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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
Impact Factor (RJIF): 6.34
TPI 2025; 14(12): 41-55

www.thepharmajournal.com
Received: 05-10-2025
Accepted: 07-11-2025

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A novel herbal facewash formulation based on green tea and tea tree oil: Formulation and performance evaluation

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DOI: https://www.doi.org/10.22271/tpi.2025.v14.i12a.26338

Abstract
Herbal cosmetics have gained significant attention due to their safety, biocompatibility, and therapeutic efficacy in skincare applications. The present study aimed to develop and optimize a novel herbal facewash formulation incorporating *Camellia sinensis* (green tea) extract and *Melaleuca alternifolia* (tea tree) oil for effective cleansing and skin protection. Green tea leaves were subjected to Soxhlet extraction using methanol, while tea tree oil was obtained by hydrodistillation. The extracts were characterized through pharmacognostic evaluation, physicochemical analysis, phytochemical screening, FT-IR, and GC-MS studies. A carbopol-based gel facewash was formulated and optimized using a Box-Behnken Design to study the effect of green tea extract, tea tree oil, and carbopol concentration on viscosity and drug content. The optimized formulation exhibited ideal physicochemical properties, including suitable viscosity (5242 cps), satisfactory drug content (78.2%), good spreadability, stable foaming ability, acceptable extrudability, and skin-friendly pH (6.3). GC-MS analysis confirmed the presence of bioactive compounds such as catechins, caffeine, and terpinen-4-ol, contributing to antioxidant and antimicrobial activities. The study concludes that the developed herbal facewash is stable, effective, and suitable for topical cosmetic application, offering a promising natural alternative for routine facial cleansing and skincare.

Keywords: Herbal facewash, *Camellia sinensis*, *Melaleuca alternifolia*, Green tea extract, Tea tree oil, Box-Behnken design, Herbal cosmetics, Phytochemical evaluation, GC-MS analysis, Topical formulation

1. Introduction
Skin care plays a vital role in maintaining personal hygiene, protecting against environmental pollutants, and preventing dermatological disorders. With increasing concerns regarding the adverse effects of synthetic cosmetic ingredients, there has been a growing inclination toward herbal and natural formulations owing to their safety, minimal side effects, and therapeutic benefits. Herbal facewash formulations not only cleanse the skin but also provide additional benefits such as antioxidant, antimicrobial, and anti-inflammatory effects. (Buffoli B, 2015) [4]. *Camellia sinensis* (green tea) is a well-known medicinal plant rich in polyphenols, flavonoids, catechins, and caffeine, which exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities. These properties make green tea particularly beneficial in managing acne, excessive sebum production, and oxidative skin damage. (Arterbery VE, 2018) [3] Similarly, Melaleuca alternifolia (tea tree) oil is widely recognized for its broad-spectrum antimicrobial, antifungal, and anti-inflammatory properties, primarily attributed to terpinen-4-ol and other monoterpenes. (Hiral S. Popaniya, 2024) [9] Tea tree oil has been extensively used in dermatological preparations for acne, skin infections, and irritation. (Nirmala, 20245) [14] Face wash is an essential skincare product specifically designed to cleanse the delicate skin of the face without stripping it of its natural moisture. Also referred to as a facial cleanser, it effectively removes dirt, excess oil, makeup, dead skin cells, and other environmental pollutants that accumulate throughout the day. (Nazeer AS, 2023) [13] Unlike regular soaps that may cause dryness or irritation, face washes are formulated to maintain the skin's natural balance while keeping it fresh, soft, and hydrated. They are commonly used as part of a daily skincare routine, usually in combination with a toner and moisturizer. (Sehgal A, 2023) [17].
Despite the availability of various herbal facewash products in the market, many lack scientific standardization, optimization, and comprehensive evaluation. Therefore, the present study was

designed to develop a scientifically validated herbal facewash formulation combining green tea extract and tea tree oil. The formulation was optimized using a statistical box-Behnken Design to ensure desirable viscosity and drug content while maintaining user acceptability. Extensive pharmacognostic, phytochemical, compatibility, and physicochemical evaluations were carried out to establish the quality, stability, and efficacy of the developed formulation.

2. Material and Method

2.1 Material: Dried green tea leaves (*Camellia sinensis*) were procured from an authenticated herbal supplier and used as the source of plant extract. Tea tree oil (*Melaleuca alternifolia*) was obtained from a certified essential oil manufacturer and was of pharmaceutical grade. Sodium lauryl sulfate, cocamidopropyl betaine, glycerin, carbopol 940, propylene glycol, methyl paraben, propyl paraben, triethanolamine, and sodium hydroxide were used as formulation excipients. Ethanol and distilled water were employed as solvents for extraction and formulation purposes. All chemicals and reagents used in the study were of analytical grade and were obtained from standard laboratory suppliers. Distilled water was used throughout the study for the preparation of solutions and formulations.

2.2 Methods

2.2.1 Drying of *Camellia sinensis* (Green tea) and *Melaleuca alternifolia* (Tea tree)

Freshly collected green tea and tea tree leaves were washed to remove impurities, blotted dry, and shade dried at room temperature in a clean, well-ventilated environment for 7-10 days. The dried leaves were then powdered and stored in airtight, amber-colored containers in a cool, dry place until further use to preserve their phytoconstituents.

2.2.2 Pharmacognostic Studies

Morphological Studies

The green tea leaves (*Camellia sinensis*) were examined to assess their macroscopic, morphological, and organoleptic properties. Morphological studies included visual, organoleptic, and macroscopic examination of the leaves, assessing characteristics such as color, shape, size, texture, odor, taste, and surface features. These parameters provided preliminary confirmation of the identity and quality of the plant materials.

Microscopic studies were performed using transverse section and powder microscopy. Thin transverse sections of fresh leaves were prepared, cleared, stained, and examined to observe characteristic anatomical features, while powder microscopy helped identify diagnostic elements such as fibers, vessels, crystals, starch grains, and trichomes, aiding in authentication and detection of adulterants.

Physicochemical Parameters

Physicochemical parameters were evaluated in accordance with standard pharmacopoeia procedures. These included determination of ash values (total ash, acid-insoluble ash, water-soluble ash, and sulphated ash), extractive values (alcohol-soluble and water-soluble), foreign matter content, loss on drying, swelling index, foaming index, and bitterness value. These parameters provided essential information regarding purity, inorganic matter, moisture content, extractable constituents, and quality consistency of the crude drugs. Overall, the pharmacognostic and physicochemical studies established standard quality parameters necessary for the safe and effective use of green tea and tea tree leaves in herbal formulation development.

2.2.3 Extraction and Percentage Yield

Extraction of plants

About 200 g of fresh *Camellia sinensis* leaves were powdered and subjected to Soxhlet extraction using methanol as the solvent. The powdered material was placed in the extraction chamber, and the solvent was heated at 70-80 °C under controlled conditions. The methanolic solvent continuously evaporated, condensed, and percolated through the plant material, facilitating efficient extraction of phytoconstituents. After completion of the extraction, the solvent was evaporated under reduced pressure, and the dried extract was collected and its percentage yield was calculated. Tea tree leaves (≈200 g) were subjected to hydro-distillation using a Clevenger-type apparatus with distilled water for 3-4 hours to obtain the essential oil. The collected oil was dried over anhydrous sodium sulfate and stored in amber-colored vials at 4-8°C until further use.

Physicochemical characters of oil

Tea Tree (Melaleuca alternifolia) leaves different physicochemical parameters were observed i.e., color, odor, appearance, refractive index, boiling point and specific gravity.

The physicochemical evaluation of Tea Tree (*Melaleuca alternifolia*) oil included assessment of organoleptic characters such as colour, odour, and taste using sensory organs. The refractive index of tea tree oil was measured at 25 ± 0.5°C using an Abbe refractometer with the sodium D line (589.3 nm). The boiling point was determined using a thermometer, reflecting the degree of unsaturation of fatty acids. Optical rotation was measured using a polarimeter to determine the rotation of plane-polarized light by the oil sample. Specific gravity was evaluated using a pycnometer by comparing the weight of a known volume of oil with an equal volume of water, and the values were calculated accordingly.

2.2.4 Phytochemical Screening

The preliminary phytochemical screening including Qualitative and Quantitative analysis of the selected *Camellia sinensis* (L.) leaves extracts was observed using the procedures A preliminary phytochemical investigation was conducted to identify the presence of various bioactive constituents in *Camellia sinensis* (L.) leaves extract using methanol. This qualitative analysis aimed to detect secondary metabolites such as alkaloids, glycosides, carbohydrates, steroids and sterols, saponins, proteins and amino acids, flavonoids, tannins, phenolic compounds, mucilage, fixed oils, and volatile oils. Accurately weighed 3 g of each dried extract was dissolved in an appropriate volume of its respective solvent (methanol). The mixtures were then filtered using Whatman No. 1 filter paper to obtain clear test solutions, which were subsequently used for phytochemical analysis.

