Preparation And Evaluation of Herbal Cosmetaceuticals from Methanolic Extract of Aegle Marmelos Fruit

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Abstract—**The present study aimed to prepare and evaluate an herbal cosmeceutical face serum containing methanolic extract of Aegle marmelos fruit (AMME) and to standardize the formulation using a validated High-Performance Thin-Layer Chromatography (HPTLC) method. The fruits of Aegle marmelos were collected, authenticated, and subjected to physicochemical evaluation according to WHO and Indian Pharmacopoeia guidelines. The methanolic extract was prepared using Soxhlet extraction, yielding 22.85% w/w of a reddish-brown semisolid extract. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, coumarins, steroids, and proteins. TLC analysis confirmed the presence of marmelosin with an Rf value of 0.46. Herbal face serum formulations were prepared and evaluated for physicochemical parameters including pH, viscosity, and spreadability. Among the prepared formulations, batch B2 was selected as the optimized formulation due to its desirable physicochemical characteristics and skin-compatible pH. Quantitative estimation of marmelosin in the extract and optimized serum formulation was performed using a validated HPTLC method. The marmelosin content was found to be 11.94% w/w in AMME and 11.80% w/w in the face serum formulation. The HPTLC method demonstrated satisfactory linearity (R^2 = 0.9499), precision, accuracy, robustness, sensitivity, and specificity in accordance with ICH guidelines. The study concluded that Aegle marmelos fruit extract possesses significant potential as a natural cosmeceutical ingredient and can be successfully incorporated into herbal face serum formulations for skincare applications.**

Index Terms—**Aegle marmelos, Herbal Cosmeceuticals, Face Serum, Marmelosin, HPTLC, Phytochemical Screening, Methanolic Extract, Herbal Skincare, Cosmeceutical Formulation, Quality Control.**

I. INTRODUCTION

The global cosmetic industry has witnessed a remarkable transformation in recent years with an increasing shift toward natural and herbal products. Consumers are becoming more conscious of the ingredients used in skincare formulations and are actively seeking safer, eco-friendly, and plant-based alternatives to synthetic cosmetics. This growing demand has led to the emergence of cosmeceuticals, a category of products that combines the aesthetic benefits of cosmetics with the therapeutic advantages of pharmaceutical ingredients. Herbal cosmeceuticals have gained significant attention due to their ability to provide skin nourishment, protection, and rejuvenation while minimizing adverse effects commonly associated with synthetic chemicals. The incorporation of medicinal plant extracts into cosmetic formulations has therefore become an important area of research and development.[1]

Skin is the largest organ of the human body and serves as the primary protective barrier against environmental stressors such as ultraviolet radiation, pollution, microbial infections, and oxidative damage. Continuous exposure to these factors accelerates skin aging and leads to various dermatological conditions including dryness, wrinkles, hyperpigmentation, inflammation, and loss of elasticity. Oxidative stress caused by the excessive generation of reactive oxygen species (ROS) is considered one of the major contributors to premature skin aging. Antioxidants derived from natural sources have demonstrated significant potential in neutralizing free radicals and protecting skin cells from oxidative damage. Consequently, the development of herbal skincare products enriched with natural antioxidants has

become a promising strategy for maintaining healthy and youthful skin.[2]

Medicinal plants represent an abundant source of bioactive compounds such as flavonoids, phenolic acids, tannins, alkaloids, coumarins, terpenoids, and glycosides. These phytoconstituents exhibit a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial, wound-healing, photoprotective, and anti-aging effects. The utilization of plant-derived compounds in cosmetic formulations not only enhances product efficacy but also improves consumer acceptance due to their natural origin. Scientific validation of traditional medicinal plants is therefore essential for the development of effective herbal cosmeceutical products.[3]

Aegle marmelos (L.) Corrêa, commonly known as Bael, belongs to the family Rutaceae and is widely distributed throughout India and Southeast Asia. The plant holds a prominent place in traditional systems of medicine such as Ayurveda, where various parts including leaves, fruits, roots, and bark are used for the treatment of numerous ailments. The fruit of Aegle marmelos is particularly rich in biologically active compounds including marmelosin, aegeline, skimmianine, coumarins, flavonoids, tannins, phenolic compounds, and essential nutrients. These constituents have been reported to possess significant antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and wound-healing activities. Such pharmacological properties make Aegle marmelos fruit a promising candidate for incorporation into cosmetic and dermatological preparations.[4, 5]

Among the various phytoconstituents present in Aegle marmelos, marmelosin has attracted considerable scientific interest due to its potent biological activities. Marmelosin exhibits antioxidant and antimicrobial properties that may contribute to skin protection and maintenance. The presence of phenolic compounds and flavonoids further enhances the free radical scavenging ability of the fruit extract, thereby helping to prevent oxidative damage to skin cells. Additionally, the anti-inflammatory activity of the extract may reduce skin irritation and support the healing of damaged tissues. These characteristics suggest that Aegle marmelos fruit extract could serve as a multifunctional ingredient in herbal skincare formulations.[6]

