Indian J. Applied & Pure Bio. Vol. 41(1), 319-330 (2026). **ISSN: 0970-2091**

Formulation of Skin Moisturizer using *Moringa oleifera* Lam. and *Ananas comosus* (L.) Merr.

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Abstract

This study investigates the formulation and evaluation of a natural skin moisturizer utilizing ethanol extracts from *Moringa oleifera* and *Ananas comosus* (pineapple) leaves. The objective was to assess the therapeutic and cosmetic potential of these plant-based ingredients through phytochemical screening, antioxidant evaluation via the DPPH assay and antimicrobial susceptibility testing. The extracts were tested against bacterial strains, including Staphylococcus aureus, using the disc diffusion method. The results indicating notable antibacterial activityparticularly from the Moringa extract. Phytochemical analysis identified key bioactive compounds such as flavonoids, tannins, alkaloids, phenolics and saponins. All known for their roles in skin nourishment and repair. The DPPH assay demonstrated strong antioxidant activity, essential for combating oxidative stress and delaying skin aging. Additionally, vitamin E content was quantified using spectrophotometric analysis.This confirming its significant contribution to the cream moisturizing and protective properties. The formulated cream underwent pH testing, skin irritation assessments and total residue analysis. The pH was within the optimal skin-friendly range (5.5–6.0)with no observed irritation. It indicating excellent dermal compatibility. Residue levels were within acceptable cosmetic limit and supporting the safety of the formulation. The findings strongly support the use of Moringa and pineapple leaf extracts in herbal skincare formulations. This offering a natural, safeand effective alternative to synthetic moisturizers with enhanced antibacterial and antioxidant benefits.

Key words : *Moringa oleifera*, *Ananas comosus*, Natural moisturizer, Antioxidant activity, Antimicrobial properties

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The demand for natural ingredients in cosmetic formulations has significantly increased due to growing awareness of the adverse effects of synthetic compounds and consumer preference for safe, eco-friendly and sustainable alternatives. Among the numerous botanicals explored, *Moringa oleifera* Lam and *Ananas comosus* (L.) Merr. (pineapple) have attracted attention for their bioactive compounds that offer dermatological benefits. *Moringa oleifera*, the most cultivated species of the Moringaceae family, is native to the sub-Himalayan regions of India, Pakistan and Nepal, but has now become widespread in Africa, Southeast Asia and South America[10]. It is a well-known plant for its nutritional and pharmacological properties, being rich in beta-carotene, vitamin C, calcium, potassium, proteins and a diverse array of phenolics, glucosinolates and antioxidants[3,4,14,20,24,26].

The leaves of *M. oleifera* have been recognized for their high antioxidant content and are increasingly used in cosmetic applications, particularly moisturizers and conditioners, due to their protective effects on skin cells against oxidative stress[18,25]. Antioxidants like beta-carotene in Moringa leaves help maintain cellular membrane integrity by neutralizing free radicals, which are key contributors to skin aging, cancer and photodamage[6,8,22]. These properties make *M. oleifera* an ideal ingredient in topical formulations aimed at enhancing skin hydration, elasticity and overall appearance[13]. Furthermore, oleic acid present in Moringa helps restore the skin barrier, making it beneficial for dry and sensitive skin types[1].

Similarly, *Ananas comosus* has a long history of use in traditional medicine particularly in South America[11]. Pineapple is a rich source of bromelain, a proteolytic enzyme found in both the fruit and stem, known for its anti-inflammatory, antimicrobial and exfoliating properties[19,23]. Bromelain, alongside alpha-hydroxy acids (AHAs) naturally present in pineapple, aids in removing dead skin cells, improving skin texture and reducing the appearance of fine lines[17,21]. The pineapple contains essential micronutrients including vitamin B1, B6, copper, calcium, potassium and dietary fiber, which contribute to skin nourishment and rejuvenation[15]. Moisturizers incorporating herbal extracts not only enhance hydration but also provide therapeutic support for conditions associated with dry or damaged skin[2,9,12]. The challenges in topical formulation development remain achieving stable delivery and maintaining antioxidant potency under varying environmental conditions[8,16]. In this context, the synergistic application of *Moringa oleifera* and *Ananas comosus* offers promising potential for the development of effective natural moisturizers. This study aims to formulate and evaluate a skin moisturizer using extracts from *M. oleifera* leaves and *A. comosus* fruit, targeting hydration, antioxidant protection and skin revitalization.