Table 1: Preliminary Phytochemical Screening of Plant Extracts

Phytochemical Class	Test Name	Reagent Used	Inference
Alkaloids	Dragendorff's test	Potassium bismuth iodide	Orange-brown precipitate
	Mayer's test	Potassium mercuric iodide	White/cream precipitate
	Hager's test	Saturated picric acid	Yellow precipitate
	Wagner's test	Iodine in potassium iodide	Reddish-brown precipitate
Glycosides	Legal test	Pyridine + sodium nitroprusside	Pink to red color
	Baljet test	Sodium picrate	Yellow to orange color
	Borntrager's test	Dil. H_2SO_4, chloroform, ammonia	Red color (anthraquinones)
Carbohydrates	Molisch's test	α-Naphthol + conc. H_2SO_4	Violet ring
	Fehling's test	Fehling's A & B	Brick-red precipitate
	Barfoed's test	Barfoed's reagent	Copper oxide precipitate
Steroids / Sterols	Liebermann-Burchard test	Acetic anhydride + H_2SO_4	Blue-green color
	Salkowski test	Chloroform + H_2SO_4	Red to cherry-red color
Saponins	Foam test	Distilled water	Stable foam (~1 cm)
Proteins	Biuret test	NaOH + $CuSO_4$	Violet color
Amino Acids	Ninhydrin test	0.2% Ninhydrin	Purple color
Flavonoids	Shinoda test	Mg turnings + HCl	Pink/red color
	Ferric chloride test	10% $FeCl_3$	Greenish-blue/violet
	Sodium hydroxide test	NaOH + HCl	Yellow color disappears
Tannins / Phenolics	Ferric chloride test	Ferric chloride	Blue-black/green color
Acidic Compounds	Sodium bicarbonate test	$NaHCO_3$	Effervescence
Mucilage	Ruthenium red test	Ruthenium red	Pink coloration
Fixed & Volatile Oils	Spot test	Filter paper	Oily stain (fixed oil)
	Sudan Red IV test	Sudan Red IV dye	Red-stained oil droplets

2.2.5 Compatibility Studies

FTIR Technique

Drug-excipient compatibility was evaluated using FTIR spectroscopy by preparing KBr pellets of the pure drug and excipients. The spectra (4000-400 cm^{-1}) were analyzed for any changes in characteristic peaks to detect possible chemical interactions.

Gas Chromatography- Mass Spectroscopy (GC-MS) analysis: GC-MS analysis of *Camellia sinensis* leaf extract and *Melaleuca alternifolia* (tea tree) essential oil was performed using a SHIMADZU GC-MS QP-2020 system equipped with an autosampler and an SH Rxi-5MS column. Helium was used as the carrier gas, and the oven temperature was programmed from 80 °C to 280 °C under controlled conditions. Samples were prepared by extracting the crude material with ethanol, and the obtained extract was analyzed over a mass range of 45-350 m/z. The chemical constituents were identified by comparing their mass spectra with NIST library databases.

2.2.6 Formulation Development

Purified water was taken in a clean beaker and, under continuous stirring, methyl paraben (as a preservative), glycerin, and propylene glycol were added and allowed to dissolve completely. Carbopol 940, serving as the gelling agent, was gradually incorporated into the aqueous phase with constant stirring to prevent lump formation and allowed to hydrate for 30-45 minutes. Separately, green tea extract was dissolved in a small volume of warm water and incorporated into the hydrated gel base. Tea tree oil, pre-mixed with a portion of propylene glycol for uniform dispersion, was added slowly. Sodium lauryl ethyl sulphate was then introduced to impart mild foaming properties. The pH of the formulation was carefully adjusted to 5.5-6.5 using triethanolamine (TEA), which also facilitated neutralization of Carbopol and conversion to a smooth, transparent gel. The final volume was adjusted to 100 g using purified water or rose water, and the mixture was thoroughly homogenized. The resulting herbal facewash gel was transferred into sterile containers for storage and labeling.

2.2.7 Optimization of Herbal Facewash by Box-Behnken Design: To develop an optimized herbal facewash formulation incorporating Green Tea Extract and Tea Tree Oil, a Box-Behnken Design (BBD) was employed using Design-Expert® software. This statistical method is well-suited for evaluating the quadratic effects of independent variables with a limited number of experiments, providing reliable optimization with three levels for each factor.

Table 2: Independent Variables and Their Levels in BBD

Variable	Level	
Independent variables	Low	High
X1: Green Tea (%)	1	3
X2: Tea Tree Oil (%)	1	2
X3: Carbopol 940 (%)	0.5	1.5
Coded Values	-1	+1
Dependent variables		
Y1= Viscosity (cps)		
Y2= Drug Content (%)		

2.3 Characterization of Herbal Facewash

2.3.1 Physical Appearance

The physical appearance of the facewash gel was examined visually for its color, clarity, consistency, and the presence of any particulate matter or air bubbles. This ensured uniformity and aesthetic appeal, which are essential for consumer acceptance.

2.3.2 Viscosity Determination

A Brookfield viscometer (MCR101, Rheoplus, Anton Paar India Pvt. Ltd.) fitted with an appropriate spindle (Spindle No. 3) was used to evaluate the viscosity of the nanoemulsion formulations at 50 rpm. The viscosity was measured at 25 ± 1°C and expressed in centipoises (cP). Three duplicates of

each determination were made, and the mean ± standard deviation was computed.

2.3.3 Drug Content
The drug content of green tea and tea tree oil in the facewash was determined using UV-Visible spectrophotometry. Accurately weighed 1 gram of formulation was dissolved in 100 mL of methanol, sonicated for 15 minutes, filtered, and analyzed at λmax specific to green tea (around 274 nm) and tea tree oil (220-230 nm). The drug content was calculated using a standard calibration curve and expressed as a percentage of the theoretical amount.

2.3.4 Spreadability
The spreadability of the facewash gel was assessed to determine ease of application. A fixed amount (1 g) of gel was placed between two horizontal glass slides, and a weight of 500 grams was placed on the top slide for 5 minutes. The diameter of the circular spread area was measured. The time taken for the slides to separate under the applied force was used to calculate spreadability using the formula:

$$\text{Spreadability} = \frac{\text{Weight (g)} \times \text{Distance (cm)}}{\text{Time (sec)}}$$

2.3.5 pH Determination
The pH of the formulations was determined by dispersing 1 gram of the gel in 10 mL of distilled water and allowing it to stand for 30 minutes with occasional stirring. The pH of this dispersion was then measured using a calibrated digital pH meter at room temperature. A pH range of 5.0 to 7.5 was considered ideal to ensure skin compatibility.

2.3.6 Foaming Test
To evaluate foaming ability and foam retention, 1 gram of the formulation was dissolved in 20 mL of distilled water in a 100 mL graduated cylinder. The mixture was shaken vigorously 10 times and the foam volume was measured immediately (initial foam volume). Subsequent measurements were recorded after 5 and 10 minutes to evaluate foam retention. A high foam retention percentage indicates longer-lasting cleansing action.

Foam retention (%) = (Foam Volume at 10 min / Initial Foam Volume) × 100

2.3.7 Swelling Index
The swelling index of the herbal facewash gel was determined by allowing 1 g of gel to absorb 10 mL distilled water for 24 hours at room temperature. The excess water was removed, and the swelling index was calculated from the weight gain to assess the gel's hydration capacity.

$$\text{Swelling Index (\%)} = \left(\frac{\text{Weight of swollen gel} - \text{Initial weight}}{\text{Initial weight}}\right) \times 100$$

2.3.8 Extrudability Test
The extrudability of the herbal facewash gel was evaluated by filling 20 g of gel into a collapsible tube and applying a 500 g weight via glass slides. The gel extruded from the nozzle within 30 seconds was collected and weighed. Extrudability was expressed as the weight of gel dispensed per unit time, indicating the formulation's consistency and ease of use.
Extrudability= Applied weight to extrude gel from tube (gm)/ Area (cm^2)

2.3.9 Checkpoint Analysis
Checkpoint analysis validates the predictive accuracy of formulation optimization models, such as the Box-Behnken Design. Formulations are prepared at selected optimal levels of key variables (e.g., green tea extract, tea tree oil, Carbopol 940) and evaluated for parameters like viscosity and drug content. Experimental results are compared with predicted values, and prediction errors are calculated. Low errors confirm the model's reliability, ensuring that the optimized formulation is statistically robust and practically effective.

2.4 Formulation and Evaluation of Optimized Batch of Facewash
Based on the results obtained through Box-Behnken Design optimization and checkpoint analysis, the most effective batch was identified and selected as the optimized formulation for herbal facewash gel. The optimized formulation consisted of Green Tea Extract, Tea Tree Oil, and Carbopol 940. These concentrations were chosen to achieve an ideal balance of drug content, stability, and other properties such as spreadability and appearance.

3. Results and Discussion
3.1 Pharmacognostic Studies
Macroscopic and Organoleptic Characteristics

Table 3: Organoleptic Description of *Camellia sinensis* (L.) leaves

Character	Fresh Leaves	Dry Leaves	Powder
Size	6-18 cm long, 2-6 cm wide	5.5-17.8 cm long and 1.7-5.8 cm	Fine powder
Shape	lanceolate or elongated-ovate	lanceolate or elongated-ovate	Particles
Color	Glossy dark green	Dark green	Dark green
Texture	Smooth	Hard	Smooth
Odour	Aromatic and Distinctive	Distinctive	Distinctive
Taste	Astringent	Astringent	Astringent

In microscopic characteristics studied transverse section, Stomatal number and powder microscopy of *Camellia sinensis* (L.) leaves.

