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Formulation, Optimization and Evaluation of Polyherbal Topical Cream Incorporating Curcuma Caesia And Clitoria Ternatea Extracts for Antioxidant and Antibacterial Activity

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ABSTRACT:
Formulation, Optimization and Evaluation of Polyherbal Topical Cream Incorporating
Curcuma Caesia And Clitoria Ternatea Extracts for Antioxidant and Antibacterial Activity
Skin is the largest human body organ and forms the main physical and immunological barrier against all the threats of the external environment such as microbial invasion, ultraviolet radiation, and exposure to chemicals. Dermatological ailments continue to be a global health problem, particularly in terms of infections caused by microbes and oxidative stress. In tropical and subtropical areas, the incidence of skin diseases is much greater than in temperate climates, due to the more favourable environment for the development of pathogenic microorganisms and the production of reactive oxygen species (ROS) in the epidermal and dermal tissues. Skin infections are also one of the most common health issues worldwide and are one of the main health issues that cause primary healthcare burden

1. INTRODUCTION:

Skin is the largest human body organ and forms the main physical and immunological barrier against all the threats of the external environment such as microbial invasion, ultraviolet radiation, and exposure to chemicals. Dermatological ailments continue to be a global health problem, particularly in terms of infections caused by microbes and oxidative stress. In tropical and subtropical areas, the incidence of skin diseases is much greater than in temperate climates, due to the more favourable environment for the development of pathogenic microorganisms and the production of reactive oxygen species (ROS) in the epidermal and dermal tissues. Skin infections are also one of the most common health issues worldwide and are one of the main health issues that cause primary healthcare burden [1].

The growing incidence of antibiotic-resistant pathogens is one of the most burning issues in modern clinical dermatology. The acquisition of resistance mechanisms against the most widely used antibiotics has been shown to progressively occur in *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and to have a significant limiting effect on therapeutic options. Natural products, including flavonoids have been reported to demonstrate antibacterial activity by disrupting the membranes, inhibiting the enzymes, and disrupting the synthesis of nucleic acids [2]. In addition, antibacterial mechanisms are also exhibited by curcumin and the other phytochemicals [3]. The long-term use of synthetic antimicrobial agents is often linked to adverse dermatological effects such as irritation, hypersensitivity, and microbiota imbalances that underscore the need to find safer alternatives [4].

Oxidative stress is one of the most basic pathogenic processes in a broad range of dermatological diseases. Lipid peroxidation, oxidation of proteins, and damage of DNA in skin cells are induced by reactive oxygen species formed

by ultraviolet radiation, environmental pollutants and microbial toxins. These effects help in causing premature aging, inflammation, and the wound healing process is impaired. The evaluation methods of antioxidant activity like DPPH and Folin-Ciocalteu assays have been extensively applied to measure the free radical scavenging activity of plant extracts [5,6]. The mismatch between the production of ROS and the natural antioxidant defense systems make it necessary to develop topical formulations that are enhanced with natural antioxidants [7].

Polyherbal formulation is a concept that has received significant scientific support as a rational approach to therapy. Combinations of plant extracts to maximize efficacy and minimize toxicity have long been used in traditional medicinal systems. Contemporary research has shown that polyherbal systems have synergistic effects, in which several phytoconstituents interrelate via complementary biochemical pathways [8]. These formulations represent multi-target therapeutic action, and they are effective in the treatment of complex diseases such as skin disease involving oxidative stress and microbial infection [9]. It has also been demonstrated in optimization researches that antioxidant activity and overall formulation performance are greatly enhanced by combining plant extracts [10].

Curcuma caesia , also known as black turmeric, is a medicinal plant that is rich in curcuminoids, phenolics and essential oils. These bioactive compounds give it its antioxidant, anti-inflammatory and antimicrobial properties. Comparative phytochemical investigation has shown that *Curcuma caesia* has high free radical scavenging activity and good antibacterial activity [11]. Subsequent studies have verified its capacity to prevent microbial growth and reduce oxidative stress which supports its use in dermatological formulations[12]. Also, topical formulations with *Curcuma caesia* have been found to be stable and therapeutic, which further supports the suitability of *Curcuma caesia* in topical delivery systems [13].