Sample Collection and Preparation :

Fresh *Moringa oleifera* leaves and *Ananas comosus* (pineapple) peels were collected from local vendors in Coimbatore. These plant parts were washed thoroughly, shade-dried and crushed into fine powders using a mechanical grinder. The powdered samples were then stored in airtight containers to preserve their phytochemical integrity for

further analysis.

Extraction Process :

To extract bioactive compounds, both polar (water, acetone, ethanol) and non-polar (chloroform, hexane) solvents were used in a 10:1 solvent-to-sample ratio. The powdered moringa leaves and pineapple peels were subjected to maceration for 72 hours at room temperature with occasional shaking. The change in solvent color was used as an indicator of successful extraction. After extraction, the mixtures were filtered and solvents were evaporated. The dried residues were collected and stored in sealed containers.

Crude Extract Preparation :

For crude extract preparation, 20 grams of each powdered sample was mixed with 50 mL of ethanol and placed on an orbital shaker for 24 hours. After shaking, the mixtures were filtered using cellulose membrane filters. The filtrates were collected and stored at refrigerated temperatures until further analysis.

Phytochemical Screening :

Standard phytochemical tests were performed on the extracts to detect the presence of secondary metabolites.

For Ananas comosus :

Alkaloids were identified using Hager's reagent, which produced an orange-red precipitate. Saponins were confirmed by a frothing test, while tannins were detected using ferric chloride, producing a red precipitate. Steroids were indicated by a reddish-brown color at the chloroform-sulfuric acid interface. Phenols gave a greenish-brown color with ferric chloride and flavonoids produced a yellow precipitate with sodium hydroxide. Glycosides were confirmed by adding acetic anhydride and sulfuric acid, leading to a color change.

For Moringa oleifera :

Flavonoids were detected with sodium hydroxide and confirmed by color change with dilute hydrochloric acid. Alkaloids were tested using Dragendorff's reagent. Tannins were confirmed using trichloromethane. Steroids showed a reddish-brown interface with chloroform and sulfuric acid. Quinones and terpenoids were confirmed by color changes with concentrated sulfuric acid and chloroform.

Antimicrobial Activity :

The antimicrobial properties of the extracts were evaluated using the well diffusion method on Mueller-Hinton Agar (MHA). Wells were created in the agar plates and filled with the extracts. The plates were inoculated with gram-positive bacteria (*Staphylococcus* sp., *Streptococcus* sp.,) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella* spp.). After incubation at 37°C for 24 hours, zones of inhibition were measured to assess antimicrobial efficacy.

Antimicrobial Susceptibility Test (Disk Diffusion Method) :

Further antimicrobial testing was performed using the disk diffusion method on Nutrient Agar. Extracts were evaporated to

remove solvents and reconstituted in sodium carboxymethyl cellulose (NaCMC) to obtain concentrations of 2%, 4% and 8% w/v. Tetracycline was used as a positive control, while NaCMC alone served as the negative control. Filter paper disks were impregnated with the test samples and placed on inoculated plates. After 24 hours of incubation at 37°C, the zones of inhibition were measured.

Antioxidant Activity (DPPH Assay) :

The antioxidant activity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A 0.0025 g DPPH solution was prepared in methanol and diluted to working concentration. Each sample (20 µL of extract) was added to 2 mL of DPPH solution and incubated in the dark for 20 minutes at room temperature. Absorbance was measured at 517 nm using a UV-Visible spectrophotometer and the percentage of radical scavenging activity was calculated.

Moisturizing Cream Formulation :

A moisturizing cream was formulated using both plant extracts. The oil phase—beeswax (8 g) shea butter (10 g) and almond oil (10 mL)—was heated to 70°C until fully melted. After slightly cooling, the aqueous phase, which included moringa (5 g) and pineapple (5 g) extracts, was added with continuous stirring to form a uniform emulsion. During the cool-down phase, lavender oil (1 mL) methylparaben (0.1 g) and zinc oxide (0.1 g) were added. The cream was mixed thoroughly and transferred into sterilized containers once cooled.