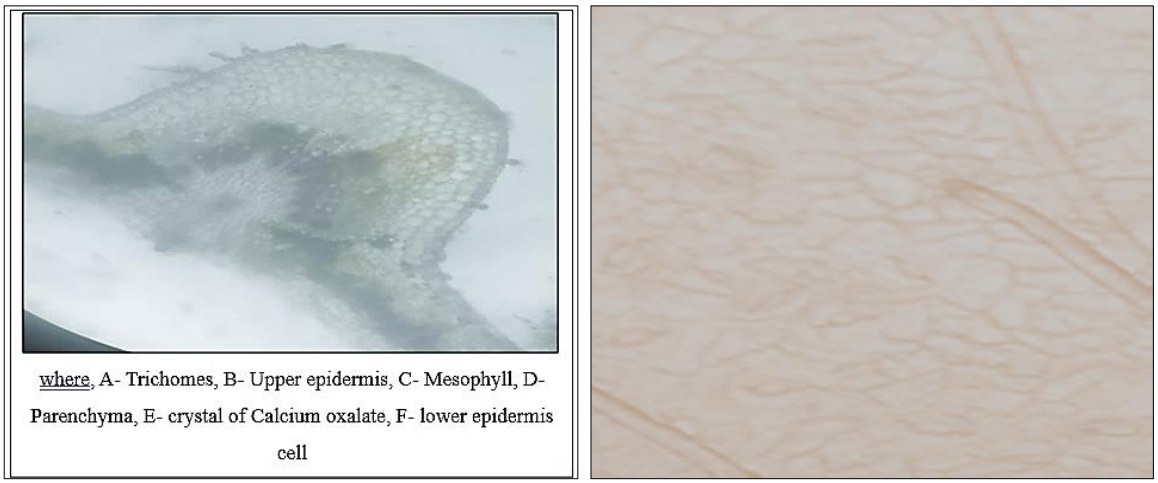
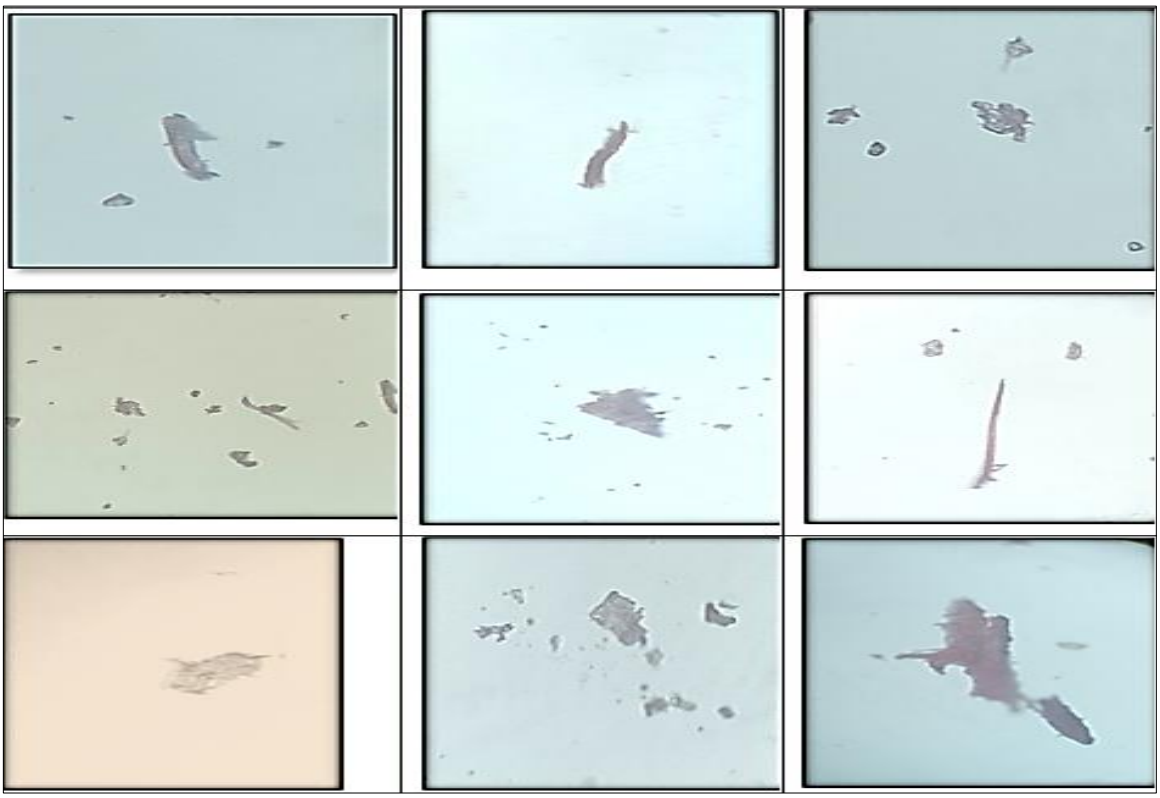


Fig 1: Transverse section observation of *Camellia sinensis* (L.) leaves by using compound microscope

Powder microscopy



a- Parenchymatous cell; b- Fiber, c- Calcium oxalate crystals, prism crystals; d- sclereid cell; e- Cork cell f- Unicellular trichome; g-Aleurone grains, h- Cells filled with mucilage, h- Thick-walled cell

Fig 2: Powder microscopy of *Camellia sinensis* (L.) leaves powder and also staining with different chemicals

3.1.1 Other physicochemical evaluation

The results of physiochemical evaluation of *Camellia sinensis* (L.) leaves like ash values, extractive values, foreign organic matter, loss on drying, swelling index, foaming index, bitterness values and Heavy metal determination are represented in the Tables 4-6.

Table 4: Determination of different Ash values

S. No.	Ash Value	Leaves (% w/w)
1	Total Ash	5.87±0.63%
2	Acid insoluble ash	0.15±0.02
3	Water soluble ash	1.04±0.13
4	Sulphated ash	0.66±0.04

Note: Data were expressed as mean± SEM of triplicates

Table 5: Determination of different extractive values

S. No.	Extractive Values	Leaves (% w/w)
1	Hot Extraction Cold Extraction	16.45±1.1 20.91±1.6
2	Alcohol soluble	27.88±3.9
3	Ether soluble	10.44±2.2

Note: Data were expressed as mean± SEM of triplicates

Table 6: Determination of other physicochemical parameters

S. No.	Tests	Leaves (% w/w)
1	Foreign organic matter	0.38 ± 0.21 %w/w
2	Loss on Drying	14.05%
3	Swelling index	1ml/g
4	Foaming index	Less than 100
5	Bitterness value	Bitter
6	Crude fiber content	27.44± 0.5 %w/w

Values: % w/w mean ± SEM; n=3

3.2 Extraction of Plant Material

The leaves of *Camellia sinensis* (L.) were subjected to successive Soxhlet extraction using methanol as the solvent at 60 °C, and the resulting extract was filtered and concentrated using a rotary vacuum evaporator. Separately, tea tree leaves were extracted by hydro-distillation using a Clevenger-type apparatus, wherein 200 g of coarsely powdered leaves were distilled with 500 mL of water for 3-4 hours. The obtained essential oil was separated, dried over anhydrous sodium sulfate, and stored in amber-colored vials under refrigerated conditions (4-8 °C) until further use.

The Soxhlet extraction of *Camellia sinensis* (L.) leaves using methanol at 60 °C yielded approximately 22 g extract from 100 g of powdered leaves, corresponding to a 22% yield, which is consistent with reported literature values. Hydro-distillation of *Melaleuca alternifolia* produced about 2.4 mL of essential oil with a 1.2% yield, falling within standard recovery ranges. The obtained oil was dried over anhydrous sodium sulfate and stored in amber vials at 4-8 °C to maintain stability.

3.2.1 Physicochemical Properties of Tea Tree Oil (*Melaleuca alternifolia*)

The extracted *Melaleuca alternifolia* oil physicochemical characteristics were observed including color, odor, taste, appearance, refractive index, boiling point and specific gravity. The results are shown in table 7.

Table 7: Physicochemical characters of *Melaleuca alternifolia* oil

S. No.	Oil Parameter	Value
1.	Color	Light Greenish
2.	Odor	Strong, characteristic, camphoraceous
3	Taste	Pungent and bitter
4	Appearance	Mobile, clear liquid
5.	Refractive index	1.475 - 1.482 (at 20°C)
6.	Boiling point	150-190°C (varies with composition)
7.	Optical Rotation	+5° to +15° (at 20°C)
8.	Specific Gravity	0.885 - 0.906 (at 20°C)

3.3 Preliminary Phytochemical Studies

Preliminary Phytochemical Studies of *Camellia sinensis* (L.) leaves results showed that the methanol extract have presence of alkaloids, saponins, phenolic and tannins, flavonoids, carbohydrates, proteins and amino acids. Numerous pharmacological actions were attributed to these secondary metabolites. These standardization parameters provided updated information *Camellia sinensis* (L.) plant.