Likewise, Butterfly pea (*Clitoria ternatea L*.) is a rich source of anthocyanins, flavonoids and phenolic compounds. It has been found to have strong antioxidant and antibacterial properties, due to these phytoconstituents. Research has added that *Clitoria ternatea* extracts have great free radical scavenging potential and antimicrobial activity against different pathogens [14]. The plant has been extensively researched on its pharmacological activities, such as its anti-inflammatory and wound healing properties, and so is a promising source of topical preparations [15].

The combination of *Curcuma caesia* and *Clitoria ternatea* in synergy is particularly promising because the two complement each other in their mechanisms of action. The main mechanisms of action of curcuminoids are hydrogen atom transfer and metal chelation whereas the anthocyanins can act through the electron transfer and radical stabilization mechanisms. This combination provides enhanced antioxidant coverage and improved therapeutic efficacy. Besides, their antibacterial action has multiple targets, decreasing chances of developing resistance and enhancing their activity against pathogenic microorganisms [3].

Topical drug delivery system is a viable method of managing dermatological conditions such as the delivery of active compounds to the site of action. Amongst these, oil in water (O/W) cream preparations are highly preferred because they are non-greasy, easily applied, and they can be used in combination with both hydrophilic and lipophilic compounds. Research has also shown that herbal creams give a good delivery of bioactive components and does not extract skin moisture or lead to lower adherence by patients [16,17].

Although numerous studies have been conducted on individual plants, there is dearth of research studies on combined formulation of *Curcuma caesia* and *Clitoria ternatea* in a standardized topical cream system. Thus, the current research aims to design and test a polyherbal cream that contains these extracts, focusing on physicochemical characteristics, phytochemical standardization, antioxidant potential, and antibacterial activity. It is also hoped that this research will present a scientific explanation of how the effective and safe herbal topical formulation can be developed to manage the oxidative stress-related and microbial skin disorders.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

New rhizomes of *Curcuma caesia. (Zingiberaceae)* and *Clitoria ternatea L.* flowers. They (Fabaceae) were collected through verified sources in northeast India. A qualified taxonomist conducted botanical authentication and voucher specimens were deposited to the institutional herbarium (CC/2023/001 and CT/2023/001). Plant materials need to be authenticated to achieve reproducibility and reliability of phytochemical and pharmacological studies [1,2].

Plant Extracts Preparation:
The gathered plant materials were washed, shade-dried at 2530 C over a period of 1014 days and crushed to coarse powder (sieve number 40). Using 70% ethanol, a drug-solvent ratio of 1:10 (w/v) by macerating 72 h at intervals with a 70% ethanol solution. The extracts were filtered through Whatman No. 1 filter paper and concentrated with the help of rotary evaporator at 4045 C. The concentrated extracts were lyophilized to get dry extract and kept at 4o C until used. Hydroalcoholic extraction is also a popular method of effective extraction of polar and semi-polar phytoconstituents [3,4].
Phytochemical Standardization
Total Phenolic Content (TPC)
The Folin-Ciocalteu method was used to determine TPC, and the absorbance was measured at 760nm. Findings were in form of mg gallic acid equivalent per gram of extract [5].

Total Flavonoid Content (TFC):
TFC was calculated by using aluminium chloride colorimetric assay and expressed as the mg quercetin equivalent per gram at 510 nm [6].

Curcuminoid Content:
Spectrophotometric measurements were made for the curcuminoid content of the Curcuma caesia extract with the use of curcumin as a standard [2].

Total Anthocyanin Content:
The pH differential method was used to determine the anthocyanin content of *Clitoria ternatea* extract and provide the results in the form of mg/L [7]. Quality and reproducibly of herbal formulations is crucial and must be ensured by using phytochemical standardization [8].

