



Development of an Enhanced Anti-Aging Formulation

Vikas Kumar Singh¹, Reenu Yadav^{2*}, Sanjay Kumar³, Phool Singh Yaduvanshi⁴, Gaurav Jain¹, Kavita R Loksh⁵

^{1,3,4,5} IES Institute of Pharmacy, IES University, Bhopal, M.P.

^{*2} Department of Pharmacy, Prestige Institute of Management and Research, Bhopal (M.P.)

Corresponding Author:

Reenu Yadav

Department of Pharmacy, Prestige Institute of Management and Research, Bhopal (M.P.)

Abstract

This study investigates the anti-aging and antimicrobial properties of fresh blueberry and banana fruits. It begins with phytochemical screening and identification of active compounds in the extracts, providing a foundation for understanding their therapeutic effects. The extracts were evaluated for physical properties, antioxidant, and anti-aging activities, leading to the development of an anti-aging herbal transdermal gel. The formulated patch was rigorously standardized and assessed for physicochemical properties, pH, antioxidant effects, and in-vitro drug diffusion. A drug release kinetic study was performed to determine the release profile of the active compounds. The results confirmed the significant anti-aging and antimicrobial potential of blueberry and banana extracts, resulting in an effective herbal patch formulation. Future research will focus on optimizing these extracts' properties, isolating bioactive compounds, conducting clinical trials, and exploring complementary natural ingredients to improve formulation efficacy and expand the range of potential cosmeceutical and pharmaceutical products.

Keyword: Anti-aging, antimicrobial, blueberry, banana, phytochemical screening, herbal transdermal patch.

1. INTRODUCTION

Aging is a natural yet intricate biological process marked by a progressive decline in physiological functions, often manifested as wrinkles, diminished skin elasticity, pigmentation changes, and oxidative damage. These changes occur due to intrinsic factors such as genetic predisposition and extrinsic factors like ultraviolet (UV) radiation, pollution, and environmental stressors. Among these, oxidative stress resulting from free radical accumulation is a significant contributor to skin aging, leading to the breakdown of collagen and elastin, which are crucial for maintaining skin integrity (Sharma et al., 2020). Consequently, there is an increasing emphasis on developing skincare formulations rich in natural antioxidants to mitigate these effects and promote healthier, younger-looking skin.

Blueberries (*Vaccinium spp.*) are widely recognized for their abundant anthocyanins, flavonoids, and polyphenols, which exhibit exceptional antioxidant, anti-inflammatory, and anti-aging properties. These compounds not only scavenge reactive oxygen species (ROS) but also stimulate collagen synthesis, improve microcirculation, and prevent photoaging (Kalt et al., 2020; Miller et al., 2019). Studies have demonstrated that the bioactive constituents of blueberries can enhance skin elasticity and reduce the depth of wrinkles, making them an essential ingredient in anti-aging formulations (Prior et al., 2018). Bananas (*Musa spp.*), often considered a natural elixir for skin health, contain essential vitamins such as A, C, and E, along with significant amounts of phenolic compounds. These phytochemicals provide a dual benefit: they combat oxidative damage and hydrate the skin, improving texture and reducing the appearance of fine lines and wrinkles (Pothavorn et al., 2022). Additionally, banana peel extracts have been shown to possess antimicrobial and antioxidant properties, further enhancing their utility in skincare applications (Sulaiman et al., 2011). In this study, blueberries and bananas have been selected for their complementary properties in combating skin aging. The synergy between the antioxidants in blueberries and the hydrating and restorative effects of bananas presents a novel approach to addressing the limitations of existing anti-aging products. The formulation aims to leverage these botanicals' potent bioactive compounds to develop an innovative and effective anti-aging skincare product.

Natural plant-based antioxidants have garnered significant attention for their potential in combating skin aging due to their ability to neutralize oxidative stress and improve skin health. Blueberries (*Vaccinium spp.*) are particularly valued for their high content of anthocyanins and flavonoids, which possess potent antioxidant and anti-inflammatory effects. Research by Kalt et al. (2020) and Prior et al. (2018) demonstrated that these compounds help in scavenging free radicals, protecting skin cells from UV damage, and enhancing collagen production, which ultimately helps in reducing the visible signs of aging. Additionally, Wu et al. (2016) found that blueberry extract can also support skin hydration, contributing to a youthful appearance.

Bananas (*Musa spp.*), a rich source of vitamins A, C, and E, along with phenolic compounds, also offer promising benefits in skincare. Pothavorn et al. (2022) found that banana extracts possess both antioxidative and hydrating properties, making them effective in preventing skin damage and improving texture. Further, Sulaiman et al.



(2011) highlighted that banana peels, known for their high levels of phenolic compounds, exhibit antioxidant, anti-inflammatory, and antimicrobial properties, which support skin healing and rejuvenation.

The synergistic effects of blueberries and bananas in skincare have been explored by various authors, including Lee et al. (2019), who suggested that combining different plant extracts can enhance the anti-aging effects, promoting better skin hydration, elasticity, and protection against oxidative damage. Given their complementary properties, blueberries and bananas offer a promising approach for developing advanced anti-aging formulations that can address the limitations of traditional skincare products.