Table 8: Preliminary Phytochemical Screening of *Camellia sinensis* (L.) leaves methanol extract

S. No.	Test	Test	Methanol Extract
1	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Hager's test	+
		Wagner's test	+
2	Carbohydrates	Molish test	+
		Benedict's test	+
		Fehling's test	+
3	Glycosides	Keller Killiani test	+
		Legal test	+

		Fluorescence test	+
4	Saponins	Frothing test	+
5	Flavonoids	Shinoda test	+
		Alkaline Reagent test	+
		Lead Acetate Test	+
		Ammonia test	+
6	Phenolic compound and tannins	Ferric chloride test	+
		Silver nitrate test	+
		Lead tetra acetic acid test	+
7	Fats	Acrolein Test	+
		Spot Test	+
		Translucent Spot Test	+
8	Sterols	Salkovaski Test	-
		Libermann Burchard's test	-
9	Protein and Amino acid	Biuret test	+
		Ninhydrin test	+

3.4 Compatibility Studies

FTIR Technique

The FTIR spectral analysis was conducted to evaluate possible interactions between the plant material and excipients. The results confirms that the drug remained chemically stable and retained its structural integrity within the system. Hence, FTIR studies supported the absence of undesirable interactions and validated the successful incorporation of the drug into the formulation matrix.

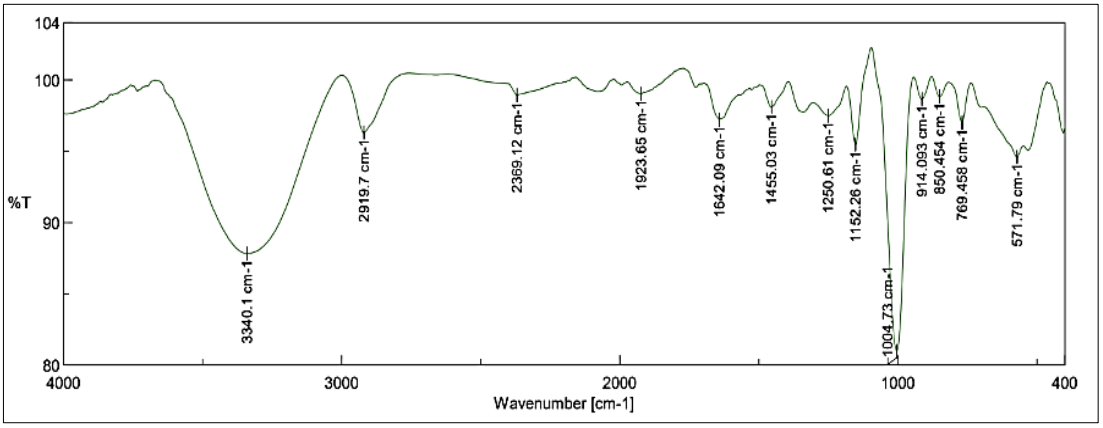


Fig 3: FTIR of Green Tea Extract

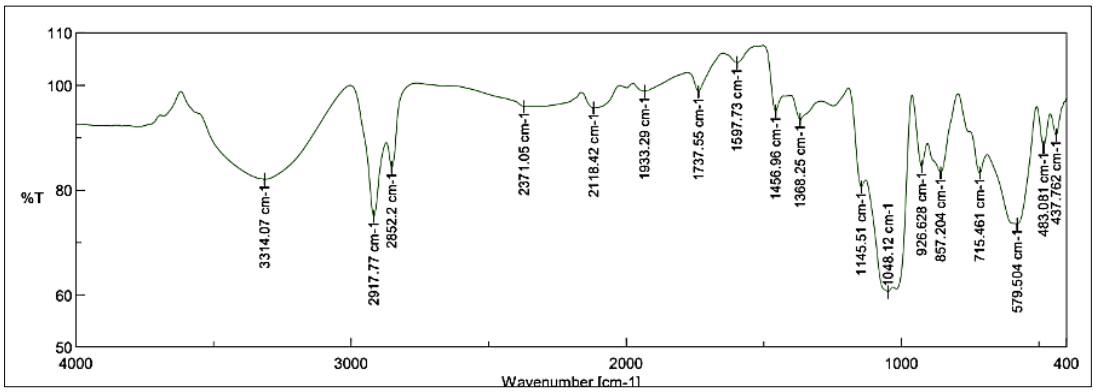


Fig 4: FTIR of Tea Tree Extract

Gas Chromatography- Mass spectroscopy

The GC chromatogram of compounds identified in *Camellia sinensis* (L.) leaves extracts are shown in figure 5. The name of the compounds identified are given in table 9.

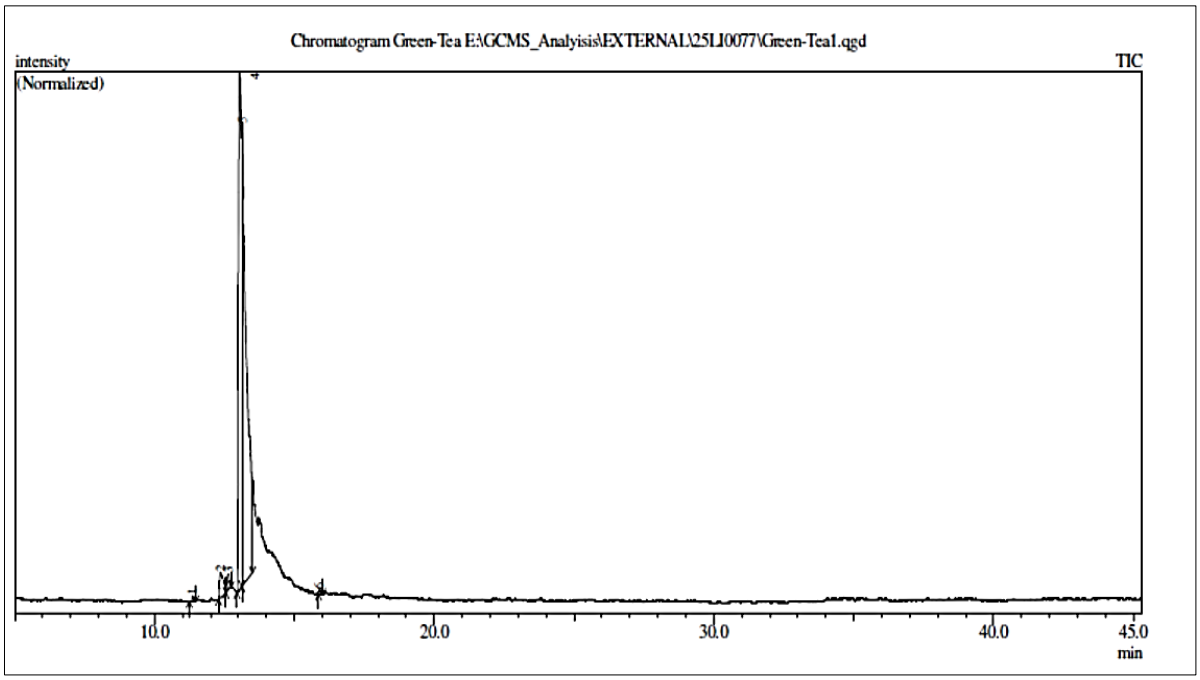


Fig 5: GC chromatogram of *Camellia sinensis* (L.) leaves extract

Table 9: Phytochemical compounds identified by GC-MS analysis of *Camellia sinensis* (L.) leaves extract

S. No.	Name of compound	RT	Peak area%	Molecular formula	Molecular weight
1	2-Methyl-5-formylfuran	6.178	0.54	$C_6H_6O_2$	110
2	2-Propanedioic acid	8.035	1.01	$C_3H_4O_4$	105
3	2-Pentadecanone	8.861	0.16	$C_{15}H_{30}O$	226
4	9,12,15-Octadecatrienoic acid-methyl ester	10.746	0.85	$C_{19}H_{34}O_2$	294
5	Decamethyl cyclo pentasiloxane	11.307	0.25	$C_{10}H_{30}O_5Si_5$	370
6	Catechin	11.823	10.43	$C_{15}H_{14}O_6$	290
7	Caffeine	13.062	15.83	$C_8H_{10}N_4O_2$	195
8	Myristic acid	14.804	1.15	$C_{14}H_{28}O_2$	228
9	Stearic acid methyl ester	15.694	0.17	$C_{19}H_{38}O_2$	280
10	Citronellyl butyrate	15.917	0.28	$C_{14}H_{26}O_2$	226
11	Heptadecanoic acid	17.290	1.03	$C_{17}H_{34}O_2$	271
12	Linoleic acid	17.832	0.81	$C_{18}H_{32}O_2$	276
13	Epicatechin-3-o-gallate	18.025	0.93	$C_{22}H_{18}O_{10}$	442
14	Docosanoic acid	18.807	2.75	$C_{22}H_{44}O_2$	340
15	Linolenic acid	19.554	3.24	$C_{18}H_{30}O_2$	280
16	Hexadecanoic acid-methyl ester	19.980	0.63	$C_{16}H_{32}O_2$	256
17	Heptadecanoic acid	20.961	0.12	C17H34O2	270
18	Palmitic acid	21.513	1.26	$C_{16}H_{32}O_2$	256
19	Oleic acid	22.075	1.93	$C_{18}H_{34}O_2$	282
20	4, 8,12,16-tetramethylheptadecan-4-olide	23.649	0.93	$C_{12}H_{40}O2$	324
21	Vitamin E	33.904	0.33	$C_{29}H_{50}O_2$	430
22	Oxirane-tetradecyl	34.126	0.27	$C_{16}H_{32}O$	240

The GC chromatogram of compounds identified in *Camellia sinensis* (L.) leaves extracts showed 22 compounds, some of these phytoconstituents are responsible of their pharmacological activities. Some bioactive phytoconstituents are catechin, caffeine, Epicatechin-3-gallate, vitamin E, citronellyl butyrate and oxirane-tetradecyl etc.