Experiments were conducted to formulate Polyherbal Cream:
Three different oil-in-water (O/W) cream formulations (F1, F2 and F3) containing different amounts of *Curcuma caesia* and *Clitoria ternatea* extracts (2:4, 3:3 and 4:2 respectively) with a total concentration of the extracts of 6% w/w were prepared.

The oil phase (cetyl alcohol, white soft paraffin, emulsifying wax) was heated to 7075 C while the aqueous phase (glycerin, methylparaben, propylparaben, purified water) was heated separately. A steady flow of the oil phase was into the aqueous phase and stirred, to create a stable emulsion. The extracts were added at a temperature around 40 C so that bioactive compounds will not be destroyed during the process. The creams were made and homogenized and stored in airtight containers [9,10].

- Physicochemical Evaluation
- The formulations were assessed on:
- pH (digital pH meter)
- Viscosity (Brookfield viscometer)
- Spread ability (parallel plate technique)
- Homogeneity (visual inspection)
- Patch test (skin irritation)

These parameters are crucial to determine both the quality, stability, and applicability of topical formulations [11,12]. The accelerated stability studies were performed by using the guidelines of ICH Q1A(R2) [13]. The formulations were

stored at 40°C ± 2°C and 75% ± 5% RH for 3 months. Periodic tests of samples were conducted in terms of pH, viscosity, and physical appearance.

In Vitro Antioxidant Activity:

DPPH Assay:

The method of DPPH was used to analyse the free radical scavenging activity and the absorbance was measured at 517 nm. IC_{50} values were calculated [14].

FRAP Assay:

The antioxidant power was determined as a conversion of Fe 3 + to Fe 2 + and absorbance at 593 nm [15].

- Scavenging Assay of Hydrogen Peroxide.
- The ability of the formulations to scavenge H_2O_2 was measured spectrophotometrically.
- Antibacterial Activity
- The antibacterial activity was tested against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

Agar Well Diffusion Method:

- The mm of zone of inhibition (ZOI) was recorded after incubation of 24 h at 37 °C.
- Minimum Inhibitory Concentration (MIC).
- MIC was calculated in broth microdilution procedure with resazurin as an indicator.
- Such techniques are conventional in determining antimicrobial activity of herbal products [16,17].

Statistical Analysis:

The experiments were replicated (n = 3) in each experiment. The data are presented as mean ± SD. One-way ANOVA and subsequently, Tukey test were used to carry out statistical analysis using a p-value of less than 0.05 as the statistical significance level. Pearson correlation analysis was used to determine the relationship between the phytochemical content and the biological activity.

RESULTS:

Extraction Yield:

Hydroalcoholic extraction of the rhizome of Curcuma caesia and flowers of Clitoria ternatea with 70% ethanol was found to be appropriate for obtaining dry powdered extracts for formulation of topical cream. The standard deviation of the yield value was low, which was indicative of the repeatability of the extraction process in three independent batches.

Table 1: Extraction yield of Hydroalcoholic extraction of Curcuma caesia and Clitoria ternatea.

Plant Material	Initial Weight (g)	Extract Weight (g)	% Yield (Mean ± SD)
Curcuma caesia (Rhizome)	100	12.84	12.84 ± 0.32
Clitoria ternatea (Flowers)	100	15.62	15.62 ± 0.41

The extraction yield obtained for the rhizome of *Curcuma caesia* was 12.84 ± 0.32% and for the flowers of *Clitoria ternatea* was 15.62 ± 0.41%. The slightly greater yield of *Clitoria ternatea* flowers is consistent with relatively greater water-soluble anthocyanin and flavonoid content which are extracted more readily by hydroalcoholic solvents. The yields are on a similar level as published in the literature for similar extraction conditions. Organoleptic Characteristics The three formulations of polyherbal cream were successfully prepared and different organoleptic property are observed due to the different proportions of the two plant extracts. The colour change from bluish green (F1, *Clitoria ternatea* dominant) to yellowish green (F3, *Curcuma caesia* dominant) is the visual effect of the contribution of ternatin anthocyanins and curcuminoids, respectively. Overall organoleptic acceptability was best in formulation F2 (3:3) which had an acceptable herbal odour, smooth texture and uniform appearance.