Face serums have become increasingly popular in modern skincare because of their lightweight texture, rapid absorption, and ability to deliver concentrated active ingredients directly to the skin. Unlike conventional creams, serums contain higher concentrations of bioactive compounds and are designed to penetrate deeper layers of the skin, resulting in enhanced efficacy. Incorporation of herbal extracts into serum formulations offers a convenient and effective approach for delivering natural antioxidants and skin-protective agents. However, successful formulation of herbal serums requires careful optimization of ingredients and comprehensive evaluation of physicochemical characteristics such as pH, viscosity, spreadability, stability, and active constituent content.

Although Aegle marmelos has been extensively studied for its medicinal properties, limited research has focused on the development and evaluation of cosmeceutical formulations utilizing its fruit extract. Furthermore, scientific data regarding the standardization and quantification of marker compounds such as marmelosin in topical cosmetic preparations remain scarce. Therefore, there is a need to develop a stable and effective herbal face serum containing methanolic extract of Aegle marmelos fruit and to evaluate its quality using validated analytical techniques.[7]

In the present study, methanolic extract of Aegle marmelos fruit was prepared and subjected to phytochemical screening and chromatographic fingerprinting. An herbal face serum was formulated using the extract and evaluated for important physicochemical parameters including pH, viscosity, and spreadability. Furthermore, the content of marmelosin in both the extract and the developed formulation was estimated using a validated HPTLC method. The study aims to establish the potential of Aegle marmelos fruit as a valuable natural ingredient for the development of herbal cosmeceutical products and to provide scientific evidence supporting its application in modern skincare formulations.[8, 9]

II. MATERIALS AND METHODS:

Collection and Authentication of Plant Material

Fresh fruits of Aegle marmelos (Linn.) were collected from local farms in Malegaon, Maharashtra, India. Herbarium specimens were prepared by pressing,

mounting, labeling, and preserving the samples according to standard botanical procedures. A voucher specimen was deposited for future reference and authentication.

Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Methanol, petroleum ether, ethyl acetate, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, ferric chloride, lead acetate, sodium hydroxide, hydrochloric acid, sulfuric acid, ninhydrin reagent, Millon's reagent, vanillin, and marmelosin standard were procured from certified chemical suppliers and used without further purification.

Preparation of Fruit Powder

The collected fruits were thoroughly washed to remove adhering dirt and impurities. The fruit pulp was separated from the pericarp, cut into small pieces, and shade-dried for 3–4 days at room temperature. The dried material was coarsely powdered using a mechanical grinder and stored in airtight containers until further use.[10,11]

Physicochemical Evaluation of Fruit Powder

The powdered fruit material was evaluated for various physicochemical parameters according to World Health Organization (WHO) guidelines and the Indian Pharmacopoeia (IP, 2014). Parameters such as foreign organic matter, loss on drying, total ash, acid-insoluble ash, water-soluble ash, and extractive values (water-soluble and alcohol-soluble) were determined to assess the quality, purity, and identity of the crude drug.[12,13]

Preparation of Methanolic Extract

The dried fruit powder was subjected to Soxhlet extraction using methanol as the extraction solvent. Approximately 100 g of coarsely powdered material was placed in a Soxhlet apparatus and extracted continuously until complete exhaustion of the plant material. The obtained extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extract was further dried to obtain a solid mass, designated as Aegle marmelos Methanolic Extract (AMME), and stored in a desiccator for further studies.[14-17]

Determination of Percentage Yield

The percentage yield of the methanolic extract was calculated by comparing the weight of the dried extract with the initial weight of the powdered fruit material used for extraction.[18]

Preliminary Phytochemical Screening

The methanolic extract of Aegle marmelos fruit (AMME) was subjected to preliminary phytochemical screening using standard qualitative chemical tests to identify the presence of major bioactive constituents. Alkaloids were detected using Dragendorff's, Mayer's, Wagner's, and Hager's tests. Flavonoids were identified by alkaline reagent, Shinoda, and Zinc-HCl tests. Steroids were evaluated using Salkowski and Liebermann–Burchard reactions, while proteins were assessed by Ninhydrin and Millon's tests. Tannins were detected using ferric chloride and lead acetate tests. The presence of saponins was confirmed by the foam formation test, coumarins by sodium hydroxide and ferric chloride tests, and glycosides by Borntrager's and Legal's tests. The appearance of characteristic color changes or precipitates was considered indicative of the respective phytoconstituents.[19,21]