Vitamin E Analysis by HPLC :

Vitamin E content was determined using High Performance Liquid Chromatography (HPLC) with a PDA detector. The samples were saponified using ethanolic KOH at 90°C for 45 minutes and extracted with hexane. The organic layer was washed with water to neutral pH, filtered through sodium sulfate and evaporated. The residues were reconstituted in methanol and filtered through 0.45 µm filters. HPLC conditions included a C18 column, methanol as the mobile phase, isocratic flow at 0.8 mL/min, 291 nm detection wavelength and 50 µL injection volume.

Skin Irritation Test :

The formulated cream was tested on three individuals by applying it to a 1-inch patch of skin and exposing it to direct sunlight for three minutes. No signs of irritation, redness, or itching were observed, indicating the product was safe for topical application.

pH Testing :

The pH of the moisturizing cream was measured using pH indicator strips. The results ranged from 5.5 to 6.0, indicating a slightly acidic to neutral formulation, suitable for maintaining healthy skin.

Phytochemical Analysis :

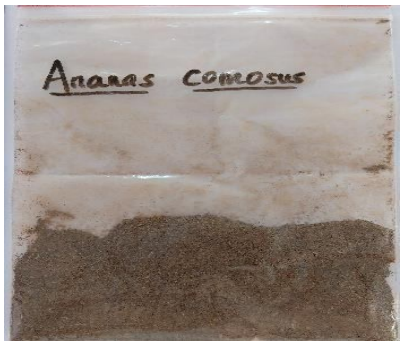
Phytochemical screening of *Moringa oleifera* and *Ananas comosus* extracts(Figure 1,2,3) confirmed the presence of multiple bioactive compounds (Table-1) known for their therapeutic, antioxidant and antimicrobial properties. The ethanolic extract of *Moringa*

oleifera revealed the presence of flavonoids, alkaloids, tannins, steroids, quinones and terpenoids. These constituents are well-documented for their strong antioxidant potential and skin-soothing effects. Similarly, the extract of *Ananas comosus* showed the presence of alkaloids, saponins, tannins, steroids, phenols, flavonoids and glycosides. The presence of saponins and glycosides in pineapple extract supports its cleansing and skin-rejuvenating properties.

Figure 1. Dried *Moringa* Leaves

Figure 2. Dried Pineapple Leaves

Figure 2. Powdered *Moringa* leaves



Powdered Pineapple leaves

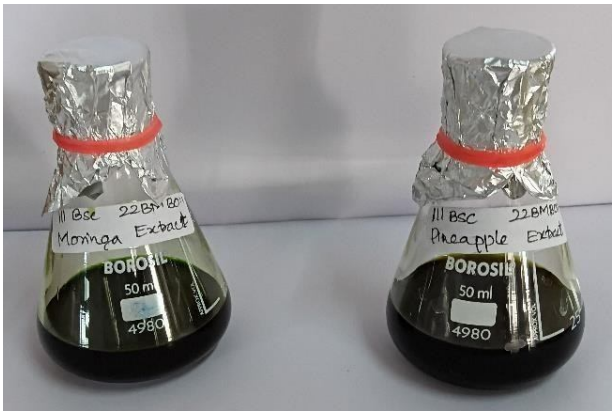


Figure 3. Crude extract

Table-1. Phytochemicalanalysis of *Moringa oleifera* and *Ananas comosus*

Phytochemicals	*Moringa oleifera*	*Ananas-comosus*
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Steroids	+	+
Quinone	+	+
Terpenoids	+	+

Antimicrobial Activity :

The antimicrobial potential of both extracts was initially assessed using well diffusion on Mueller Hinton Agar against Pseudomonas aeruginosa. The zone of inhibition was measured across increasing concentrations (25 µL, 50 µL, 75 µL and 100 µL) showing a concentration-dependent increase in antimicrobial efficacy (Figures 4). This indicates that both Moringa and Pineapple possess bioactive compounds capable of suppressing the growth of common skin pathogens.

