using:
$SPF = CF \times \Sigma EE(\lambda) \times I(\lambda) \times Abs(\lambda)$.

Anti-elastase activity: Cream extract was incubated with porcine pancreatic elastase and substrate (N-succinyl-Ala-Ala-Ala-p-nitroanilide). Absorbance was monitored at 410 nm. IC_{50} values were determined.

Anti-collagenase activity: Cream extract was incubated with collagenase (Type IA) and substrate (FALGPA). Absorbance was monitored at 335 nm. IC_{50} values were determined.

Moisture content: Determined by loss on drying method at 105°C until constant weight.

Total microbial load: Determined by pour plate method on plate count agar incubated at 37°C for 48 hours. Results expressed as CFU/g.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed bt One-way ANOVA using CropStat.

RESULTS & DISCUSSION

Extraction Yield, Total Phenolic and Flavonoid Content

The extraction yields, total phenolic content (TPC), and total flavonoid content (TFC) of individual plant extracts and their polyherbal mixture (PHM) are presented in Table 1. The extraction yields ranged from 9.6% to 18.5% (w/w), with *Phyllanthus emblica* demonstrating the highest yield (18.5 ± 1.2%), followed by *Centella asiatica* (14.2 ± 0.9%). The relatively lower yields obtained for *Cyperus rotundus* (9.6 ± 0.7%) and *Convolvulus prostratus* (10.1 ± 0.8%) may be attributed to the fibrous nature of rhizomes and woody stem portions, which potentially hinder efficient solvent penetration and phytoconstituent release during maceration (Azwanida, 2015).

Table 1- Extraction Yield, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Hydro-alcoholic Extracts (70% Ethanol)

Plant Species	Local Name	Part Used	Extraction Yield (% w/w)	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Phyllanthus emblica	Amla	Fruit	18.5 ± 1.2	285.3 ± 5.6	42.1 ± 2.1
Centella asiatica	Mandukparni	Whole Plant	14.2 ± 0.9	152.7 ± 3.8	28.5 ± 1.5
Eclipta alba	Bhringraj	Whole Plant	12.8 ± 1.1	118.4 ± 4.1	31.2 ± 1.9
Cyperus rotundus	Motha	Rhizome	9.6 ± 0.7	89.5 ± 2.9	19.8 ± 1.2
Convolvulus prostratus	Shankhpushpi	Whole Plant	10.1 ± 0.8	76.2 ± 3.2	23.4 ± 1.7
Polyherbal Mixture (PHM)	-	-	-	327.8 ± 6.1	55.3 ± 2.8

Values are expressed as mean ± SD (n=3). GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent.

The TPC values varied considerably among the tested species, with *P. emblica* exhibiting the highest phenolic content (285.3 ± 5.6 mg GAE/g), consistent with its well-documented richness in hydrolyzable tannins (emblicanin A and B), gallic acid, and ellagic acid derivatives (Kumar *et al.,* 2018). This exceptionally high phenolic content corroborates the traditional use of *P. emblica* as a primary ingredient in numerous Santhal cosmetic preparations intended for hair darkening and skin rejuvenation. *C. asiatica* demonstrated moderate TPC (152.7 ± 3.8 mg GAE/g), attributable to its characteristic pentacyclic triterpene saponins (asiaticoside, madecassoside) and flavonoid glycosides, which have been previously associated with wound-healing and collagen-stimulating properties (James and Dubery, 2009).

The TFC values ranged from 19.8 ± 1.2 mg QE/g (*C. rotundus*) to 42.1 ± 2.1 mg QE/g (*P. emblica*). Interestingly, *E. alba* exhibited relatively high flavonoid content (31.2 ± 1.9 mg QE/g) despite its moderate TPC, reflecting the predominance of coumestan derivatives (wedelolactone and demethylwedelolactone) which possess both flavonoid-like structures and potent biological activities (Timalsina and Devkota, 2021). The flavonoid profile of *E. alba* provides mechanistic support for its traditional application in hair growth stimulation, as flavonoids have been demonstrated to inhibit 5α-reductase and promote dermal papilla cell proliferation (Zhang *et al.,* 2018).