Thin Layer Chromatography (TLC) Analysis

Preliminary thin-layer chromatography (TLC) analysis of AMME was carried out to evaluate the phytochemical profile of the extract. TLC plates coated with silica gel G were used as the stationary phase, while a mixture of petroleum ether and ethyl acetate (5:5 v/v) served as the mobile phase. The methanolic extract and standard marmelosin solution were separately dissolved in methanol and applied onto the TLC plates. After chromatographic development, the plates were dried and sprayed with vanillin–sulphuric acid reagent, followed by heating at 105°C for 5–10 min to visualize the separated components. The chromatograms were examined under UV light at 254 nm and 366 nm, as well as under daylight. The retention factor (Rf) values and color characteristics of the resolved spots were recorded and compared with the standard marker compound for phytochemical identification.[22-24]

Formulation and Evaluation of Facial Serum

The facial serum was formulated using a carefully selected blend of ingredients aimed at providing

hydration, nourishment, stability, and preservation. Key emollients like almond oil (2–4%) and olive oil (5–9%) were included for deep moisturization and skin barrier support. Aloe vera (15%) promoted collagen production and skin healing, while glycerine (25–30%) acted as a strong humectant to retain moisture. Emulsifiers such as Tween 20 and Span 80 (1–10% each) ensured a uniform mixture of oil and water phases. Glyceryl monostearate (0.2%) improved spreadability and moisture retention. Preservatives like methylparaben (0.5–1%) and antioxidants like BHT (0.2–1%) protected the formulation from microbial growth and oxidation. Water served as the base solvent, and a small quantity of perfume was added for a pleasant fragrance. The combination of these ingredients resulted in a stable, effective, and user-friendly facial serum.[25-28]

Formulation of Herbal Face Serum

The herbal face serum was formulated using the methanolic extract of Aegle marmelos fruit (AMME) by employing a factorial design approach. Five different formulations (B1–B5) were prepared by varying the concentrations of olive oil, almond oil, and glycerine while maintaining constant amounts of extract, aloe vera gel, glyceryl monostearate, and methyl paraben. Olive oil and almond oil were incorporated as emollients, glyceryl monostearate as an emulsifying agent, glycerine as a humectant, aloe vera as a moisturizing agent, and methyl paraben as a preservative. Distilled water was added to obtain a final volume of 30 mL for each formulation.[29-30]

Table 1: Formulation Table (Batch wise)

Ingredients	B1	B2	B3	B4	B5
Oil Phase					
Olive oil (gm)	2.7	1.5	2.4	2.1	1.8
Almond oil (gm)	1.2	1.0	0.8	0.6	0.9
Glyceryl monostearate (gm)	1.36	1.36	1.36	1.36	1.36
Span 80 (ml)	q.s.	q.s.	q.s.	q.s.	q.s.
Perfume (ml)	q.s.	q.s.	q.s.	q.s.	q.s.
Aqueous Phase					
Extract (gm)	1.5	1.5	1.5	1.5	1.5
Aloe vera (gm)	4.5	4.5	4.5	4.5	4.5
Methyl paraben (gm)	0.3	0.3	0.3	0.3	0.3
Glycerine (gm)	9	8	6	7	7.5
Tween 20 (ml)	q.s.	q.s.	q.s.	q.s.	q.s.
Water (ml)	q.s. 30ml	q.s. 30ml	q.s. 30ml	q.s. 30ml	q.s. 30ml

Preparation of Face Serum

The oil phase consisting of olive oil, almond oil, glyceryl monostearate, span 80, and perfume was accurately weighed and heated to 75 ± 2°C in a water bath. Simultaneously, the aqueous phase containing AMME, aloe vera gel, glycerine, methyl paraben, Tween 20, and distilled water was heated to the same temperature. The aqueous phase was slowly added to the oil phase with continuous stirring to form an emulsion. Stirring was continued for 1 h at 75°C to ensure uniform mixing and stabilization of the formulation. The prepared serum was allowed to cool to room temperature, and the final volume was adjusted with distilled water. The formulations were then stored in suitable containers for further evaluation.[31]

Evaluation of Face Serum Formulation[32-36]

A. Viscosity

The viscosity of the prepared face serum formulations was determined using a Brookfield viscometer equipped with spindle No. 62LV at 100 rpm and maintained at 30°C. Approximately 50 mL of serum was used for each measurement, and the viscosity values were recorded in centipoise (cP).

B. Spreadability

Spreadability was determined by placing 1 mL of serum between two glass plates and applying a specified weight. The diameter of the spread formulation was measured, and spreadability was calculated using standard procedures. This test was performed to evaluate the ease of application of the serum on the skin.

C. pH Determination

The pH of the serum formulations was measured using a calibrated digital pH meter. The electrode was immersed in the formulation, and the pH was recorded after stabilization of the reading. Measurements were performed in triplicate, and the mean value was reported.

D. Optimization of Formulation

All prepared batches (B1–B5) were evaluated for viscosity, pH, and spreadability. Based on the obtained results, the optimized formulation was selected by considering acceptable physicochemical characteristics, stability, and ease of application. Among the tested formulations, batch B2 exhibited the most desirable properties and was therefore selected as the optimized herbal face serum formulation for further studies.