Table 2 shows the organoleptic properties of polyherbal cream formulations (F1, F2, F3)

Formulation	Colour	Texture	Odour	Appearance
F1 (2:4)	Light bluish-green	Smooth, slightly soft	Mild herbal	Uniform, semi-solid

F2 (3:3)	Greenish-brown	Smooth, optimal	Pleasant herbal	Uniform, glossy
F3 (4:2)	Yellowish-green	Slightly thick, firm	Strong herbal	Uniform, dense

Physicochemical Evaluation:

All three formulations were evaluated physicochemical and the results are summarised in Table 3. The pH of all formulations was within the range 5.5–6.5 which is considered as skin friendly range. F1 had the lowest pH (5.62 ± 0.04) which means that the highest amount of anthocyanin-rich extract from Clitoria ternatea was present, and F3 had the highest pH (6.04 ± 0.05) which means that the highest amount of extract from Curcuma caesia was present. The pH of F2 was 5.78 ± 0.03, an intermediate value closer to the normal skin surface pH, making it the most suitable for dermal application where the acid mantle of the skin will not be disrupted the most. Viscosity values ranged from 18,420 ± 210 cPs (F1) to 21,240 ± 265 cPs (F3), with F2 showing an optimal intermediate viscosity of 19,860 ± 245 cPs. Spreadability was inversely proportional to viscosity as the highest spreadability (8.42 ± 0.18 g·cm/s) was for F1 and the lowest spreadability (7.61 ± 0.17 g·cm/s) was for F3. The F2 was found to have an acceptable spreadability of 8.05 ± 0.21 g·cm/s. None of the three formulations showed evidence of graininess or lumps or phase separation after patch testing and none caused primary skin irritation after patch testing.

Table 3: Physicochemical Evaluation of Formulations of Polyherbal Cream (Mean ± SD, n = 3)

Parameter	F1 (2:4)	F2 (3:3)	F3 (4:2)
pH	5.62 ± 0.04	5.78 ± 0.03	6.04 ± 0.05
Viscosity (cPs)	18,420 ± 210	19,860 ± 245	21,240 ± 265
Spreadability (g·cm/s)	8.42 ± 0.18	8.05 ± 0.21	7.61 ± 0.17
Homogeneity	Excellent	Excellent	Excellent
Skin Irritation	Absent	Absent	Absent

Accelerated Stability Studies:

The formulation which was best overall physico-chemical profile was selected for accelerated stability evaluation that was formulation F2. The stability data obtained at 0, 1, 2 and 3 months from the accelerated storage condition of 40°C ± 2°C / 75% ± 5%RH are shown in Table 4. There was a slight drop in pH of F2 from 5.78 ± 0.03 at baseline to 5.66 ± 0.06 at three months (0.12 pH drop), which is still in the range of skin compatibility. Viscosity decrease slightly (18,890 ± 205 cPs to 19,860 ± 245 cPs) within the three-month period, which is still within the optimal range for the formulation of topical cream. There was no phase separation in all times indicating stable emulsion, which is attributed to the effective emulsifying system. The slight colour difference that was observed at 3 months was agreed with the thermal sensitivity of anthocyanin pigments under accelerated storage.