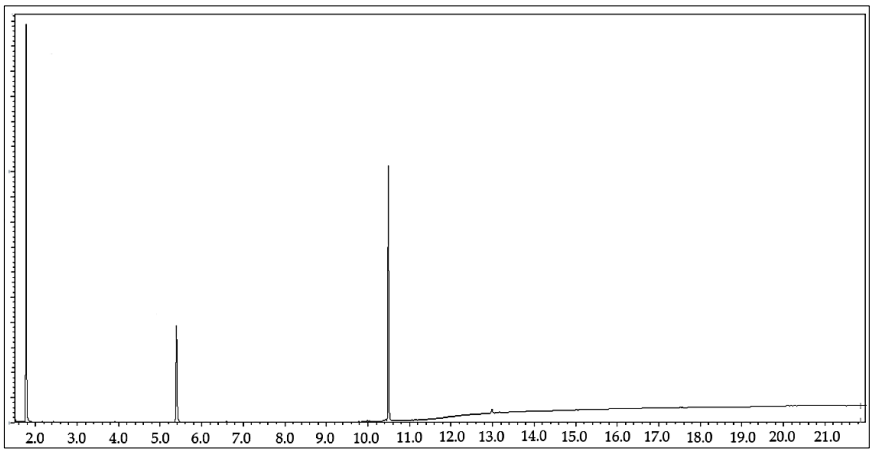


Fig 6: GC chromatogram of *Melaleuca alternifolia* essential oil

Table 10: Phytochemical compounds identified by GC-MS analysis of *Melaleuca alternifolia* essential oil

S. No.	Name of Compound	Retention Time (RT, min)	Peak Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	Terpinen-4-ol	17.8	41.2	$C_{10}H_{18}O$	154.25
2	γ-Terpinene	15.6	19.7	$C_{10}H_{16}$	136.23
3	α-Terpinene	16.2	6.3	$C_{10}H_{16}$	136.23
4	p-Cymene	14.8	8.9	$C_{10}H_{14}$	134.22
5	1,8-Cineole (Eucalyptol)	13.7	4.5	$C_{10}H_{18}O$	154.25
6	α-Terpineol	18.4	3.6	$C_{10}H_{18}O$	154.25
7	Limonene	13.4	3.2	$C_{10}H_{16}$	136.24
8	Sabinene	11.6	2.1	$C_{10}H_{16}$	136.23
9	α-Pinene	9.8	1.9	$C_{10}H_{16}$	136.23
10	δ-3-Carene	10.2	1.7	$C_{10}H_{16}$	136.23

Terpinen-4-ol is the primary active compound responsible for the antimicrobial and anti-inflammatory effects of tea tree oil. Compounds like γ-terpinene, α-terpinene, and p-cymene contribute to fragrance and synergistic bioactivity. Minor constituents like α-pinene, sabinene, and limonene enhance the antioxidant and penetration-enhancing properties of the oil.

3.5 Characterization of Heral Facewash

The prepared herbal facewash gel formulations were subjected to a comprehensive evaluation to assess their physicochemical properties, user acceptability, and biological efficacy. The evaluation was carried out using standard procedures as described below:

3.5.1 Viscosity Determination

The formulated herbal facewash gels showed acceptable and uniform physical appearance across all batches, with color variations from pale to dark green depending on extract concentration. Most formulations were clear with smooth consistency, while slight turbidity and thinner texture were observed in batches with lower Carbopol content. No particulate matter was detected, and minimal air entrapment was noted in a few batches. Viscosity analysis revealed values ranging from 4311 ± 22 to 6156 ± 24 cP, reflecting the effect of varying concentrations of extracts and Carbopol 940.

ANOVA for Quadratic Model

Table 11: Response 1: Viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.705E+06	9	4.117E+05	51.65	0.0002	significant
A-Green Tea	42340.50	1	42340.50	5.31	0.0694	
B-Tea Tree	4.876E+05	1	4.876E+05	61.16	0.0005	
C-Carbolpol 940	1.679E+06	1	1.679E+06	210.63	< 0.0001	
AB	3.266E+05	1	3.266E+05	40.97	0.0014	
AC	6642.25	1	6642.25	0.8332	0.4032	
BC	2.642E+05	1	2.642E+05	33.14	0.0022	
A^2	4.317E+05	1	4.317E+05	54.15	0.0007	
B^2	5.018E+05	1	5.018E+05	62.95	0.0005	
C^2	75680.10	1	75680.10	9.49	0.0274	
Residual	39857.92	5	7971.58			
Lack of Fit	17677.25	3	5892.42	0.5313	0.7046	not significant
Pure Error	22180.67	2	11090.33			
Cor Total	3.745E+06	14				

The statistical model was significant, with a Model F-value of 51.65 and only a 0.02% probability of occurring due to noise, indicating good model reliability. Significant model terms included B, C, AB, BC, and the quadratic terms A^2, B^2, and

C^2, while non-significant terms may be reduced to improve the model. The Lack of Fit was non-significant (F = 0.53), confirming an adequate fit of the model to the experimental data. The Predicted R^2 of 0.9112 is in reasonable agreement with the Adjusted R^2 of 0.9702; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio.