E. Estimation of Marmelosin by HPTLC

The content of marmelosin in the methanolic extract of Aegle marmelos fruit (AMME) and the optimized face serum formulation (AMME-F) was determined using a validated High-Performance Thin-Layer Chromatography (HPTLC) method. Prior to validation, chromatographic conditions were optimized to obtain suitable separation and fingerprint profiles of the extract and formulation. Marmelosin was used as the reference standard. Specificity was confirmed by comparing the retention factor (Rf) values and spectral characteristics of the extract and formulation with those of the standard compound.

F. Sample Preparation

For extract analysis, 10 mg of AMME was accurately weighed and dissolved in 1 mL of methanol. For standard preparation, 0.1 mg of marmelosin was dissolved in methanol. For formulation analysis, 10 mL of face serum was dispersed in water and extracted with ethyl acetate. The ethyl acetate layer was concentrated to 1 mL and used for HPTLC analysis.

G. HPTLC Chromatographic Conditions

Chromatographic separation was performed on precoated silica gel 60 F254 HPTLC plates using petroleum ether:ethyl acetate (5:5 v/v) as the mobile phase. Sample application was carried out using a CAMAG Linomat applicator. After development, the plates were derivatized with vanillin–sulphuric acid reagent and scanned densitometrically at 310 nm. Fingerprinting and quantification were performed using CAMAG software.

Table 2: HPTLC Chromatographic Conditions for Marmelosin Estimation

Sr. No.	Parameter	Condition
1	Stationary Phase	Pre-coated Silica Gel 60 F254
2	HPTLC Plate Size	20 × 10 cm, 0.2 mm thickness
3	Mobile Phase	Petroleum Ether: Ethyl Acetate (5:5 v/v)
4	Spraying Reagent	Vanillin–Sulphuric Acid Reagent
5	Chamber Saturation Time	15 min
6	Detection Wavelength	310 nm
7	Light Source	Deuterium Lamp
8	Slit Dimension	6 × 0.45 mm
9	Scanning Speed	20 mm/s
10	Band Length	8 mm
11	Solvent Migration Distance	8 cm
12	Temperature	25 ± 2°C
13	Syringe Volume	100 µL
14	Spray Gas	Nitrogen Gas
15	Application Rate	0.2 µL/s
16	Sample Applicator	CAMAG Linomat 5
17	Scanner	CAMAG TLC Scanner
18	Documentation System	REPROSTAR 5

H. Method Validation

The developed HPTLC method was validated according to ICH guidelines for linearity, specificity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). Linearity was evaluated over a concentration range of 200–1200 µg/band. Precision was assessed through intra-day and inter-day studies, while accuracy was determined by recovery studies at 80%, 100%, and 120% levels. Robustness was evaluated by introducing deliberate changes in chromatographic parameters including slit width, wavelength, scan speed, and saturation time.

I. Statistical Analysis

All experimental studies were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The obtained data were analyzed using appropriate statistical methods to ensure reliability and reproducibility of the results.

III. RESULTS AND DISCUSSION

Pharmacognostic Evaluation of Aegle marmelos Fruit

The pharmacognostic evaluation of Aegle marmelos fruit was performed to establish its identity and quality prior to extraction and formulation development. The fruit exhibited characteristic morphological features including a round to slightly pear-shaped or oblong appearance with a diameter ranging from 5–25 cm. The outer shell was hard, thick, woody, and grey in color, while the inner pulp was yellow and aromatic. The fruit possessed a characteristic rose-like odor and a sweet marmalade-like taste. These observations were consistent with the standard descriptions reported for A. marmelos fruits and confirmed the authenticity of the collected plant material. Such pharmacognostic characteristics serve as important diagnostic parameters for identification and quality assessment of crude herbal materials.

Table 3: Pharmacognostic Characteristics of Aegle marmelos Fruit

Sr. No.	Parameter	Observation
1	Shape	Round, slightly pear-shaped or oblong
2	Size	5–25 cm diameter
3	Texture	Hard thick shell, woody
4	Colour	Woody grey outer shell with yellow pulp
5	Odour	Characteristic, rose-like
6	Taste	Marmalade-like

Physicochemical Evaluation of Fruit Powder

Physicochemical parameters were evaluated according to WHO guidelines and Indian Pharmacopoeia standards to determine the quality, purity, and stability of the powdered fruit material. The powder showed no detectable foreign organic matter, indicating its purity. The ethanol-soluble and water-soluble extractive values were found to be 18% and 30%, respectively, demonstrating the presence of appreciable amounts of extractable phytoconstituents. Total ash content (1.65%) and acid-insoluble ash (0.9%) were within pharmacopeial limits, suggesting minimal contamination with inorganic materials. The loss on drying was recorded as 7.5%, indicating acceptable moisture content and reduced susceptibility to microbial growth during storage. Overall, all physicochemical parameters were found to comply with WHO and IP specifications, confirming the suitability of the fruit powder for further extraction and formulation studies.