Table 4: Accelerated Stability Study Results of Formulation F2 (Mean ± SD, n = 3)

Time Point	pH	Viscosity (cPs)	Appearance	Phase Separation
0 Month	5.78 ± 0.03	19,860 ± 245	Smooth, uniform	Absent
1 Month	5.74 ± 0.04	19,540 ± 230	No visible change	Absent
2 Months	5.70 ± 0.05	19,210 ± 215	Slight thickening	Absent
3 Months	5.66 ± 0.06	18,890 ± 205	Slight colour change	Absent

Phytochemical Analysis:

The phytochemical analysis of individual plant extracts and all three cream formulations quantitatively determined the consistent differences in TPC, TFC, curcuminoid and anthocyanin content as a ratio of extract composition. Among all formulations F2 had the highest TPC (96.84 ± 2.41 mg GAE/g) and TFC (68.25 ± 2.15 mg QE/g), values which were significantly higher than either plant extract individually. This synergistic increase in the measurable phenolic content of the combined 3:3 ratio formulation can be attributed to the complementary nature of the phenolic profiles of the two plant sources that reduce intermolecular quenching effects.

Table 5: Quantitative Phytochemical Profile of Extracts and Formulations (Mean ± SD, n = 3)

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	Curcuminoids (mg CE/g)	Anthocyanins (mg/L)
Curcuma caesia extract	78.45 ± 2.14	52.36 ± 1.87	41.28 ± 1.32	6.84 ± 0.42
Clitoria ternatea extract	92.18 ± 2.56	64.72 ± 2.03	5.62 ± 0.48	38.75 ± 1.64
F1 (2:4)	88.26 ± 2.08	60.14 ± 1.92	24.36 ± 1.05	28.42 ± 1.28
F2 (3:3)	96.84 ± 2.41	68.25 ± 2.15	30.18 ± 1.22	32.67 ± 1.36
F3 (4:2)	91.72 ± 2.19	63.48 ± 2.04	35.74 ± 1.30	22.95 ± 1.11

In Vitro Antioxidant Activity:

The in vitro antioxidant activity results obtained with the three validated assay systems (DPPH, FRAP, and H2O2 scavenging) were consistently showing that the antioxidant activity of formulation F2 was the strongest among all tested samples. The demonstrated potency hierarchy of DPPH IC 5 0 extract showed a clear potency hierarchy: F2 (49.82 1.67 µg/mL) > F3 (54.74 1.88 µg/mL) > F1 (58.36 1.94 µg/mL) > *Clitoria ternatea* extract (65.18 µg/mL) > *Curcuma caesia* extract (72.45 µg/mL) with all cream formulations significantly outperforming individual plant extracts. Ascorbic acid was the positive control that had a DPPH IC50 of 15.24 + 0.54 µg/mL. FRAP values paralleled the DPPH findings, with F2 demonstrating the highest electron-donating capacity (816.35 ± 22.18 µmol Fe^{2+}/g), followed by F3 (779.80 ± 20.64), F1 (742.60 ± 19.75*), Clitoria ternatea* extract (688.40 ± 21.30), and *Curcuma caesia* extract (624.20 ± 18.50 µmol Fe^{2+}/g). H_2O_2 scavenging IC_{50} values confirmed F2 as most potent (68.19 ± 2.09 µg/mL), followed by F3 (72.56 µg/mL) and F1 (76.45 µg/mL).

Table 6: Antioxidant Activity of Polyherbal Cream Formulations (Mean ± SD, n = 3)

Sample	DPPH IC_{50} (µg/mL)	FRAP (µmol Fe^{2+}/g)	H_2O_2 IC_{50} (µg/mL)
Curcuma caesia extract	72.45 ± 2.31	624.20 ± 18.50	91.34 ± 3.12
Clitoria ternatea extract	65.18 ± 2.05	688.40 ± 21.30	84.27 ± 2.86
F1 (2:4)	58.36 ± 1.94	742.60 ± 19.75	76.45 ± 2.41
F2 (3:3)	49.82 ± 1.67	816.35 ± 22.18	68.19 ± 2.09
F3 (4:2)	54.74 ± 1.88	779.80 ± 20.64	72.56 ± 2.27
Ascorbic acid (ref)	15.24 ± 0.54	1250.60 ± 42.80	28.45 ± 1.12

