AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Phytochemical Evaluation and Dermatological Safety Assessment of *Nardostachys jatamansi Root and Curcuma caesia Rhizome Extracts*

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ABSTRACT

This study aimed to evaluate the phytochemical profile and antimicrobial potential of extracts derived from *Curcuma caesia* rhizomes and *Nardostachys jatamansi* roots. A total of 250 g of Curcuma caesia and 80 g of Nardostachys jatamansi were subjected to solvent extraction. The yields of Curcuma caesia extracts were 2.22% in petroleum ether and 7.15% in ethanol. Phytochemical screening of the ethanolic extracts confirmed the presence of several bioactive constituents, including alkaloids, flavonoids, terpenoids, tannins, phenolics, saponins, glycosides, and proteins. The total phenolic content was measured at 171 mg/g for *Curcuma caesia* and 393 mg/g for *Nardostachys jatamansi*, expressed in gallic acid equivalents. Similarly, the total flavonoid content was 175 mg/g and 410 mg/g, respectively, in terms of rutin equivalents. A polyherbal gel was formulated using these extracts, with the G3 combination formulation showing superior performance in antimicrobial activity tests, as evidenced by a larger zone of inhibition. Furthermore, dermal safety assessment revealed no skin irritation, indicating its suitability for topical application. Overall, the findings support the potential of this herbal gel as a safe and effective natural antimicrobial agent.

Keyword: *Curcuma caesia, Nardostachys jatamansi,* antimicrobial, polyherbal gel

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Received 22 August 2025, Accepted 05 November 2025

Please cite this article as: Singh S *et al*., Phytochemical Evaluation and Dermatological Safety Assessment of Nardostachys jatamansi Root and Curcuma caesia Rhizome Extracts. American Journal of PharmTech Research 2025.

INTRODUCTION

Herbal medicine, a key component of traditional healthcare systems such as Ayurveda, Traditional Chinese Medicine, and Unani, relies on the therapeutic properties of plants to treat and prevent various ailments (Ekor, M. et.al 2014)[1]. With growing interest in natural and holistic approaches to health, herbal medicine continues to gain global recognition for its efficacy, safety, and minimal side effects.

Herbal extracts are concentrated preparations obtained from medicinal plants, rich in bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolics. These extracts serve as the foundation for many natural remedies and modern phytopharmaceuticals. In dermatology, herbal extracts are increasingly used for their anti-inflammatory, antimicrobial, antioxidant, and skin-soothing effects, making them valuable ingredients in cosmetic and therapeutic formulations. (Heinrich et.al 2018)[2]

Herbal extracts have gained significant attention in dermatology and cosmetology due to their bioactive compounds, minimal side effects, and therapeutic potential. (Mukherjee, P. K et.al.2012)[3] *Nardostachys jatamansi* (commonly known as spikenard) and *Curcuma caesia* (black turmeric) are two traditionally used medicinal plants in Ayurveda and folk medicine, valued for their anti-inflammatory, antioxidant, and antimicrobial properties.

Nardostachys jatamansi

Nardostachys jatamansi root extract is known for its soothing, anti-aging, and skin-calming effects, making it a promising ingredient in formulations for sensitive or irritated skin. Similarly, *Curcuma caesia* rhizome extract possesses potent anti-inflammatory and wound-healing properties, attributed to its rich phytochemical profile, including curcuminoids and essential oils. (Joshi, H et. al. 2006)[4] Medicinally, *N. jatamansi* is renowned for its neuroprotective, anti-inflammatory, antioxidant, antidepressant, and sedative properties. It has been traditionally used to treat conditions such as insomnia, anxiety, epilepsy, and mental fatigue. In dermatology, it is valued for its skin-soothing, anti-aging, and wound-healing effects. Its root extract is often incorporated into herbal formulations for treating skin irritation, improving complexion, and promoting overall skin health. (Dwivedi, V. N et.al.2017)[10]