Table 4: Physicochemical Parameters of Aegle marmelos Fruit Powder

Sr. No.	Parameter	Result (%)	Standard Limit
1	Foreign Organic Matter	0	< 1.0%
2	Ethanol Soluble Extractive	18	10–20% w/w
3	Water Soluble Extractive	30	30–45% w/w
4	Total Ash	1.65	< 5%
5	Acid Insoluble Ash	0.9	< 1%
6	Water Soluble Ash	1.05	—
7	Loss on Drying	7.5	< 8%

Extraction of Aegle marmelos Fruit Pulp

Methanolic extraction of Aegle marmelos fruit pulp was carried out using the Soxhlet extraction method. The extraction process yielded a reddish-brown semisolid extract designated as Aegle marmelos Methanolic Extract (AMME). The percentage yield obtained was 22.85% w/w, indicating efficient extraction of methanol-soluble bioactive constituents. The comparatively high yield may be attributed to the presence of coumarins, flavonoids, phenolics, tannins, and other polar phytoconstituents known to be readily extracted by methanol. The semisolid consistency of the extract facilitated its incorporation into the face serum formulation.

Table 5: Extraction Yield of Aegle marmelos Fruit Pulp

Solvent	Extract Code	Colour	Yield (% w/w)	Consistency
Methanol	AMME	Reddish Brown	22.85	Semisolid

Preliminary Phytochemical Screening

Preliminary phytochemical screening of AMME revealed the presence of several important classes of secondary metabolites. Alkaloids were confirmed by

Dragendorff's, Mayer's, and Hager's tests, while flavonoids were identified through sulfuric acid and Shinoda tests. Steroids, proteins, tannins, saponins, coumarins, and glycosides were also detected through their respective characteristic reactions. The presence of these phytoconstituents is significant because many of them possess antioxidant, anti-inflammatory, antimicrobial, and skin-protective activities. Flavonoids and phenolic compounds are known for their free radical scavenging properties, whereas tannins and coumarins contribute to antimicrobial and astringent effects. These findings support the potential application of A. marmelos fruit extract in herbal cosmeceutical formulations.

Table 6: Preliminary Phytochemical Screening of AMME

Sr. No.	Phytochemical Class	Test Performed	Observation	Result
1	Alkaloids	Dragendorff's Test	Brown precipitate	+
		Mayer's Test	Cream precipitate	+
		Hager's Test	Yellow precipitate	+
2	Flavonoids	Sulfuric Acid Test	Orange-red colour	+
		Shinoda Test	Red colour	+
3	Steroids	Salkowski Test	Reddish-brown colour	+
		Liebermann–Burchard Test	Reddish ring at junction	+
4	Proteins	Ninhydrin Test	Purple colour	+
		Millon's Test	Red coloured solution	+
5	Tannins	Ferric Chloride Test	Deep blue-black colour	+
		Lead Acetate Test	White precipitate	+
6	Saponins	Foam Formation Test	Persistent foam	+
7	Coumarins	Sodium Hydroxide Test	Yellow colour	+
		Ferric Chloride Test	Yellow coloration	+
8	Glycosides	Borntrager's Test	Red/Pink ammoniacal layer	+
		Legal's Test	Pink-red colour	+

Note: (+) Present

Thin Layer Chromatography (TLC) Screening

Thin-layer chromatography (TLC) was performed to identify the presence of marmelosin in the methanolic extract of Aegle marmelos fruit pulp (AMME) by comparing its chromatographic profile with that of the standard marmelosin. The chromatogram of standard marmelosin exhibited a single spot with an Rf value of 0.46, appearing light brown under UV light at 254 nm and dark yellow under UV light at 366 nm. The methanolic extract (AMME) showed three distinct spots with Rf values of 0.17, 0.46, and 0.67, indicating the presence of multiple phytoconstituents. Importantly, one of the extract spots exhibited the same Rf value (0.46) and fluorescence characteristics as the standard marmelosin. This observation confirmed the presence of marmelosin in the methanolic extract. The additional spots observed in the extract correspond to other phytochemical constituents present in the fruit pulp. Thus, TLC served as a simple and effective preliminary analytical tool for the identification of marmelosin and phytochemical profiling of AMME.

Table 7: TLC Screening of Standard Marmelosin and AMME

Track	Sample	Spot No.	UV 254 nm	UV 366 nm	Daylight	Rf Value
1	Standard Marmelosin	1	Light Brown	Dark Yellow	Not Visible	0.46
2	AMME	1	Brown	Light Yellow	Not Visible	0.17
2	AMME	2	Brown	Dark Yellow	Not Visible	0.46
2	AMME	3	Brown	Blue	Not Visible	0.67

The matching Rf value of 0.46 between the standard marmelosin and the extract confirmed the presence of marmelosin in AMME. The occurrence of additional spots in the extract indicated the complexity of the phytochemical composition of A. marmelos fruit pulp.