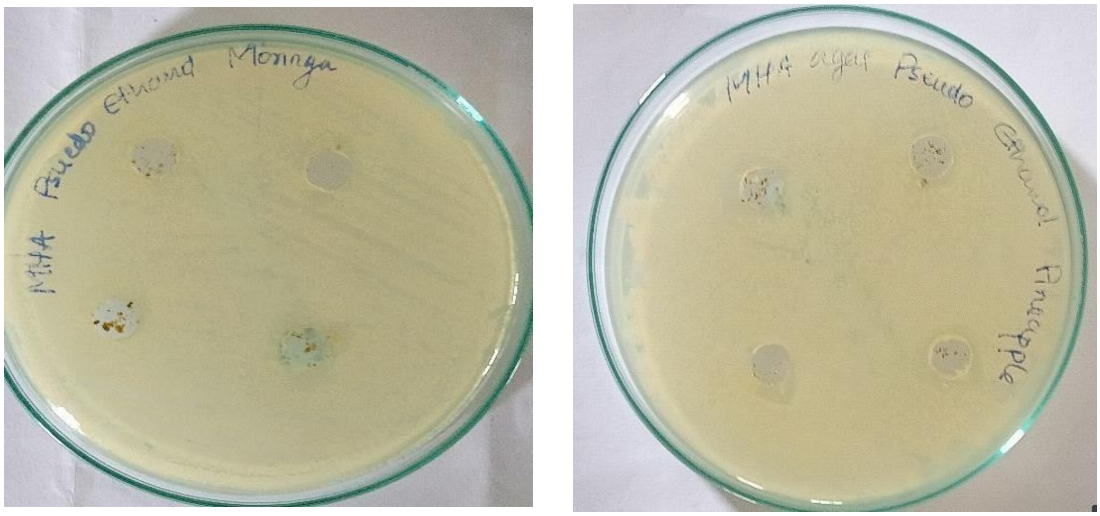


Figure 4. Antimicrobial activity of *Moringa oleifera* and *Ananas comosus*

Antimicrobial Susceptibility Testing :

Further evaluation using the antimicrobial susceptibility method demonstrated antibacterial activity of both extracts against *Staphylococcus aureus* (Figure 5). For *Moringa oleifera*, the zones of inhibition at concentrations of 2%, 4% and 8% were 2.5 mm, 2.7 mm and 2.4 mm respectively, with the highest activity observed at 4% (Table 3). The positive control (tetracycline) exhibited a 2.8 mm inhibition zone, while the NaCMC negative control showed minimal activity (1.5 mm). For *Ananas comosus*, inhibition zones were 2.2 mm (2%) 2.4 mm (4%) and 2.6 mm (8%) indicating a progressive increase in efficacy with concentration (Table-2). These results affirm the potential of both extracts as natural antibacterial agents suitable for topical applications.

Table-2. Shows the Antimicrobial activity of the extracts

Concentration (b/v)	Zone of Inhibition(mm) against *Staphylococcusaureus*	
	Moringa oleifera	*Ananas comosus*
2%b/v	2.5 mm	2.2 mm
4%b/v	2.7 mm	2.4 mm
8%b/v	2.4 mm	2.6 mm
Positive Control	2.8 mm	2.8 mm
NegativeControl	1.5 mm	1.4 mm