Curcuma caesia

Curcuma caesia, commonly known as black turmeric, is a rare and valuable medicinal plant belonging to the Zingiberaceae (ginger) family. Native to India and parts of Southeast Asia, the plant is easily recognized by its bluish-black rhizome and distinct aromatic properties. Traditionally used in Ayurveda and folk medicine, *C. caesia* is known for its diverse therapeutic

applications**.** (Shrivastava, A. et.al.2022)[35.] The rhizome of *Curcuma caesia* is rich in bioactive compounds such as curcuminoids, essential oils, flavonoids, and terpenoids, which contribute to its anti-inflammatory**,** antioxidant**,** antimicrobial**,** analgesic**,** and wound-healing properties. It has been used to treat conditions like skin infections, pain, respiratory disorders, and joint inflammation**. (** Chakraborty, S et.al.2024)[33]

This study focuses on the phytochemical evaluation and dermatological safety assessment of these two extracts to explore their potential as natural, skin-friendly ingredients in topical applications. By analyzing their bioactive constituents and skin irritancy profile, the research aims to support their safe and effective use in cosmetic or therapeutic skincare products**.** (Rai, M et.al.2020)[31]

MATERIALS AND METHOD

Soxhlet extraction:

Dried powered of*Curcuma Caesia* (Rhizome) and *Nardostachys jatamansi* (root) were successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using ethanol solvent system at 40-60ºC temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container (Evans et. al., 2019, 2009 and Alara et. al., 2019). Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of *Curcuma Caesia* and *Nardostachys jatamansi* by using standard procedures (Kokate et. al., 2006).

Quantitative Phytochemical estimation-

Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. Ethanol extract of *Curcuma Caesia* and *Nardostachys jatamansi* (0.2 mL from stock solution) was mixed separate in test tubes with 2.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 7.5% Na_2CO_3 solution was added to the mixture and volume make up to 7 ml with deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 25°C, after which the absorbance was read at 760 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20 to 100µg/ml). The

estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample. (Saeed et. al., 2012).

Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminum chloride method (Chang et. al., 2002). In a 10 ml test tubes, 0.5 ml of ethanol extract of *Curcuma Caesia* and *Nardostachys jatamansi* , 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) was mixed separate in test tubes. After 5 min, 2 ml of NaOH (4%) was added and volume up to 5ml with deionized distilled water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoid was made using rutin standard solution (20 to 100µg/ml) under the same procedure as earlier described. The total flavonoid was expressed as milligrams of rutin equivalents per g of dried sample**.** (Senguttuvan et. al., 2014).

Polyherbal gel formulation

Formulation of Placebo Gel (Control formulation) For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water along with methyl paraben, propyl paraben and glycerine kept for overnight. Take the extracts of Curcuma *Caesia* and *Nardostachys jatamansi* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring for 10 minutes. (Kumar et. al., 2020)[24].

Evaluation of Herbal gels (Chacko et. al., 2025, Bishi et. al., 2025 and Patel et. al., 2023).

Organoleptic properties of gel

Physical appearances of the formulated Poly herbal gels were evaluated by visual perception and with the help of simple microscope. The sample was placed on glass slide with the help of cover slip. Then the slide was observed under microscope and the physical appearances of gels were studied. All the formulation of gels was stored in container and they are visually observed to identify for their appearance of any type of aggregates in the gel formulations. The prepared formulations of gel were microscopically observed to find out the presence of any unwanted particulate. All the formulations of gel were determined for fulfill the obligation of free from unwanted particulate matter

Measurement of pH

A digital pH meter is used to find out the pH of gel formulations. In a clean beaker with 50 mL of distilled water the 1 gm of gel were dissolved properly and kept it in a beaker for 2 hr's. The pH of each formulation was investigated in triplicate and the average reading was recorded.

Viscosity

Viscosity of gel was measured by use of Brookfield viscometer (LVDV-II+ Pro). The sufficient quantity of herbal gel was filled in sample holder separately. The height of the gel was filled in the sample holder should sufficiently allow to dip the spindle. Viscosities of the gels were recorded by adjusting the rotating speed of the spindle at 50 rpm.