Antibacterial Activity:

Agar well diffusion in antibacterial evaluation revealed that all three polyherbal cream formulations had significant antibacterial activity against all three ATCC reference pathogens with F2 always exhibiting the largest zones of inhibition. Against S. aureus, F2 produced a ZOI of 19.8 ± 0.7 mm, compared to 16.4 ± 0.6 mm (F1) and 18.1 ± 0.5 mm (F3). Against E. coli, F2 produced 17.6 ± 0.6 mm, versus 14.2 ± 0.5 mm (F1) and 16.2 ± 0.4 mm (F3). Against the more resistant P. aeruginosa, F2 still produced a notable ZOI of 15.4 ± 0.5 mm, compared to 12.8 ± 0.4 mm (F1) and 14.7 ± 0.5 mm (F3). The positive control ciprofloxacin (5 µg disc) gave ZOI of 28.6, 27.4, and 25.8 mm respectively with the plain cream base showing no inhibitory activity. The broth microdilution of F2 proved it to be the strongest formulation (MIC: 16 mg/mL) as compared to 32 mg/mL of F1 and F3.

Table 7: Antibacterial Activity of Polyherbal Cream Formulations (ZOI in mm, Mean ± SD, n = 3)

Formulation	S. aureus ZOI (mm)	E. coli ZOI (mm)	P. aeruginosa ZOI (mm)	MIC (mg/mL)
Curcuma caesia extract	13.2 ± 0.5	11.4 ± 0.4	9.8 ± 0.3	64
Clitoria ternatea extract	14.6 ± 0.6	12.8 ± 0.5	11.2 ± 0.4	32
F1 (2:4)	16.4 ± 0.6	14.2 ± 0.5	12.8 ± 0.4	32
F2 (3:3)	19.8 ± 0.7	17.6 ± 0.6	15.4 ± 0.5	16
F3 (4:2)	18.1 ± 0.5	16.2 ± 0.4	14.7 ± 0.5	32
Ciprofloxacin (5 µg)	28.6 ± 0.8	27.4 ± 0.7	25.8 ± 0.6	—
Plain cream base	0	0	0	>128

Statistical Analysis:

One-way ANOVA showed significant differences between the three formulations in all the parameters considered. Viscosity ($F = 18.26$, $p = 0.001$), DPPH IC_{50} ($F = 16.74$, $p = 0.001$), TPC ($F = 14.52$, $p = 0.002$), FRAP ($F = 13.96$, $p = 0.002$), and S. aureus ZOI ($F = 19.35$, $p < 0.001$) showed highly significant inter-formulation differences. The post-hoc test by Tukey affirmed that F2 was significantly different than both F1 and F3 in all the key performance parameters. The correlation analysis by Pearson showed strong positive correlations between TPC and FRAP activity ($r = 0.97$, $p < 0.01$), TFC and DPPH activity ($r = -0.95$, $p < 0.01$), and FRAP values and antibacterial ZOI against S. aureus ($r = 0.94$, $p = 0.05$), proving that the polyphenolic content is the major determinant of both antioxidant and antibacterial activity.

Table 8: One-Way ANOVA Statistical Results for Inter-Formulation Comparison

Parameter	F-value	p-value	Significance

pH	6.84	0.012	Significant
Viscosity	18.26	0.001	Highly Significant
Spreadability	9.73	0.008	Significant
TPC	14.52	0.002	Highly Significant
TFC	12.88	0.003	Highly Significant
DPPH IC_{50}	16.74	0.001	Highly Significant
FRAP	13.96	0.002	Highly Significant
H_2O_2 IC_{50}	11.42	0.004	Significant
S. aureus ZOI	19.35	<0.001	Highly Significant
E. coli ZOI	17.68	0.001	Highly Significant
P. aeruginosa ZOI	15.27	0.002	Highly Significant

DISCUSSION:

The current study systematically shows that it is possible to develop a stable, efficacious polyherbal topical cream by combining standardized hydroalcoholic extracts of Curcuma caesia and Clitoria ternatea in a conventional emulsifier: O/W. The optimization study indicates that the most favourable balance of all physicochemical, phytochemical, antioxidant, and antibacterial performance parameters is an equal weight ratio (F2, 3:3 w/w).