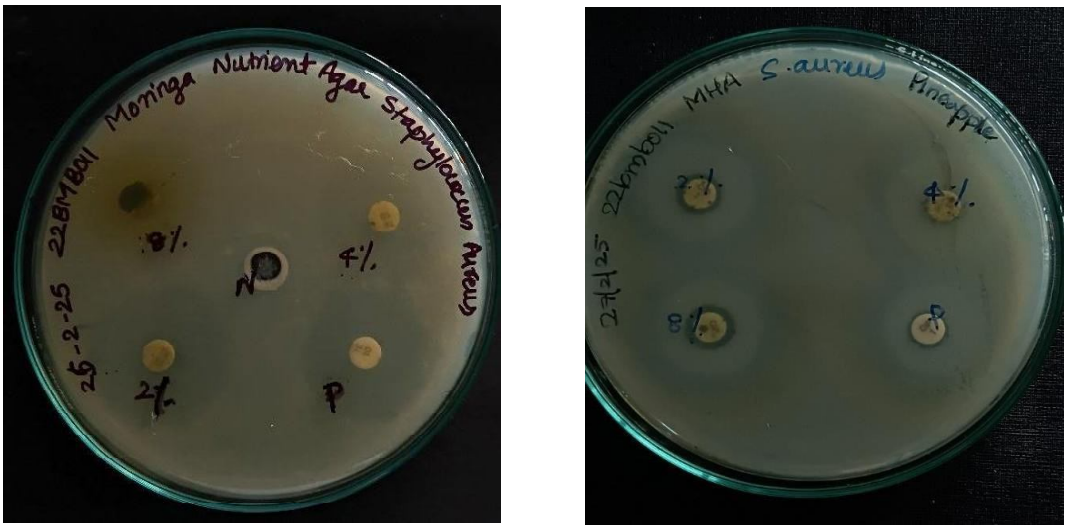


Figure 5. Antimicrobial susceptibility testing of *Moringa oleifera* and *Ananas comosus*

Antioxidant Activity :

The DPPH radical scavenging assay was employed to determine the antioxidant capacity of both extracts (Figures 6).*Moringa oleifera* exhibited significantly higher antioxidant activity (80%–90%) compared to *Ananas comosus*, which showed a moderate range (15%–30%). The absorbance of the Moringa extract was recorded at 0.130 nm, markedly lower than that of pineapple extract at 0.899 nm and compared against a control blank of 1.287 nm. The high antioxidant potential of Moringa can be attributed to its rich content of flavonoids, polyphenols and vitamin E. These findings support its role in neutralizing oxidative stress, thereby helping to reduce skin aging and inflammation.

Moisturizing Cream Formulation and Evaluation :

A moisturizing cream was successfully formulated using the ethanol extracts of both

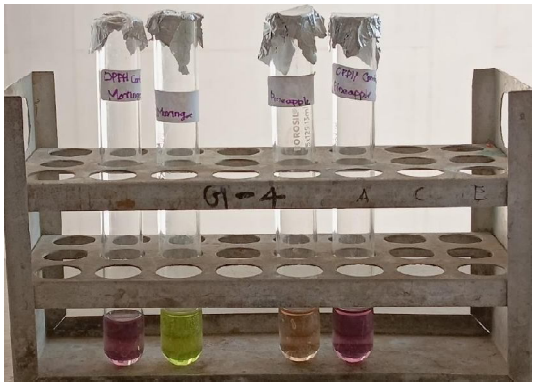


Figure 6. Antioxidantactivity *Moringa oleifera* and *Ananas comosus*

Figure 7. Skin moisturizing cream

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Sponsored by Ministry of Food Processing Industries, Government of India.

TEST REPORT

Test Report No:2025/03/25/SSFTL/24-25/NN-539/001	Issue Date:28.03.2025	Page 1 of 1

CUSTOMER DETAILS	
Customer Name & Address	**Ms. HANNA VINOY** Hindusthan College of Arts & Science, Coimbatore.
Customer Reference	Test Request dt 25.03.2025

SAMPLE DETAILS			
Product Category	Cosmetics & Essential Oils	Sample Code	SSFTL/24-25/NN-539/001
Sample Name	Moisturizing Cream	Sample Conditions at Receipt	Good
Sample Description	Sample in Glass Container	Sample Received on	25.03.2025
Sample Quantity	17g	Test Commenced on	26.03.2025
Sampled by	Drawn by Customer	Test Completed on	28.03.2025
Sampling Procedure	---	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS – CHEMICAL PARAMETERS

Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Total Residues	Inhouse Method	%	87.36
2	Skin Irritation Test	Inhouse Method	-	Passes
3	Vitamin E	Inhouse Method	mg/kg	553