Evaluation of Herbal Face Serum Formulations

Five different face serum formulations (B1–B5) were prepared and evaluated for physicochemical parameters including spreadability, viscosity, and pH. Spreadability is an important parameter influencing the ease of application of topical formulations. Among

the tested batches, B5 exhibited the highest spreadability (81.84 g·cm/s), whereas B1 showed the lowest value (27.97 g·cm/s). Viscosity values ranged from 1050 to 1169.6 cP, indicating acceptable consistency suitable for topical application. The pH values of all formulations ranged between 4.81 and 5.13, which is compatible with the normal physiological pH of human skin and minimizes the risk of irritation.

Among all formulations, Batch B2 demonstrated an optimum balance between spreadability (55.9 g·cm/s), viscosity (1121.3 cP), and pH (4.81). Therefore, B2 was selected as the optimized formulation for further analytical and stability studies. The selected formulation exhibited desirable aesthetic characteristics, smooth texture, and satisfactory skin compatibility, making it suitable for cosmeceutical application.

Table 8: Evaluation of Face Serum Formulations

Parameter	B1	B2	B3	B4	B5
Spreadability (g·cm/s)	27.97	55.90	52.90	54.43	81.84
Viscosity (cP)	1169.6	1121.3	1051.0	1088.3	1050.0
pH	5.128	4.81	5.08	4.87	5.07

The pH values of all formulations were within the acceptable range for topical application. Batch B2 was selected as the optimized formulation due to its favorable viscosity, adequate spreadability, and skin-compatible pH.

HPTLC Analysis and Quantification of Marmelosin

HPTLC Fingerprinting and Specificity

HPTLC fingerprinting was performed to establish the identity and specificity of marmelosin in both AMME and the optimized face serum formulation (AMME-F). Spectral overlay studies at 310 nm demonstrated a close match between the chromatographic profiles of standard marmelosin (SM), AMME, and AMME-F. The similarity in retention behavior and spectral characteristics confirmed the presence of marmelosin in both the extract and formulated product. These findings validated the specificity of the developed HPTLC method for marmelosin analysis.

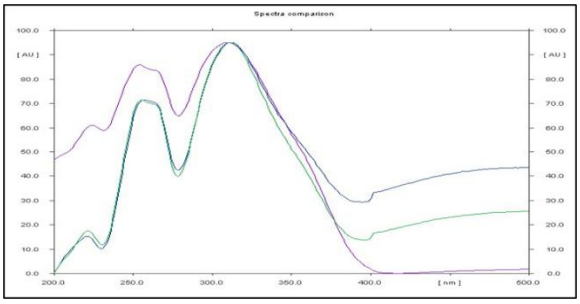

Figure 1: Spectral overlay of tracks specificity @ 310nm

Linearity Study

The linearity of the HPTLC method was evaluated over a concentration range of 20–120 ng/band. A good linear relationship was observed between concentration and peak area. The calibration curve generated for marmelosin exhibited a correlation coefficient (R^2) of 0.9499, indicating acceptable linearity within the selected concentration range.

The obtained calibration curve demonstrated satisfactory linearity over the selected concentration range, supporting the suitability of the developed HPTLC method for quantitative estimation of marmelosin.

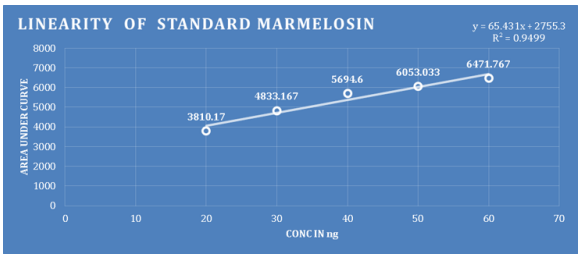

Figure 2: Photo Documentation of Linearity curve assay

Quantitative Estimation of Marmelosin in AMME and AMME-F

Quantification of marmelosin was carried out using the calibration curve generated from standard marmelosin. Based on the peak area obtained for AMME, the concentration of marmelosin was calculated to be 11.94% w/w of the methanolic extract. Similarly, quantitative estimation of the optimized face serum formulation (AMME-F) revealed a marmelosin content of 11.80% w/w.

The slight reduction in marmelosin content observed in the formulation compared to the extract may be attributed to formulation processing and distribution of the active constituent within the formulation matrix.

However, the difference was minimal, indicating that the formulation process did not significantly affect the stability or retention of marmelosin.

Table 9: Quantitative Estimation of Marmelosin

Sample	Marmelosin Content (% w/w)
AMME (Methanolic Extract)	11.94
AMME-F (Face Serum Formulation)	11.80

The results demonstrated successful incorporation and retention of marmelosin in the developed herbal face serum. The comparable marmelosin content between the extract and formulation confirmed the effectiveness of the formulation process and highlighted the potential of AMME as a valuable cosmeceutical ingredient.