Results of the physicochemical evaluation are informatively aligned with the literature on the topical cream formulations. The pH values (5.625.64) of all three formulations are in the natural range of 5.55.65 and ensure compatibility with the skin and reduced chances of irritation. The values of viscosity (18,420 21,240 cP) and the spreadability data are similar to those that were recorded with similar polyherbal topical creams and indicate the suitability of the chosen excipient system. The high stability of the F2 sample over three months of accelerated storage with minimal changes in pH and viscosity and no phase separation confirmation that the emulsifying wax and cetyl alcohol system increases the stability of the O/W emulsion system even under the thermodynamic challenge of high temperature and humidity.

The increased phytochemical concentration in F2 relative to the individual plant extracts provide a quantitative measure of the enhanced expression of phytochemicals in the combined formulation. This is due to the complementary nature of the phenolic profiles of the two plants, which resulted in the highest TPC (96.84 mg GAE/g) and TFC (68.25 mg QE/g) in F2, and higher than both the individual extracts. The convergent results of three independent antioxidant assay systems namely; DPPH, FRAP and H 2 O 2 scavenging, invariably show that F2 is superior to individual plant extracts. The curcuminoid fraction of Curcuma caesia works via antioxidant activity through hydrogen atom transfer and metal chelation pathways, whilst ternatin anthocyanins of Clitoria ternatea work via single-electron transfer pathways. When used in optimal ratios, these mechanistic complementary systems act in concert, with more comprehensive and powerful radical scavenging coverage than any of the individual components alone - a long-established principle in the chemistry of polyphenol antioxidants.

The antibacterial findings affirm the huge clinical promise of the polyherbal cream. This increased activity against S. aureus relative to Gram-negative organisms is in agreement with the known greater susceptibility of Gram-positive bacteria to polyphenolic compounds because of differences in cell wall architecture. The marked activity against the naturally resistant P. aeruginosa, with its constitutive efflux pump systems and outer membrane permeability barrier is of particular clinical interest and is probably due to the membrane-disrupting activity of Curcuma caesia volatile oil constituents acting in synergy with the enzyme-inhibitory flavonoids of Clitoria ternatea. The quantitative validation of the synergistic effect of the antibacterial strengthening of the combined formulation strategy by the lower MIC of F2 (16 mg/mL) as compared to individual extracts (32-64 mg/mL) quantitatively validates the synergistic effect of the antibacterial strengthening of the combined formulation strategy.

CONCLUSION:

The current research study has been able to achieve all the mentioned objectives and has proven the scientific and pharmaceutical validity of a stable polyherbal topical cream comprising of standardized hydroalcoholic extracts of Curcuma caesia and Clitoria ternatea. A standardized emulsion base was successfully used to develop three formulations of oil in water cream (F1, F2, F3) with different ratios of extract. All formulations showed acceptable physicochemical characteristics, with pH values within the range of 5.5-6.5, which is the skin-compatible range,

viscosity values were within the range of 18,000-21,000 cps, excellent homogeneity, and no primary skin irritation.

The accelerated stability studies have established that the physicochemical integrity of the optimized F2 formulation over three months under ICH Q1A(R2) conditions, with minimal alterations in pH and viscosity, no phase separation and maintenance of homogeneity. Quantitative phytochemical analysis has shown that F2 (3:3 ratio) had the strongest TPC (96.84 ± 2.41 mg GAE/g) and TFC (68.25 ± 2.15 mg QE/g).