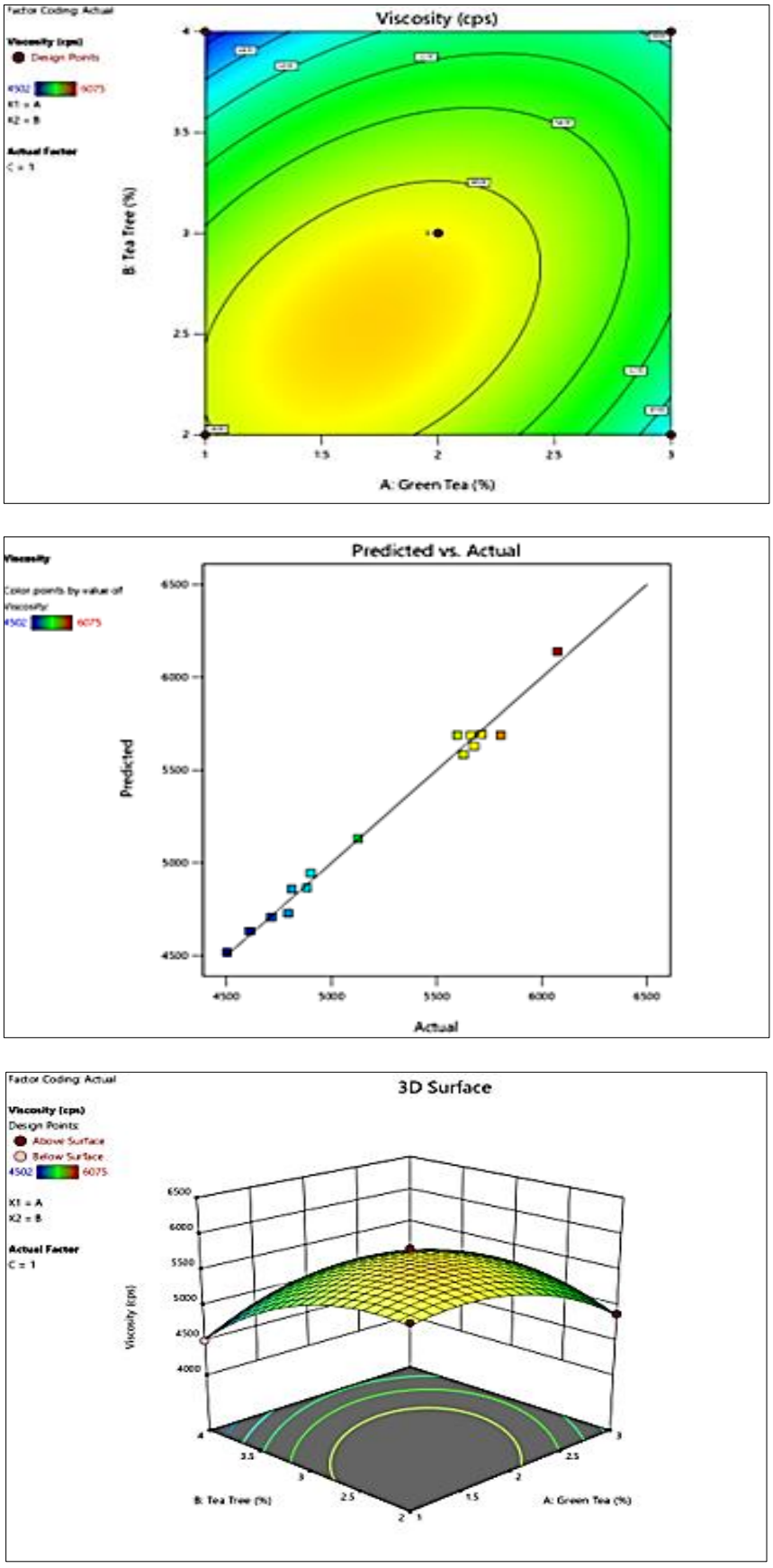
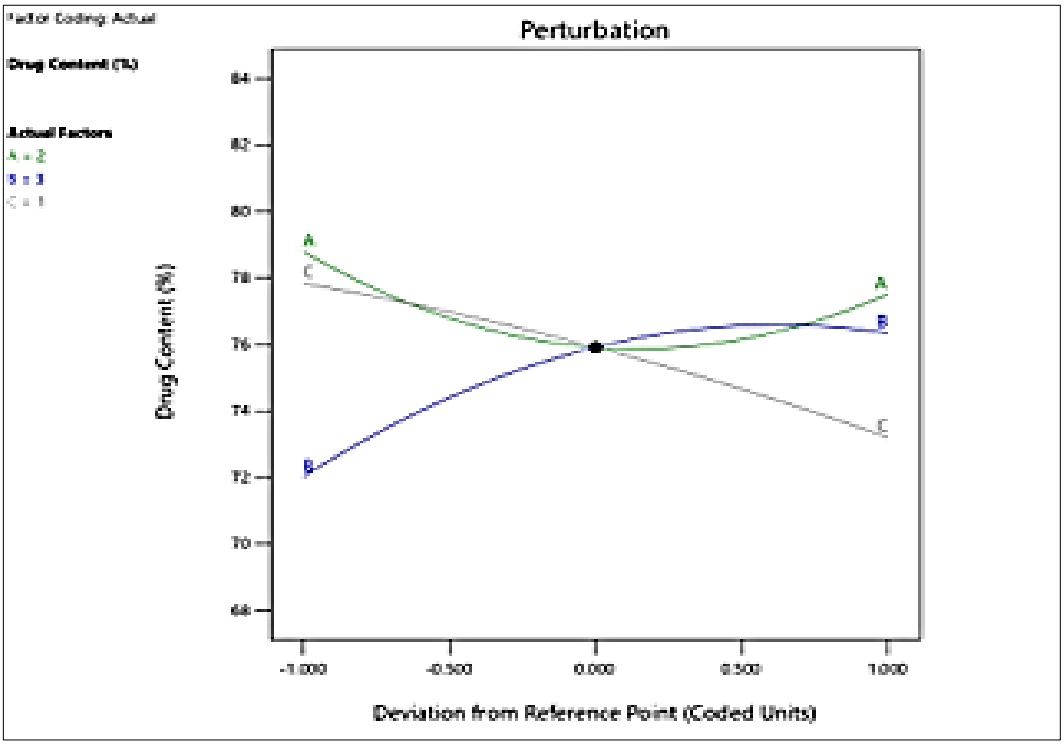
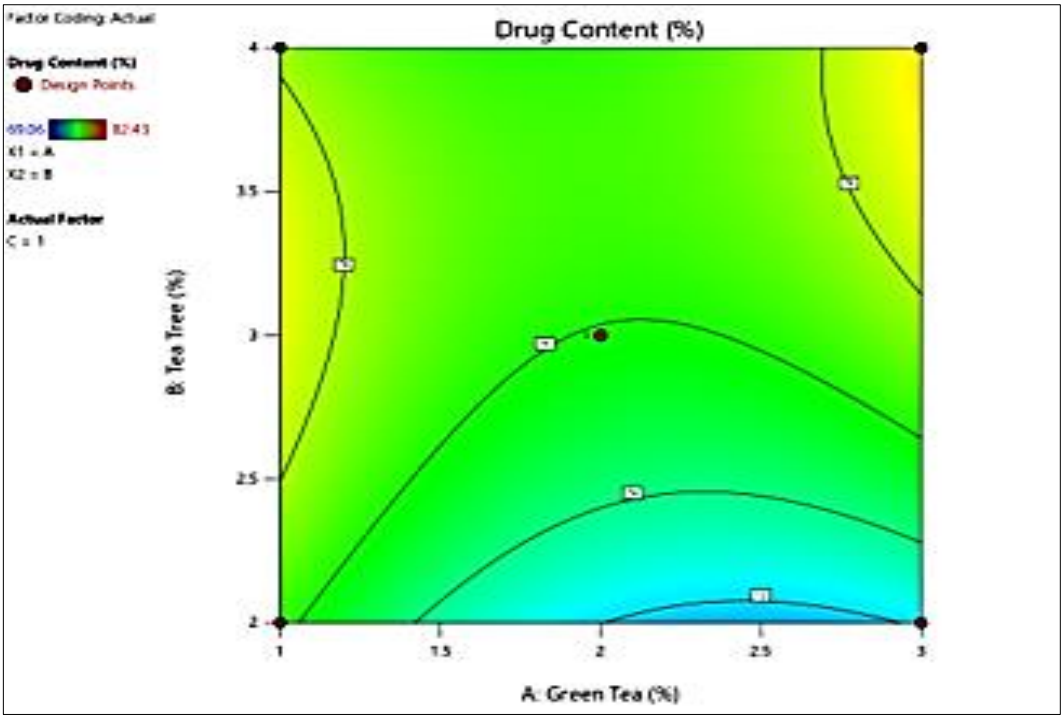


Fig 7: Plots obtained for Response 1

3.5.2 Drug Content Determination

The drug content of the formulations ranged from 69.06% to 82.43%, with F1, F2, and F15 showing the highest values, indicating better drug incorporation. Lower drug content in F6 and F9 may be due to formulation variables, though overall results confirmed acceptable and satisfactory drug distribution. The model was statistically significant with an F-value of 6.16 and a low probability (2.97%) of occurring due to noise. Model terms B, C, and BC were significant, while non-significant terms may be reduced to improve model efficiency. The Lack of Fit was non-significant (F = 2.25), indicating an adequate and reliable fit of the model to the experimental data. The fit statistics indicate good model adequacy with a low standard deviation (1.89), high R^2 (0.9173), acceptable coefficient of variation (2.49%), and adequate signal-to-noise ratio (Adeq Precision = 8.52), though the negative predicted R^2 suggests limited predictive capability.