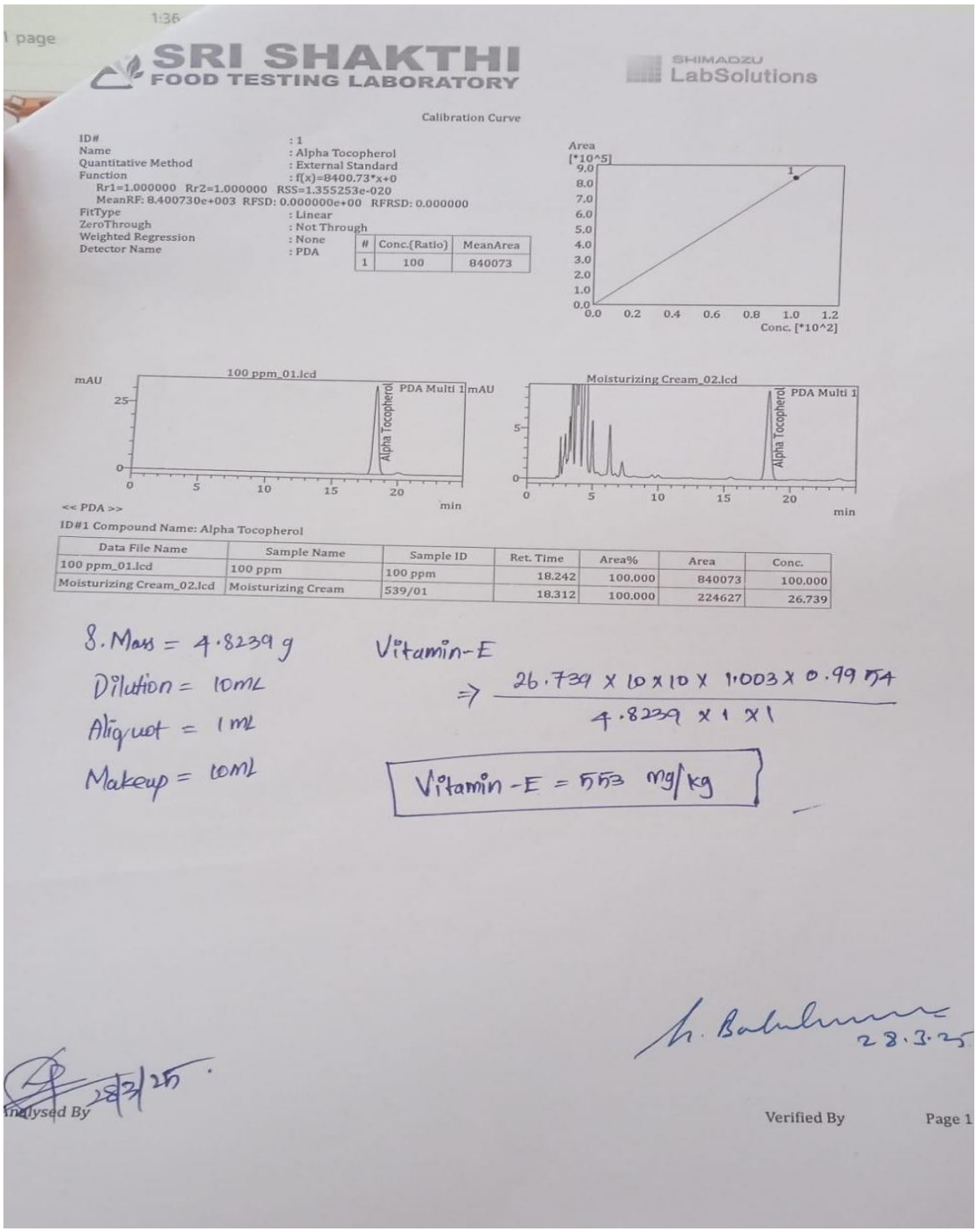
Remarks:
- Result Related Only to the Sample Tested.
- Instrument Used: HPLC – PDA.

End of Report

Checked by 26/3/25
Name: Sathesh Kumar.C
Designation: Chemist

Sri Shakthi Food Testing Laboratory Coimbatore

28.3.25
Authorized Signatory
Name : K.Balasubramanian
Designation : Chief Chemist and Quality Manager



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SHIMADZU
LabSolutions

Calibration Curve

ID# : 1
Name : Alpha Tocopherol
Quantitative Method : External Standard
Function : f(x)=8400.73*x+0
Rr1=1.000000 Rr2=1.000000 RSS=1.355253e-020
MeanRF: 8.400730e+003 RFSD: 0.000000e+00 RFRSD: 0.000000
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None
Detector Name : PDA

#	Conc.(Ratio)	MeanArea
1	100	840073

Area [*10^5]
Conc. [*10^2]