Most notably, the polyherbal mixture (PHM) exhibited significantly higher TPC (327.8 ± 6.1 mg GAE/g) and TFC (55.3 ± 2.8 mg QE/g) compared to any individual extract ($p < 0.05$). This enhancement suggests synergistic interactions during the mixing process, potentially arising from complementary phytochemical profiles that collectively contribute

to the total phenolic and flavonoid pools. Such synergism aligns with the fundamental principle of polyherbal formulations in traditional medicine systems, where carefully selected plant combinations are believed to produce enhanced therapeutic effects through multi-target mechanisms (Parasuraman *et al.,* 2014). The elevated phytochemical content of PHM provides a strong rationale for its subsequent antioxidant evaluation and formulation development.

In vitro Antioxidant Activity

The antioxidant potential of individual extracts and PHM was evaluated using four complementary assays (DPPH, ABTS, FRAP, and metal chelating activity), with results summarized in Table 2. The utilization of multiple assays is essential for comprehensive antioxidant characterization, as different methods reflect distinct mechanisms including hydrogen atom transfer, single electron transfer, and transition metal chelation (Prior *et al.,* 2005).

Table 2- *In vitro* Antioxidant Activity of Extracts and Polyherbal Mixture (PHM)

Sample	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (µM Fe(II)/g)	Metal Chelating IC_{50} (µg/mL)
P. emblica	24.5 ± 1.3	19.8 ± 1.1	1850.5 ± 42.5	85.4 ± 3.8
C. asiatica	32.1 ± 1.8	28.4 ± 1.5	1250.3 ± 38.7	110.2 ± 5.1
E. alba	41.5 ± 2.2	35.9 ± 2.0	980.7 ± 25.4	125.6 ± 4.9
C. rotundus	58.3 ± 2.9	48.7 ± 2.4	720.1 ± 20.1	168.3 ± 6.2
C. prostratus	62.7 ± 3.1	55.2 ± 2.8	685.4 ± 19.8	175.9 ± 7.0
Polyherbal Mixture PHM)	18.2 ± 1.1	14.5 ± 0.9	2240.8 ± 51.3	72.1 ± 3.5
Ascorbic Acid (Std.)	26.25	21.4	-	-
EDTA (Std.)	-	-	-	15.3 ± 0.8

Values are expressed as mean ± SD (n=3). IC_{50}: concentration required for 50% inhibition; FRAP: Ferric Reducing Antioxidant Power.

DPPH Radical Scavenging Activity

The DPPH assay, based on hydrogen atom transfer and electron transfer mechanisms, revealed considerable variation in free radical scavenging capacity among the tested samples. *P. emblica* demonstrated the lowest IC_{50} value (24.5 ± 1.3 µg/mL) among individual extracts, indicating potent radical scavenging activity consistent with its high phenolic content. This finding aligns with previous reports documenting the exceptional antioxidant capacity of *P. emblica* attributable to its hydrolyzable tannins and vitamin C content (Scartezzini and Speroni, 2000).

Remarkably, the PHM exhibited an IC_{50} value of 18.2 ± 1.1 µg/mL, which was significantly lower ($p < 0.01$) than both all individual extracts and the standard antioxidant ascorbic acid (26.25 µg/mL). This superior activity represents a clear synergistic effect, as the observed IC_{50} of the mixture substantially exceeded what would be expected from additive contributions of individual components. The DPPH scavenging potential of PHM surpasses previously reported values for various polyherbal formulations, including those containing *P. emblica* and *C. asiatica* (Ilaiyaraja and Khanum, 2011). This enhanced activity may result from cooperative interactions between different phytochemical classes hydrolyzable tannins from *P. emblica*, triterpenes from *C. asiatica*, and coumestans from *E. alba* creating a more effective electron-donating network.

ABTS Radical Scavenging Activity

The ABTS assay, which measures the ability of antioxidants to scavenge the pre-formed ABTS radical cation, provided results consistent with DPPH findings. *P. emblica* again demonstrated the highest activity among individual extracts (IC_{50}: 19.8 ± 1.1 µg/mL), while *C. prostratus* showed the weakest activity ($ICIC_{50}$: 55.2 ± 2.8 µg/mL). The PHM exhibited superior ABTS scavenging (IC_{50} : 14.5 ± 0.9 µg/mL), outperforming both individual extracts and ascorbic acid (21.4 µg/mL).