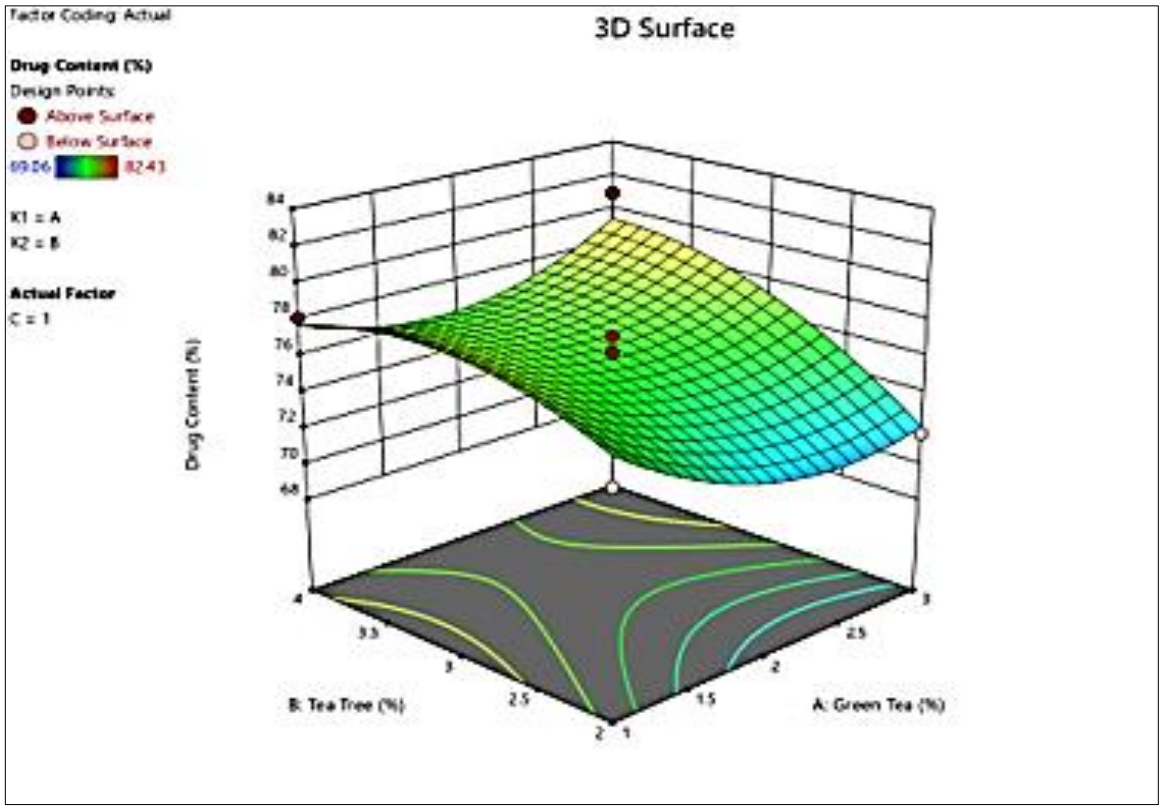


Fig 8: Plots Obtained for Response 2

3.5.3 Spreadability

Among the formulations, F12 exhibited the highest spreadability (19.66 ± 0.21 g·cm/s), indicating excellent ease of application and likely a lower viscosity, which enhances user experience. This was followed by F10 (18.61 ± 0.32 g·cm/s) and F11 (17.14 ± 0.09 g·cm/s), also demonstrating good spreadability. On the other hand, F7 (10.37 ± 0.26 g·cm/s) and F5 (10.51 ± 0.13 g·cm/s) showed the lowest spreadability, potentially due to higher viscosity or suboptimal gel structure, which may make them less favorable in terms of user comfort and application ease.

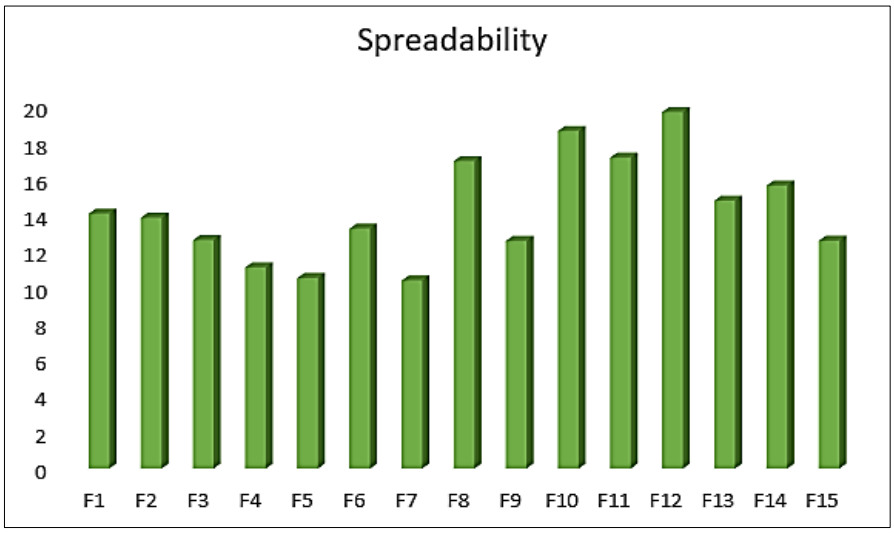


Fig 9: Graphical Representation of Spreadability Value of 15 Formulations

3.5.4 pH Determination

Regarding pH, all formulations were within the acceptable skin-friendly range of 6.1 to 6.8, which is ideal for maintaining skin's natural acid mantle and preventing irritation. F1 showed the lowest pH (6.1 ± 0.02), making it slightly more acidic and potentially suitable for oily or acne-prone skin. Conversely, F4, F5, and F9 had the highest pH values (6.8), remaining within acceptable limits and indicating balanced formulations safe for daily use.

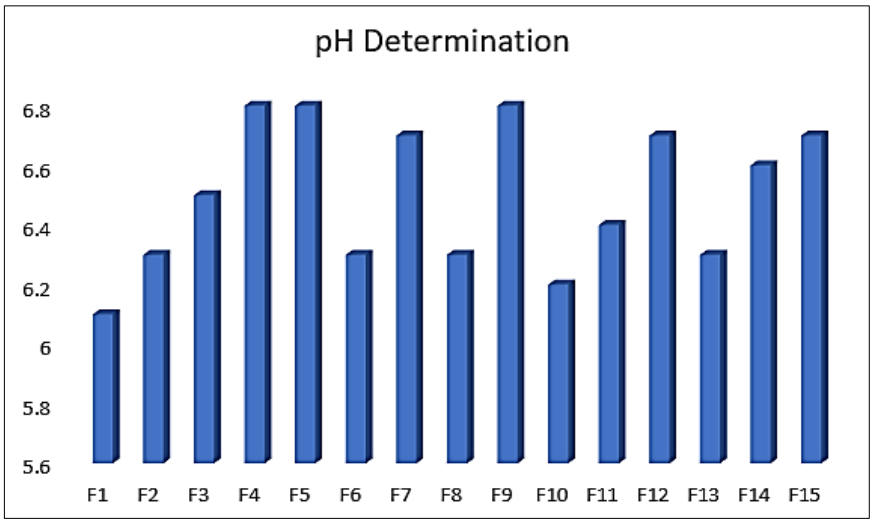


Fig 10: pH Determination of Prepared Batches (F1-F15)

3.5.5 Foaming Test: The foaming ability and foam retention data of the 15 formulated herbal facewashes indicate satisfactory performance across all batches. Initial foam volumes ranged from 40 mL (F5) to 53 mL (F12), suggesting a generally good foaming capacity attributable to the presence of surfactants and herbal ingredients. Foam retention after 10 minutes was consistently high, ranging from 81.6% (F13) to 89.6% (F4), indicating stable foam formation and lasting cleansing action. Formulations F2, F8, and F12 demonstrated superior initial foam volume and retention, highlighting their potential for enhanced user experience.

3.5.6 Swelling Index: The swelling index evaluation of the fifteen herbal facewash gel formulations (F1-F15) demonstrated satisfactory water absorption capacity across all batches, indicating good hydration potential and gel matrix integrity. The swelling index ranged from 10.12% (F5) to 13.86% (F11). Notably, formulations F11 (13.86%), F13 (13.64%), F10 (13.53%), and F14 (13.45%) showed the highest swelling indices, suggesting superior water-retaining capacity which may enhance skin adherence and prolonged application benefits. On the other hand, F5 (10.12%) and F7 (10.55%) exhibited the lowest swelling values, which could indicate relatively lower gel hydration or slightly less robust gelling matrices.

3.5.7 Extrudability Test: The extrudability results of the fifteen formulated herbal facewash batches reveal moderate to excellent performance in terms of ease of product dispensing. The extruded weight values ranged from 3.8 g to 4.7 g, while the corresponding extrudability values ranged from 19.39 to 23.98 g/cm². Among the formulations, F11 exhibited the highest extrudability (23.98 g/cm²), indicating superior ease of extrusion, followed by F6 (23.47 g/cm²) and F2 and F10 (22.96 g/cm² each). On the other hand, F5 showed the lowest extrudability (19.39 g/cm²), suggesting relatively higher consistency or stiffness, which may affect dispensing ease. The formulations such as F3, F7, and F13 showed moderate extrudability ranging between 21.9 to 22.5 g/cm², reflecting acceptable user-friendly characteristics.

3.5.8 Checkpoint Analysis: The desirability ramp illustrates the optimized levels of independent variables, including green tea extract, tea tree oil, and Carbopol 940, selected to achieve the targeted formulation characteristics. The optimized formulation predicted a viscosity of approximately 5253.96 cP and drug content of 75.98%, falling within the desired range. An overall desirability value of 1.000 confirms the robustness and suitability of the optimized formulation.
The contour plots demonstrate the combined effect of green tea extract and tea tree oil on desirability, viscosity, and drug content at a fixed Carbopol 940 concentration of 0.647%. An optimal formulation at approximately 2.17% green tea and 2.78% tea tree oil achieved maximum desirability (1.0), ideal viscosity (~5253.96 cps), and satisfactory drug content (~75.98%). Overall, the plots confirm a well-balanced and optimized herbal facewash formulation with desirable physicochemical properties.

3.6 Evaluation of Optimized Batch of Herbal Facewash
The evaluation of the optimized batch of herbal facewash demonstrated favorable physicochemical and performance characteristics suitable for topical cosmetic application. The formulation exhibited a viscosity of 5242 cps, indicating an ideal gel consistency that is neither too runny nor too stiff, ensuring ease of application and user comfort. The drug content was 78.2%, suggesting effective incorporation of the active constituents (green tea and tea tree oil) into the formulation. The physical appearance was aesthetically appealing, showing a light green, smooth, and transparent gel that was free from grittiness and air bubbles, highlighting good formulation stability and uniformity. In terms of spreadability, the gel showed a value of 14.77 ± 0.38 g·cm/s, reflecting a smooth application profile over the skin. The pH of 6.3 ± 0.41 lies within the ideal skin-compatible range (5.0-7.0), confirming its mildness and suitability for routine facial use. The extrudability was measured at 4.3 g/30 sec with a force value of 21.94 g/cm², indicating optimal ease of dispensing from the container without excessive force or leakage. The absence of grittiness further confirms the fine texture and homogeneity of the formulation. Lastly, the swelling index of 1.83 ± 0.07% reflects a moderate water uptake capacity, ensuring balanced hydration without excessive dilution during use. Overall, the optimized batch shows promising features for consumer acceptability and skincare benefits.