100 ppm_01.lcd
PDA Multi 1 mAU
Alpha Tocopherol
min

Moisturizing Cream_02.lcd
PDA Multi 1
Alpha Tocopherol
min

<< PDA >>

ID#1 Compound Name: Alpha Tocopherol

Data File Name	Sample Name	Sample ID	Ret. Time	Area%	Area	Conc.
100 ppm_01.lcd	100 ppm	100 ppm	18.242	100.000	840073	100.000
Moisturizing Cream_02.lcd	Moisturizing Cream	539/01	18.312	100.000	224627	26.739

8. Mass = 4.8239 g
Dilution = 10mL
Aliquot = 1 mL
Makeup = 10mL

Vitamin-E

$$\Rightarrow \frac{26.739 \times 10 \times 10 \times 1.003 \times 0.9954}{4.8239 \times 1 \times 1}$$

Vitamin-E = 553 mg/kg

28.3.25

Analysed By 28/3/25

Verified By

Page 1

Figure 8. Test report of the prepared moisturizer

Moringa and Pineapple (Figure 7). The cream displayed a stable, homogenous texture with no signs of phase separation or discoloration. Skin irritation tests conducted on three individuals under controlled conditions revealed no signs of itching, redness, or inflammation following 3 minutes of sun exposure, confirming its safety for topical use. The pH of the final formulation ranged from 5.5 to 6.0, which is optimal for maintaining the skin's natural barrier function.

Vitamin E and Skin Compatibility Testing :

High-Performance Liquid Chromatography (HPLC) analysis confirmed a Vitamin E content of 553 mg/kg in the final formulation (Figure 8) indicating a significant antioxidant reservoir that supports skin hydration and barrier repair. The test report showed a total active residue value of 87.36%, validating the retention of key bioactive compounds after formulation. This high concentration underscores the potential formulation effectiveness in enhancing skin texture and reducing oxidative stress.

The combined use of *Moringa oleifera* and *Ananas comosus* extracts in skincare offers synergistic benefits. *Moringa,* rich in vitamin E, flavonoids and essential fatty acids, provides deep hydration and serves as a protective barrier against environmental stressors. Its antioxidant activity supports skin cell regeneration and reduces the signs of premature aging. Pineapple, on the other hand, is a potent source of bromelain and vitamin C, which contribute to mild exfoliation, collagen synthesis and anti-inflammatory effects. Together, these extracts offer enhanced antimicrobial, antioxidant and moisturizing properties.The antimicrobial assays confirmed their ability to inhibit common skin pathogens like *Staphylococcus aureus* and Pseudomonas aeruginosa, making the formulation suitable for sensitive or acne-prone skin. The antioxidant activity, particularly that of Moringa, suggests excellent free radical scavenging capabilities, while the pH balance and vitamin E content of the formulation ensure skin compatibility and hydration. Overall, this study validates the formulations efficacy and supports the use of these plant-based ingredients as natural, sustainable alternatives to synthetic skincare agents.

This study demonstrated the effectiveness of *Moringa oleifera* and *Ananas comosus* extracts as promising natural ingredients for skincare formulations. Phytochemical analysis confirmed the presence of several beneficial bioactive compounds such as flavonoids, alkaloids, phenols, saponins and glycosides, which are known for their antioxidant, antimicrobial and anti-inflammatory properties. Both extracts exhibited antimicrobial activity against Staphylococcus aureus and Pseudomonas aeruginosa, suggesting their ability to inhibit common skin pathogens. Antioxidant testing revealed that *Moringa oleifera* had significantly higher radical scavenging activity compared to *Ananas comosus*, attributed to its rich vitamin E and polyphenol content. A moisturizing cream was formulated using these extracts, which showed good physical stability, optimal pH (5.5–6.0) and no skin irritation, making it suitable for topical application. HPLC analysis confirmed a high vitamin E content (553 mg/kg) and the retention of 87.36% of bioactive compounds further validated the formulation's effectiveness. Gel lotion formulations containing 3–5% Moringa leaf and pineapple extracts

were physically stable and enhanced skin moisture, with the 5% formulation showing the highest moisturizing effect, highlighting the potential of these antioxidant-rich extracts for topical skin care applications[5]. The combined use of *Moringa oleifera* and *Ananas comosus* offers a multifunctionalplant-based alternative to synthetic skincare agents by providing hydration, antioxidant protection and antimicrobial defense. It supporting their future application in natural cosmetic and dermatological products.

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