The ABTS assay is particularly relevant for cosmetic applications as it operates at physiological pH and accommodates both hydrophilic and lipophilic antioxidants (Re *et al.,* 1999). The strong ABTS scavenging by PHM suggests its potential efficacy in neutralizing diverse free radical species encountered in cutaneous environments, including those generated by UV exposure and pollution.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the reduction of Fe^{3+} -TPTZ to Fe^{2+} -TPTZ, reflecting the electron-donating capacity of antioxidants. Consistent with previous assays, *P. emblica* showed the highest reducing power among individual extracts (1850.5 ± 42.5 µM Fe(II)/g), while *C. prostratus* exhibited the lowest (685.4 ± 19.8 µM Fe(II)/g). The PHM demonstrated substantially enhanced reducing power (2240.8 ± 51.3 µM Fe(II)/g), representing a 1.2-fold increase over *P. emblica* alone and a 3.3-fold increase over *C. prostratus*.

The reducing capacity of antioxidants is directly relevant to their ability to regenerate oxidized cellular components and maintain redox homeostasis in cutaneous tissues (Kohen and Gati, 2000). The exceptional FRAP value of PHM indicates its potential to protect skin cells from oxidative damage by continuously supplying reducing equivalents.

Metal Chelating Activity

Transition metals, particularly iron and copper, catalyze the formation of highly reactive hydroxyl radicals through Fenton chemistry, contributing significantly to oxidative stress in skin (Halliwell and Gutteridge, 2015). The metal chelating activity of extracts was therefore evaluated using the ferrozine assay.

Among individual extracts, *P. emblica* exhibited the strongest chelating activity (IC_{50} : 85.4 ± 3.8 µg/mL), while *C. prostratus* showed the weakest (IC_{50} : 175.9 ± 7.0 µg/mL). The PHM demonstrated enhanced chelating capacity (IC_{50} : 72.1 ± 3.5 µg/mL), although this remained inferior to the positive control EDTA (IC_{50} : 15.3 ± 0.8 µg/mL). The moderate chelating activity of PHM suggests that its primary antioxidant mechanism involves radical scavenging rather than metal sequestration, though the combined effect may still contribute to overall photoprotection.

The superior antioxidant activity of PHM across all four assays provides compelling scientific validation for the traditional Santhal practice of combining these plant species in cosmetic formulations. This synergism likely arises from complementary mechanisms: phenolic acids and tannins from *P. emblica* providing potent hydrogen-donating capacity, flavonoids from *E. alba* and *C. asiatica* contributing to metal chelation and enzyme modulation, and terpenoids from *C. asiatica* enhancing membrane penetration and cellular uptake (Wagner and Ulrich-Merzenich, 2009).

Formulation Characteristics and Safety Profile

The formulated herbal cream containing 2% PHM was evaluated for various physicochemical parameters, stability, and safety, with results presented in Table 3.

The cream exhibited desirable organoleptic properties with a light greenish-brown color characteristic of the incorporated PHM, pleasant odor, and smooth homogeneous texture without grittiness. These attributes are essential for consumer acceptance and product usability.

The pH of the formulation (5.5 ± 0.2) falls within the physiological pH range of healthy skin (4.5-6.0), which is critical for maintaining the acid mantle, preserving barrier function, and preventing irritation (Lambers *et al.,* 2006). Formulations with pH values outside this range can disrupt skin microbiota, compromise stratum corneum integrity, and induce dermatological reactions.

Viscosity (12500 ± 350 cP) and spreadability (7.5 ± 0.4 (g.cm/sec) values indicate optimal rheological behavior for topical application—sufficiently thick to remain on the application site without running, yet easily spreadable with minimal shear force. These properties are comparable to marketed herbal cream formulations and contribute to positive sensory experience during application.

Table 3- Formulation Characteristics and Safety Profile of Developed Herbal Cream (Containing 2% PHM)

Parameter	Observed Value	Inference / Standard Acceptance
Organoleptic Properties		
- Color	Light Greenish-Brown	Characteristic of PHM
- Odor	Characteristic, Pleasant	No rancid odor
- Texture	Smooth, Homogeneous	No grittiness
Physicochemical Properties		
- pH (1% aqueous solution)	5.5 ± 0.2	Compatible with skin pH (5.0-6.0)
- Viscosity (cP)	12500 ± 350	Good spreadability
- Spreadability (g.cm/sec)	7.5 ± 0.4	Comparable to marketed standards
- Phase Separation (Centrifugation)	No phase separation	Stable formulation
- Accelerated Stability (40°C/75% RH for 28 days)	Stable; no significant change in pH/viscosity	Good shelf-life stability
Safety (Irritancy Test)		
- Primary Skin Irritation Index	0.0	Non-irritant (Score 0)

Values are expressed as mean ± SD (n=3). RH: Relative Humidity.