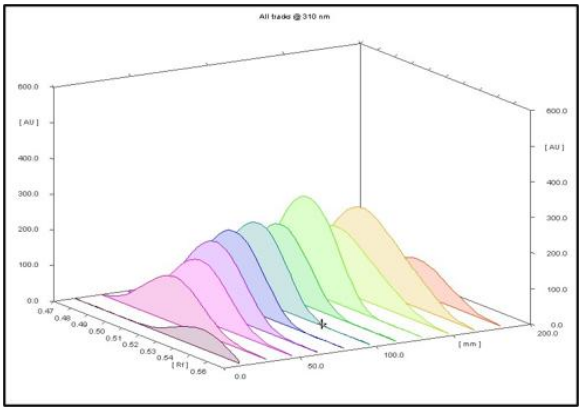

Figure 3: 3D Assay by Linearity of SM, AMME and AMME-F tracks Linearity @310nm

Overall, TLC and HPTLC analyses confirmed the presence of marmelosin as a major marker compound in Aegle marmelos fruit extract and the developed face serum. The validated HPTLC method proved suitable for qualitative and quantitative analysis, ensuring standardization and quality control of the herbal cosmeceutical formulation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the developed HPTLC method for the estimation of marmelosin was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ). The chromatographic analysis was performed at a wavelength of 310 nm, where marmelosin exhibited a distinct peak with an Rf value of 0.46. The LOD and LOQ values obtained were 0.0915 ng/band and 0.30 ng/band, respectively, indicating the high sensitivity of the developed analytical method. These results demonstrate that the HPTLC method is capable of detecting and quantifying very low concentrations of marmelosin with acceptable accuracy and precision, making it suitable for routine quality control and standardization studies.

Table 10: LOD and LOQ of Standard Marmelosin

Sr. No.	Parameter	Result
1	Detection Wavelength	310 nm
2	Rf Value	0.46
3	Regression Equation	Y = 65.431x + 2755.3
4	Correlation Coefficient (R^2)	0.9499
5	LOD (ng/band)	0.0915
6	LOQ (ng/band)	0.30

The low LOD and LOQ values obtained indicate excellent sensitivity of the HPTLC method for marmelosin determination. The method can reliably detect and quantify trace quantities of the marker compound, which is advantageous for standardization and quality assessment of herbal extracts and formulations.

Precision Study

Precision studies were carried out to evaluate the reproducibility and reliability of the developed HPTLC method. Precision was assessed in terms of intra-day and inter-day variability by analyzing standard marmelosin at different concentration levels. The results were expressed as percentage relative standard deviation (%RSD).

Intra-Day Precision

Intra-day precision was determined by analyzing three concentration levels (1 ng, 3 ng, and 5 ng) during morning, afternoon, and evening sessions on three consecutive days. The average concentrations obtained ranged from 15.80 to 15.89 ng for the 1 ng level, 27.83 to 28.08 ng for the 3ng level, and 58.28 to 58.57ng for the 5ng level. The %RSD values ranged from 0.47% to 2.11%, indicating excellent repeatability of the method within the same day. According to ICH guidelines, %RSD values below 2% are generally considered acceptable for analytical methods, and the obtained values demonstrate good precision and reproducibility.

Table 11: Intra-Day Precision Study

Day	Concentration Level	Average Peak Area	SD	%RSD	Calculated Conc. (ng)
Day 1	1 ng	1719.54	17.14	0.99	15.82
	3 ng	4592.72	63.72	1.38	28.08
	5 ng	6588.64	139.06	2.11	58.57
Day 2	1 ng	1721.01	8.15	0.47	15.80
	3 ng	4579.57	72.54	1.58	27.88
	5 ng	6577.14	137.93	2.10	58.41
Day 3	1 ng	4474.18*	53.66	1.19	15.89
	3 ng	4576.66	68.85	1.50	27.83
	5 ng	6568.99	136.11	2.07	58.28

Observed peak area value reported during analysis.

The low %RSD values obtained during intra-day studies indicate excellent repeatability and consistency of the developed HPTLC method. The method produced reproducible results across different time intervals within the same day, confirming its suitability for routine analysis.

Inter-Day Precision

Inter-day precision was evaluated by comparing the results obtained on three different days. The average concentrations obtained were 58.53 ng, 27.93 ng, and 58.42 ng, respectively. The %RSD values for Day 2 and Day 3 were found to be 0.19% and 0.15%, respectively, indicating excellent reproducibility between different analytical runs. Although Day 1 exhibited higher variability, the overall precision data demonstrated that the developed HPTLC method is capable of producing consistent results over multiple days.