Swelling Index

For the determination of swelling index we take 1gm quantity of gel and then it was filled in a clean and dry (50 mL) beaker, the beaker hold 10 mL of distilled water. The samples were retained in a beaker for period of time and then after some time kept out the gel from beaker and put into a dry or clean place for some times and weight it again to calculate and find out how much percent of the gel was swelled. We can calculate the swelling index by applying this formula:

Swelling Index (SI)=(Wt−W0)/ W0×100

Where: W0= Initial weight of the dry sample = Weight of the swollen sample at time, SI = **Swelling Index (usually expressed as a percentage)**

Spreadability

For the identification of Spreadability of gel formulations the Spreadability apparatus which contain two glass slides of (20×20 cm) is used. We take 1gm of gel and placed it on one slide. The second slide was placed over the gel and due to this the gel was pressed and spreaded between two glass slides. After that, the 100 gm of weight was placed over the top slide to press the gel freely and it will give us thin layer. The weight was removed and 20 gm weight was tied to the upper slide carefully. The total time taken by top slide and the traveled distance of slide were examined.28 the whole procedure was performed three times and the average time of three trials was used for further calculations. The following formula was used to find out the Spreadability:

Spreadability (S)=M×L/T

Where, S = Spreadability m = weight tied on top slides l = length of the glass slide t = time in sec

Antibacterial study

For evaluation of antibacterial activity, 24 h fresh culture of bacteria was suspended in sterile water to obtain a uniform suspension of microorganism. Determination of zone of inhibition Antibacterial activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.1 mL bacterial suspension having a uniform turbidity at temperature of 40°C. In a sterile petri dish having an internal diameter of 8.5 cm was taken, 20 mL of culture medium was poured into it. Care was taken to form a uniform thickness of the medium in different plates. Wells were made aseptically with cork borer having 6 mm diameter after complete solidification of liquefied inoculated medium. In each of these plate extract and

formulation were placed carefully. Plates were kept for pre diffusion for 30 min at room temperature; then the plates were incubated at 37°C for 24 h and the zones of inhibition were measured. (Balouiri et. al., 2016).

Skin irritation study OECD. (2024).

Three healthy wistar rat per group were obtained from licensed suppliers and acclimated to the laboratory. Rats, were individually housed in cages and received feed on a daily basis; tap water was available ad libitum. The backs of the animals were clipped free of fur with a hair remover cream 24 hours before application of the sample. A 200 mg sample of the test article was then applied to each site. Animals were returned to their cages. At 24 and 72 hours after test article application, the test sites were examined for dermal reactions in accordance with the FHSA (Federal Hazardous Substances Act) recommended Draize scoring criteria. The Primary Irritation Index (P.I.I.) of the test article was calculated following test completion. As defined in CFR (Code of Federal Regulation), a material producing a P.I.I. score of greater than or equal to 5.00 would be considered positive; the material would be considered a primary irritant to the skin**.** (Singh et. al., 2019)[27]

The test article, G1 gel, G2 gel, and G3 gel, 0.2 gm dose of the test sample were applied to the intact skin of rats and left in place for 24 hours. Test sites were graded for erythema and edema at 24 and 72 hours after sample application. Under the conditions of this test, the test article would not be considered a primary irritant to the skin. The Primary Irritation Index was calculated to be 0.00. (each group contain three animals n=3)

RESULTS AND DISCUSSION

Plant Collection

The plant were selected and collected from the local nursery. The plant authentified by the institute.

Table 1: Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Curcuma Caesia*	Rhizomes	250.00 gm
2.	*Nardostachys jatamansi*	Root	80 gm

Percentage yield

Table 2: Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Curcuma Caesia*	Petroleum Ether	Dark yellow to brown	200.00 gm	4.456	2.22
2.	*Curcuma Caesia*	Ethanol	Brown	195.50gm	13.980	7.150

3.	*Nardostachys jatamansi*	Petroleum Ether	Dark yellow to brown	80 gm	0.899	1.12
4.	*Nardostachys jatamansi*	70% Ethanol	Brown	80gm	8.369	10.46

Qualitative Phytochemical Analysis of different extracts

Table 3: Phytochemical analysis of *Curcuma Caesia* Extracts

S. No.	Experiment	Result	
		Petroleum ether	Ethanol
Test for Carbohydrates			
1.	Molisch's Test	+	+
2.	Fehling's Test	+	+
3.	Benedict's Test	+	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	+
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	+	+
2.	Biuret's Test	+	+
Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	+	-
3.	Borntrager's Test	-	+

Table 4: Phytochemical analysis of *Nardostachys jatamansi* Extract

S. No.	Experiment	Result	
		Petroleum ether	Hydroalcoholic
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
Test for Terpenoids			