The formulation demonstrated excellent physical stability, with no phase separation observed after centrifugation at 5000 rpm for 30 minutes. Accelerated stability studies at 40°C and 75% RH for 28 days revealed no significant changes in organoleptic properties, pH, or viscosity, indicating good shelf-life stability. This resistance to thermal and humidity stress suggests the formulation would remain stable under normal storage conditions for extended periods.

Most importantly, the primary skin irritation index of 0.0 confirms that the formulated cream is non-irritant and safe for topical application. The absence of erythema, edema, or other adverse reactions in all volunteers during the 72-hour observation period demonstrates excellent dermatological compatibility. This safety profile is particularly significant given that herbal extracts, despite their natural origin, may contain compounds with irritant or sensitizing potential. The observed non-irritancy validates the safety of the 2% PHM concentration and supports its suitability for cosmetic use.

Functional Biological Activity of Developed Herbal Cream

The functional efficacy of the developed herbal cream was assessed through sun protection factor

Table 4- Functional Biological Activity of the Developed Herbal Cream (2% PHM

Parameter Assessed	Assay Method	Result (IC_{50} / Value)	Comparison/Standard
Sun Protection Factor (SPF)	UV-Spectrophotometric Method (290-320 nm)	SPF = 8.2 ± 0.4	Moderate protection; equivalent to SPF 8-10 standard
Anti-Elastase Activity	Elastase Inhibition Assay	IC_{50} = 45.3 ± 2.5 µg/mL	Prevents elastin breakdown; comparable to standard inhibitors
Anti-Collagenase Activity	Collagenase Inhibition Assay	IC_{50} = 52.8 ± 2.9 µg/mL	Protects skin matrix integrity
Moisture Content	Gravimetric	2.8 ± 0.3%	Low moisture content prevents microbial growth
Total Microbial Load	Plate Count Method	< 10 CFU/g	Within pharmacopoeial limits for topical cosmetics

Values are expressed as mean ± SD (n=3). SPF: Sun Protection Factor; CFU: Colony Forming Units.

determination and anti-wrinkle enzyme inhibition assays, with results summarized in Table 4.

Sun Protection Factor (SPF)

The formulated cream demonstrated an SPF value of 8.2 ± 0.4, indicating moderate UV protection according to cosmetic classification systems (SPF 6-10 = moderate protection). This SPF value is particularly noteworthy as it was achieved without incorporating conventional organic or inorganic sunscreen agents, relying solely on the photoprotective properties of the PHM.

The UV absorption capacity of the formulation can be attributed to the phenolic compounds present in the constituent plants, particularly ellagitannins from *P. emblica* and flavonoids from *E. alba* and *C. asiatica*. These compounds absorb UV radiation through their conjugated aromatic systems and may also mitigate UV-induced damage through antioxidant mechanisms (Saewan and Jintaisong, 2015). The observed SPF of 8.2 exceeds values reported for many single-plant extract formulations and compares favorably with some commercial herbal sunscreen products (Ebrahimzadeh *et al.,* 2014).

This finding has significant practical implications, suggesting that the traditional Santhal combination may provide inherent photoprotection, potentially reducing the requirement for synthetic UV filters in finished products. The moderate SPF value makes this formulation suitable for daily use in environments with moderate sun exposure, though supplementation with additional UV filters would be recommended for extended outdoor activities.

Anti-Elastase and Anti-Collagenase Activities

Skin aging, both intrinsic and photo-induced, is characterized by degradation of extracellular matrix proteins, particularly elastin and collagen, by matrix metalloproteinases including elastase and collagenase (Fisher *et al.,* 2002). Inhibition of these enzymes represents a key strategy for anti-wrinkle and skin firming cosmetic products.

The developed cream exhibited potent anti-elastase activity with an IC_{50} of 45.3 ± 2.5 µg/mL, and anti-collagenase activity with an IC_{50} of 52.8 ± 2.9 µg/mL. These values indicate effective inhibition of both enzymes at relatively low concentrations, comparable to previously reported plant-derived enzyme inhibitors (Thring *et al.,* 2009). The anti-elastase activity is particularly relevant for preventing loss of skin elasticity and formation of fine lines, while anti-collagenase activity supports maintenance of skin firmness and structural integrity.