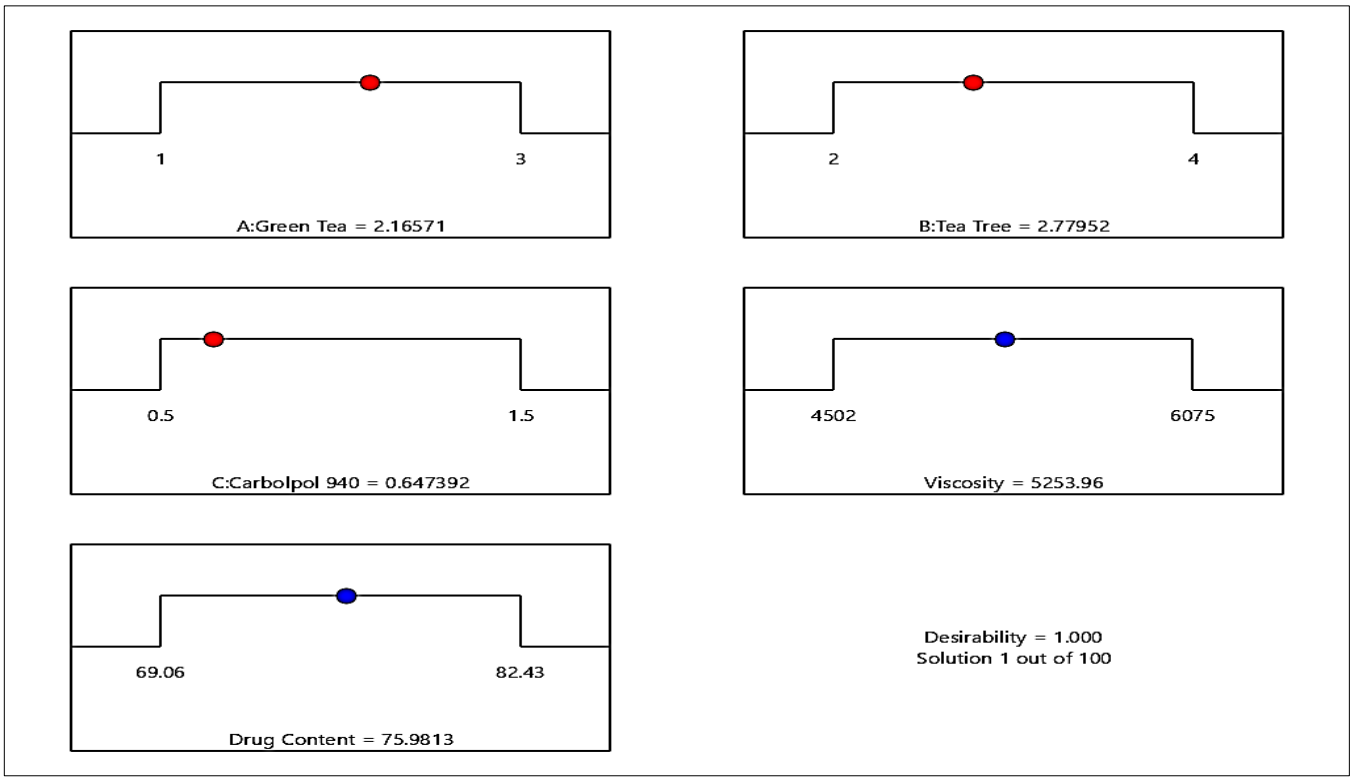


Fig 11: Ramp of desirability for the optimization process. The desired ramp depicts the levels of variables and anticipated values for the dependent variables of the optimized formulation

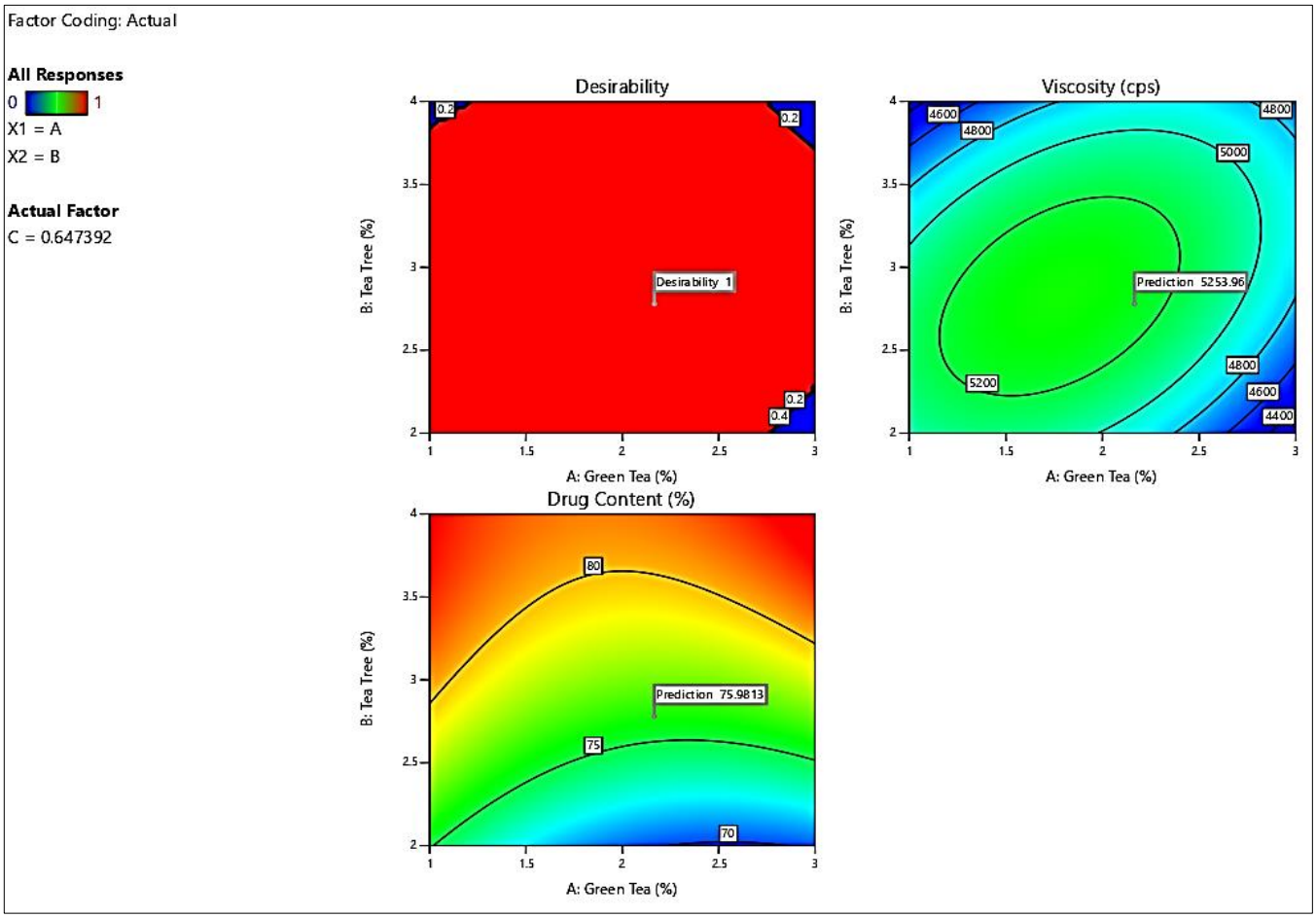


Fig 12: Contour graph of predicted responses and desirability

Conclusion

The present investigation successfully demonstrated the formulation, optimization, and evaluation of a novel herbal facewash containing *Camellia sinensis* extract and *Melaleuca alternifolia* essential oil. Comprehensive pharmacognostic and phytochemical studies confirmed the authenticity, purity, and presence of bioactive constituents in the selected plant materials. GC-MS analysis further validated the presence of key phytoconstituents such as catechins, caffeine, vitamin E, and terpinen-4-ol, which contribute to antioxidant and antimicrobial activities. The herbal facewash formulation was systematically optimized using Box-Behnken Design, resulting in an optimized batch with desirable viscosity, satisfactory drug content, skin-compatible pH, good spreadability, stable foaming properties, and acceptable extrudability. FT-IR studies confirmed the absence of chemical incompatibility between active ingredients and excipients, indicating formulation stability. Overall, the optimized herbal facewash formulation exhibited excellent physicochemical and performance characteristics suitable for routine cosmetic use. The synergistic combination of green tea extract and tea tree oil offers enhanced cleansing, antimicrobial protection, and skin-soothing benefits. The developed formulation can be considered a safe, effective, and promising herbal alternative to conventional synthetic facewash products and has potential for further clinical and commercial development.

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