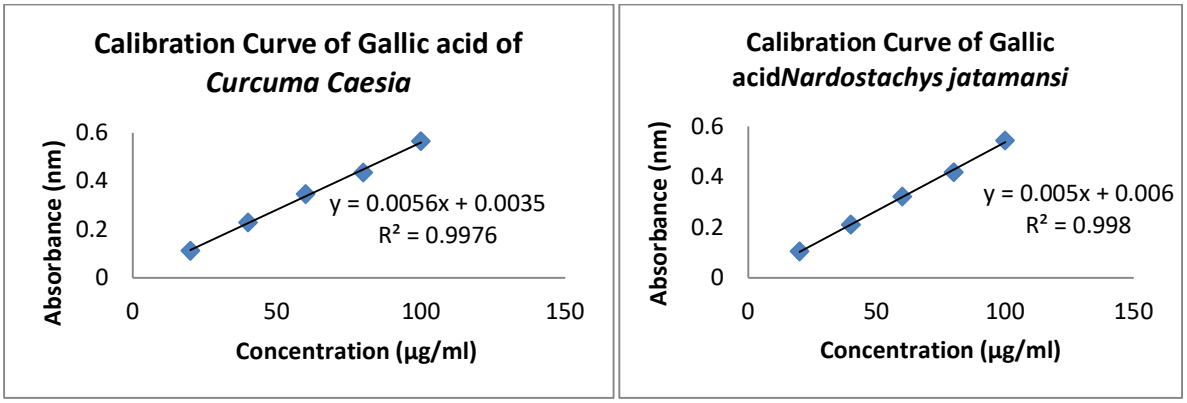
1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	-	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+
3.	Million's Test	-	_
Test for Glycosides			
1.	Legal's Test	-	+

Quantitative Phytochemical analysis of extracts of Curcuma Caesia and Nardostachys jatamansi -

Total Phenolic Content (TPC) Estimation of extracts of Curcuma Caesia and Nardostachys jatamansi -

Table 5: Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm) *Curcuma Caesia*	Absorbance (nm) *Nardostachys jatamansi*
1.	20	0.112	0.105
2.	40	0.229	0.211
3.	60	0.347	0.322
4.	80	0.436	0.419
5.	100	0.566	0.545



Graph 1: represent standard curve of Gallic acid

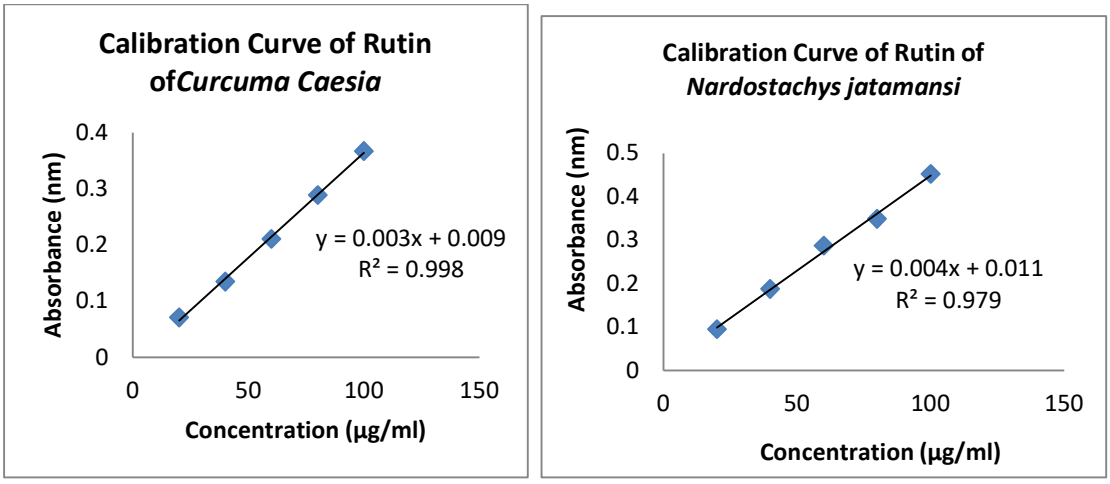
Table 6: Total Phenolic Content in ethanolic extract

Total Phenolic content (mg/gm equivalent to Gallic acid)		
Extract	***Curcuma Caesia***	***Nardostachys jatamansi***
Absorbance (Mean±SD)	0.338±0.01	0.3204 ±0.004
TPC	171.794	393

Total Flavonoid Content (TFC) Estimation of extracts of Curcuma Caesia and Nardostachys jatamansi