Table 12: Inter-Day Precision Study

Parameter	Day 1	Day 2	Day 3
Average Peak Area	6885.26	4582.98	6578.27
SD	8951.02	8.56	9.88
%RSD	130.00	0.19	0.15
Concentration (ng)	58.53	27.93	58.42

Inter-day precision studies demonstrated that the developed HPTLC method provides reproducible results under normal laboratory conditions. The very low %RSD values observed for Day 2 and Day 3 indicate excellent method precision and robustness. The overall findings confirm the reliability of the developed analytical method for quantitative estimation of marmelosin in both the methanolic extract and face serum formulation.

The precision studies collectively demonstrated that the developed HPTLC method is reproducible, reliable, and suitable for routine quality control analysis of Aegle marmelos methanolic extract and herbal cosmeceutical formulations.

Accuracy Study (% Recovery)

The accuracy of the developed HPTLC method was evaluated by performing recovery studies at three concentration levels, namely 80%, 100%, and 120% of the known amount of standard marmelosin added to the methanolic extract of Aegle marmelos (AMME). Recovery studies are important for assessing the ability of an analytical method to accurately measure an analyte in the presence of formulation excipients and matrix components.

In the present study, a fixed amount of AMME (23.87 ng) was spiked with known quantities of standard marmelosin corresponding to 80%, 100%, and 120% levels. The percentage recovery was calculated by comparing the amount of marmelosin recovered after analysis with the amount added. The results showed a recovery of 100% at all three concentration levels, indicating excellent accuracy of the developed method. The low standard deviation values further confirmed the reproducibility and reliability of the assay. These findings demonstrate that the HPTLC method is capable of accurately quantifying marmelosin in the presence of extract constituents without interference.

Table 13: Recovery Study of Marmelosin in AMME

Recovery Level (%)	Amount of AMME (ng)	Standard Added (ng)	Total Standard Taken (ng)	Total Standard Obtained (ng)	% Recovery
80	23.87	19.10	42.97	42.98	100
100	23.87	23.87	47.75	47.75	100
120	23.87	28.65	52.52	52.53	100

Robustness Study

Robustness studies were carried out to evaluate the reliability of the developed HPTLC method under small but deliberate variations in chromatographic conditions. Parameters such as mobile phase composition, saturation time, scan speed, slit width, and detection wavelength were varied individually while maintaining other conditions constant. The robustness study was performed using standard marmelosin (S-AM) at a concentration of 3 µL, and each experiment was conducted in triplicate.

The average peak area values obtained under different chromatographic conditions ranged from 4200.9 to 4547.33. The corresponding %RSD values varied between 1.15% and 1.54%, which are well within acceptable analytical limits. The low %RSD values indicate that minor variations in chromatographic parameters did not significantly affect the analytical response. These results confirm that the developed HPTLC method is robust and capable of providing reliable and reproducible results under routine laboratory conditions.

Table 14: Robustness Study of HPTLC Method

Parameter	Average Peak Area	SD	%RSD	Amount Detected (ng)
Mobile Phase (Petroleum Ether: Ethyl Acetate)	4321.33	49.54	1.15	22.09
Saturation Time (20 min)	4317.13	66.37	1.54	27.38
Scan Speed (40 mm/s)	4292.50	52.22	1.22	23.49
Slit Width (6 × 0.3 mm)	4547.33	58.07	1.28	23.86
Wavelength (320 nm)	4200.90	58.14	1.38	23.93

Table 15: Robustness Data for Standard Marmelosin (n = 3)

Sample	Concentration	Mobile Phase	Saturation Time	Scan Speed	Slit Width	Wavelength
S-AM	3 µL	4286.8	4268.3	4262.1	4497.5	4150.7
S-AM	3 µL	4378.1	4392.7	4352.8	4611.1	4264.6
S-AM	3 µL	4299.1	4290.4	4262.6	4533.4	4187.4

IV. CONCLUSION

The present study successfully developed and evaluated an herbal cosmeceutical face serum containing the methanolic extract of Aegle marmelos fruit (AMME). Physicochemical characterization of the fruit pulp and extract confirmed its quality and suitability for formulation development. Preliminary phytochemical screening revealed the presence of important bioactive constituents, including flavonoids, alkaloids, tannins, saponins, glycosides, coumarins, and phenolic compounds. TLC analysis confirmed the presence of marmelosin as a characteristic marker compound. The formulated face serum exhibited desirable physicochemical properties, including acceptable pH, viscosity, spreadability, and aesthetic characteristics, indicating its suitability for topical application. A validated HPTLC method was successfully employed for the quantification of marmelosin in both the methanolic extract and the developed face serum formulation. The method demonstrated satisfactory linearity, accuracy, precision, sensitivity, and robustness in accordance with ICH guidelines. Marmelosin content was found to be comparable in the extract and formulation, confirming the stability and effective incorporation of the bioactive constituent into the serum. Overall, the findings suggest that Aegle marmelos fruit extract is a promising natural ingredient for herbal cosmeceutical formulations and may serve as an effective source of antioxidant and skin-protective agents for future skincare applications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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