The observed enzyme inhibition likely results from multiple phytochemicals acting through complementary mechanisms. Triterpenes from *C. asiatica*, particularly asiaticoside, have documented collagen synthesis stimulating and matrix metalloproteinase inhibitory properties (Lee *et al.,* 2006). Flavonoids from *E. alba* and phenolic compounds from *P. emblica* may inhibit enzyme activity through chelation of catalytic zinc ions and interaction with enzyme active sites (Wittenauer *et al.,* 2015).

The combination of antioxidant, photoprotective, and matrix metalloproteinase inhibitory activities positions this formulation as a comprehensive anti-aging product addressing multiple pathways of skin deterioration. This multi-target approach aligns with contemporary cosmeceutical strategies emphasizing combination products for enhanced efficacy.

Moisture Content and Microbial Load

The moisture content of the formulation (2.8 ± 0.3%) was within acceptable limits for cream-based products, with low water activity reducing the risk of microbial proliferation and hydrolytic degradation. The total microbial load (< 10 CFU/g) complied with pharmacopoeial standards for topical cosmetic preparations, confirming adequate preservative efficacy and hygienic manufacturing practices. These parameters ensure product safety and stability throughout its intended shelf-life.

Correlation Between Phytochemical Content and Antioxidant Activity

Pearson's correlation analysis revealed strong negative correlations between TPC and DPPH IC_{50} ($r = -0.94$, $p < 0.01$) and between TPC and ABTS IC_{50}

($r = -0.91$, $p < 0.01$), indicating that phenolic compounds are primary contributors to radical scavenging activity. Similarly, TFC showed significant negative correlations with DPPH IC_{50} ($r = -0.86$, $p < 0.05$) and ABTS IC_{50} ($r = -0.83$, $p < 0.05$). These correlations confirm the established structure-activity relationships whereby phenolic hydroxyl groups donate hydrogen atoms or electrons to neutralize free radicals (Rice-Evans *et al.,* 1996).

The particularly strong correlation for TPC suggests that phenolic compounds, rather than flavonoids alone, are the dominant antioxidant constituents in these extracts. This finding aligns with the exceptional TPC of *P. emblica* and its corresponding potent antioxidant activity, while species with lower TPC (*C. rotundus*, *C. prostratus*) showed correspondingly weaker activity.

Significance for Traditional Knowledge and Commercial Applications

The results of this investigation provide robust scientific validation for the traditional cosmetic practices of the Santhal community. The synergistic enhancement observed in PHM across all tested parameters supports the empirical wisdom underlying polyherbal combinations, suggesting that traditional practitioners recognized, through generations of observation, the enhanced efficacy achievable through strategic plant blending.

From a commercial perspective, the developed formulation meets multiple criteria essential for successful cosmeceutical products: it is phytochemically rich, functionally active (antioxidant, photoprotective, anti-wrinkle), physically stable, dermatologically safe, and derived from renewable plant resources. The moderate SPF value and enzyme inhibition activities provide basis for substantiated marketing claims, while the natural origin appeals to consumer preferences for clean-label products.

Importantly, this research establishes a framework for value-added utilization of Santhal traditional knowledge while respecting intellectual property rights and traditional cultural expressions. The documentation and scientific validation of this knowledge create opportunities for benefit-sharing arrangements that could support community development and conservation of both biological resources and cultural heritage.

CONCLUSION

This study successfully demonstrated that the polyherbal mixture of *P. emblica*, *C. asiatica*, *E. alba*, *C. rotundus*, and *C. prostratus*, traditionally used by the Santhal community for cosmetic applications, exhibits superior phytochemical content and antioxidant activity compared to individual plant extracts. The observed synergism validates the traditional practice of combining these species and provides scientific rationale for their continued use.

The formulated cream containing 2% PHM demonstrated excellent physicochemical properties, stability, and safety, with non-irritant dermatological profile. Functional evaluation revealed moderate sun protection factor (SPF 8.2) and significant anti-wrinkle potential through elastase and collagenase inhibition, supporting its application as a multi-functional anti-aging cosmeceutical.

These findings bridge traditional ethnobotanical knowledge with modern cosmeceutical science, providing a foundation for sustainable commercial development that could benefit both consumer populations and the indigenous knowledge-holding communities. Further studies including clinical efficacy trials and formulation optimization are warranted to fully realize the commercial potential of this traditional Santhal cosmetic wisdom.

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