Table 7: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm) *Curcuma Caesia*	Absorbance (nm) *Nardostachys jatamansi*
1.	20	0.071	0.095
2.	40	0.135	0.187
3.	60	0.211	0.287
4.	80	0.289	0.349
5.	100	0.367	0.452



Graph 2: Represent standard curve of Rutin

Table 8: Total Flavonoid Content in ethanolic extract

Total Flavonoid content (mg/gm equivalent to Rutin)		
Extracts	***Curcuma Caesia***	***Nardostachys jatamansi***
Absorbance (Mean ± SD)	0.2146±0.015	0.274 ±0.003
TPC	175.7264957	410.9375

POLYHERBAL GEL

Formulation of Placebo Gel (Control formulation) For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water along with methyl paraben, propyl paraben and glycerine kept for overnight. Take the extracts of Ocimum *Curcuma Caesia* and *Nardostachys jatamansi* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring for 10 minutes

Figure 1: G1 shows gel of *curcuma cassia*, G2 shows gel of *Nardostachys jatamansi* and G3 shows the combination of both extract

Table 9: composition of the polyherbal gel

Ingredients	G1	G2	G3
Curcuma cassia	500 mg	-	500mg
Nardostachys jatamansi	-	500 mg	500mg
Carbopol 940	2%	2%	2%
Propylene glycol	5 mL	5 mL	5 mL
Methyl paraben	0.15 g	0.15 g	0.15 g
Propyl paraben	0.30 g	0.30 g	0.30 g
Triethanolamine	5 mL	5 mL	5 mL
Water	q. s	q. s	q. s

Evaluation of the polyherbal gel

Organoleptic properties

The prepared gel was inspected visually for colour, clarity, and homogeneity against a black and white background.

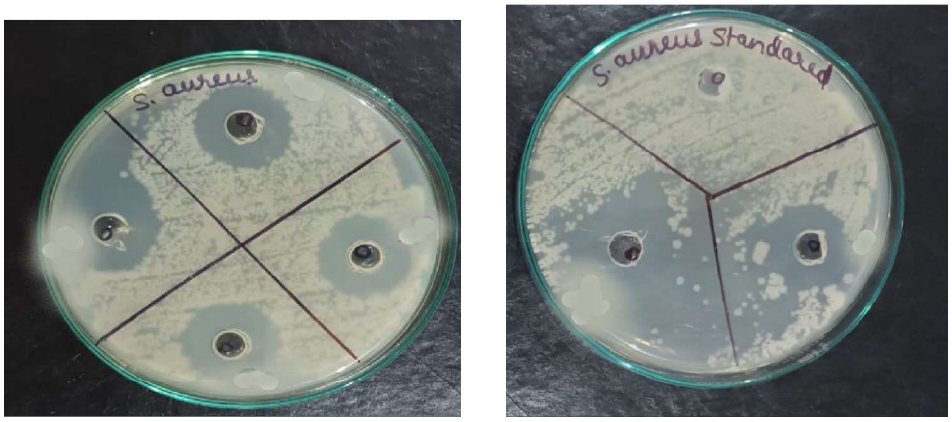
Table 10: Physical Property of the formulation

Parameters	G1	G1	G2
Visual appearance	opaque	opaque	opaque
Consistency	Uniform mixture	Uniform mixture	Uniform mixture
Color	Slightly yellow in shade	Slightly yellow in shade	Slightly yellow in shade
Greasy	Non greasy (non sticky)	Non greasy (non sticky)	Non greasy (non sticky)
Texture	Smooth in touch	Smooth in touch	Smooth in touch
Homogeneity	homogenous	homogenous	Homogenous
Grittiness	Non gritty	Non gritty	Non gritty

Table 11: Represent the evaluation parameter of the gel loaded with isolated compound

Parameters	G1	G2	G3
pH	**7.1 ±0.024**	**7.2 ±0.015**	**7.0±0.056**
Viscosity cps	1289±0.25	1282±0.15	1390±0.051
Swelling Index (%)	54.54±0.33	52.60±0.81	58.33±0.59
Spreadability	39.2±0.02	39.14 ±0.03	38.7±0.005

Antimicrobial Result



(A) (B)

Figure 2: S.aureus zone of inhibition study of extracts (B) and formulation (A)