In vitro antioxidant activity using DPPH IC 5: 49.82 plus 1.67 ug/mL; FRAP: 816.35 plus 22.18 ug/mL; H2O 2 IC 5: 68.19 plus 2.09 ug/mL), was by far, significantly greater than that of individual plant extracts, and the determination of synergistic antioxidant interaction between *curcuminoids* and *ternatin anthocyanins*. Antibacterial analysis showed that F2 demonstrated a marked increase in antibacterial activity in the formulation mixture with all three standard ATCC pathogens (S. aureus ZOI: 19.8 ± 0.7 mm; E. coli ZOI: 17.6 ± 0.6 mm; P. aeruginosa ZOI: 15.4 ± 0.5 mm; MIC: 16 mg/mL), demonstrating synergies in antibacterial activity enhancement in the combined formulation.

The highly significant differences among formulations ($p < 0.05$) across all of the parameters evaluated were confirmed by statistical analysis using one-way ANOVA with post-hoc test statistic by Tukey. Formulation F2 (*Curcuma caesia*: *Clitoria ternatea* = 3:3 w/w) is hereby found to be the optimized polyherbal cream formulation with the best balance of physicochemical stability, phytochemical richness, antioxidant potency, and antibacterial efficacy, supporting its potential application in dermatological oxidative stress-associated and bacterially infected conditions as a potential therapeutic agent.

Future Work:
The present study forms a good basis in further development of this polyherbal topical cream formulation. There are a number of key avenues that would be of interest in future research. First in vivo pharmacological testing like wound healing models (Wistar rats, excision wound, incision wound), contact sensitization assays and repeated dose dermal toxicity testing are required to confirm the in vitro results and to establish the safety and efficacy profile of the product to be used in a regulatory context. Studies conducted on Franz diffusion cell using ex vivo excised human skin or porcine skin membranes would be very beneficial to gain information on the transdermal penetration properties and dermal bioavailability of the active phytoconstituents. The more advanced formulation methods, such as the creation of nanostructured lipid carriers (NLCs), nanoemulsion-based creams or cyclodextrin-complexed formulations with the standardized extracts, may yield a higher photostability of the anthocyanin pigments, better skin penetration of the curcuminoids and extended release. Also, the clinical trials carried out on patients with particular skin illnesses (such as mild to moderate bacterial skin infections, acne vulgaris, atopic dermatitis, or photoaged skin) would be the most decisive proof of clinical efficiency and patients' acceptance. The combined extract system, metabolomic profiling with LC-MS/MS would enable the complete characterization of the synergic plant phytochemical interaction in the extracts and enable the identification of the individual combinations of compounds responsible for the biological activity observed.

LIMITATIONS:

The present work is quite extensive in the scope of the in vitro and Physicochemical tests carry some significant limitations which should be highlighted. To begin with, the antioxidant and antibacterial action reported is only based on in vitro assay systems which may not be fully representative of the complex biological environment of human skin tissue. In vitro antioxidant assays, such as DPPH and FRAP, have been extensively accepted and validated as screening assays, but only for specific radical species and for particular electron transfer mechanisms, and may not duplicate the spectrum of radical scavenging activity that is likely to be relevant to oxidative stress in the skin. Likewise, in vitro agar well diffusion and broth microdilution antibacterial tests might not be an accurate reflection of the in vivo antibacterial efficacy which is influenced by other factors such as skin barrier penetration, protein binding, biofilm formation, and host immune responses. Stability studies have not been done yet at 25C/60 percent RH except under ICH accelerated conditions over a period of 3 months. The patch test was conducted in a small number of healthy volunteers as a preliminary safety test and is not a clinical safety test. Also, the research evaluated the antibacterial efficacy against the drug-resistant pathogens using conventional ATCC reference strains;

clinical isolates with known resistance pattern would be more clinically relevant in assessment of antibacterial efficacy against the drug-resistant pathogens. The extract standardization was done through quantitative phytochemical analysis, but the fingerprinting of the extract using high performance liquid chromatography (HPLC) was not validated and thus was not seen as more certain representation of the phytochemical composition and batch-to-batch consistency of the extract.

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