Table 12: Zone of inhibition of the formulations

Test Sample	Test Microorganism	Average Zone of Inhibition (mm) ± Standard Deviation	Result Interpretation
Formulation G1	*Staphylococcus aureus* (Gram-positive)	32.0 ± 1.6	Good antimicrobial activity
Formulation G2		36.7± 1.2	Good antimicrobial activity
Formulation G3		39.5± 1.8	High antimicrobial activity
Negative Control (Gel Base)		0	No activity

Table 13: Zone of inhibition of the extracts

Test Sample	Test Microorganism	Average Zone of Inhibition (mm) ± Standard Deviation	Result Interpretation
Extract 1 (*Curcuma caesia*)	*Staphylococcus aureus* (Gram-positive)	28.26± 1.1	Good antimicrobial activity
Extract 2 (*Nardostachys jatamansi)*		29.11± 1.25	Good antimicrobial activity
Extract 3 (*Nardostachys jatamansi*+ *Curcuma caesia)*		31.89± 1.7	High antimicrobial activity

Skin irritation study (draize test)

Table 14: showing the draize score of formulation G1

Time (hrs)	PDII (G1) (erythma score + edema score / 6)	PDII (G2) (erythma score + edema score / 6)	PDII (G3) (erythma score + edema score / 6)
0	0.37	0.276	0.376
Shaved animals			
24	0.11	0.16	0.055
Formulation applied			
48	0.055	0.11	0
72	0	0	0

Primary dermal irritation index (PDII)

DISCUSSION

The present study aimed to formulate and evaluate a polyherbal gel using ethanolic extracts of *Curcuma caesia* rhizome and *Nardostachys jatamansi* root. Extraction yields showed a higher percentage for ethanol (7.15%) compared to petroleum ether (2.22%) for *Curcuma caesia*, indicating better solubility of phytoconstituents in ethanol. Phytochemical analysis confirmed the presence of several active compounds, including alkaloids, flavonoids, terpenoids, phenolics, tannins, and saponins, all of which are known to contribute to antimicrobial and therapeutic properties. Quantitative analysis revealed that *Nardostachys jatamansi* had significantly higher total phenolic (393 mg GAE/g) and flavonoid content (410 mg RE/g) compared to *Curcuma caesia* (171 mg GAE/g and 175 mg RE/g, respectively), suggesting that it may contribute more strongly to the antioxidant and antimicrobial potential of the formulation.

Three gel formulations were developed and evaluated, including a combination formulation (G3). Visual and microscopic assessments showed that all gels were free from particulate matter, smooth in texture, and had a uniform appearance. Loading the gels with extracts changed the appearance from clear and colorless to yellowish and opaque, without affecting homogeneity. The pH of all gels was within the acceptable dermal range (7.0–7.2), minimizing the risk of skin irritation. Among the tested formulations, G3 demonstrated the highest viscosity, swelling index, and spreadability, indicating better physical characteristics and user acceptability compared to G1 and G2 (G3 > G1 > G2 for all parameters). Antimicrobial testing revealed that the G3 formulation exhibited the largest zone of inhibition, confirming that the combination of both plant extracts produced a synergistic effect. This aligns with the phytochemical results, where a diverse range of bioactive compounds was identified in both plants. The skin irritation study conducted over 72 hours showed no signs of redness, inflammation, or other adverse effects in any test subjects. The Primary Irritation Index (PII) and Primary Dermal Irritation Index (PDII) scores were both zero, indicating the gel is safe for topical use. Overall, the study supports the potential of a combined herbal gel formulation (G3) as an effective and non-irritant topical antimicrobial product.

CONCLUSION

The present study successfully demonstrated the formulation and evaluation of a polyherbal gel using ethanolic extracts of *Curcuma caesia* and *Nardostachys jatamansi*. Phytochemical analysis confirmed the presence of various bioactive compounds contributing to the antimicrobial and antioxidant potential of the extracts. Among the developed formulations, the combination gel (G3) exhibited superior physicochemical properties, including optimal viscosity, spreadability, swelling index, and pH suitable for dermal application. The antimicrobial study further confirmed the

enhanced effectiveness of the G3 formulation, while skin irritation tests established its safety for topical use. Overall, the results suggest that the formulated polyherbal gel is a promising candidate for use as a natural, safe, and effective antimicrobial topical preparation.

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