2. MATERIAL & METHODS

The materials used in this study include blueberries and bananas as the primary botanical ingredients, which are sourced from their respective suppliers. The formulation also incorporates various chemicals such as petroleum, ethanol, and chloroform, which serve as solvents or carriers. Carbopol (g) is used as a thickening agent, while propylene glycol (ml) functions as a humectant. Potassium sorbate (ml) is added as a preservative to ensure the stability of the formulation, and isopropyl myristate (ml) is utilized for enhancing skin penetration. Cremophor is included as an emulsifier. All other chemicals used in the study are of analytical grade to ensure the quality and purity of the formulation.

Collection

In May 2021, fresh blueberries and bananas were purchased from local market suppliers in Chhatarpur, with blueberries sourced from an organic produce supplier and bananas from a wholesale supplier. Botanical identification is crucial for the safety and efficacy of natural plant-based drugs. In May 2021, fresh blueberries (*Vaccinium* spp.) and bananas (*Musa* spp.) were sourced from verified suppliers in Delhi. Authentication of the plant material, with specimen voucher no. 864/19, was carried out by Dr. Manjusa Saxena at Govt. Maharaja College, Chhatarpur (M.P.).

Extraction

After authentication by Dr. Manjusa Saxena, the blueberries were carefully cut into small, uniform pieces in a controlled environment to maintain fruit integrity and avoid contamination. These pieces were then prepared for drying, extraction, or powdering as needed for further processing. For extraction, the blueberries were subjected to continuous extraction using a Soxhlet apparatus with 500 ml of 95% methanol at 30-40°C for 6 hours. The process efficiency was monitored by checking for residual material in the thimble and evaporating a few drops of the extract to ensure completeness. After extraction, the solvent was evaporated, and the remaining extract was concentrated to obtain a dry residue. The percentage yield was calculated using the formula:

Percentage yield = (Weight of extract / Weight of powdered drug) x 100 (% w/w)

Physicochemical Characteristics of Extracts

Determination of Total Ash

Two grams of the crude drug were placed in a silica crucible for ash content determination. The sample was incinerated in a muffle furnace at 450°C until a white ash was formed, indicating the removal of carbon. After cooling, the crucible was weighed, and the ash percentage was calculated based on the air-dried weight of the original drug using the formula:

Total ash = $\frac{\text{Weight of ash obtained}}{\text{Weight of powdered drug taken}} \times 100$ (% w/w)

Determination of Acid Insoluble Ash

The total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid to determine acid-insoluble ash. The insoluble matter was collected in a Gooch crucible or on ashless filter paper, washed with hot water, and ignited in a furnace until it reached a constant weight. The final weight of the acid-insoluble ash was recorded after cooling.

Determination of Water Soluble Ash

The total ash was boiled with 25 ml of water for 5 minutes to determine water-soluble ash content. The insoluble matter was collected, washed with hot water, and ignited at 450°C for 15 minutes. After cooling, the weight was recorded, and the water-soluble ash content was calculated by subtracting the weight of the insoluble matter from the total ash weight.

Determination of Extractive Value

Determination of Alcohol Soluble Extractive

Five grams of air-dried crude drug powder were macerated with 100 mL of alcohol in a closed flask for 24 hours, with shaking every six hours. After an additional 18 hours, the mixture was filtered. From the filtrate, 20 aliquots of 5 mL each were evaporated to dryness, and the residue was dried at 105°C to a constant weight. The alcohol-soluble extractive percentage was calculated based on the weight of the air-dried drug.

Determination of Water Soluble Extractive

Five grams of air-dried crude drug powder were macerated with 100 mL of water in a closed flask for 24 hours, shaking every six hours. After 18 additional hours, the mixture was filtered. From the filtrate, 20 aliquots of 5 mL each were evaporated to dryness, and the residue was dried at 105°C to a constant weight. The water-soluble extractive percentage was calculated based on the weight of the air-dried drug.

**Determination of Moisture Content (Loss on Drying)**

Approximately 10 grams of crude material, cut into 3 mm pieces without preliminary drying, were weighed and placed in a tarred evaporation dish. The material was dried at 105°C for 5 hours and weighed. Drying continued, and the weight was recorded at 1-hour intervals until the weight difference between two successive weighings was less than 0.25%. Constant weight was achieved when two consecutive weighings, after drying for 30 minutes and cooling in a desiccator, showed a difference of no more than 0.01 g.

Quantitative Chemical Test

The following methods were applied:

Alkaloids:

- Mayer's Test: White/yellow precipitate with potassium mercuric iodide indicates alkaloids.
- Dragendorff's Test: Orange/reddish-brown precipitate with bismuth nitrate solution indicates alkaloids.
- Wagner's Test: Brown/reddish-brown precipitate with iodine solution indicates alkaloids.
- Hager's Test: Yellow crystalline precipitate with picric acid indicates alkaloids.

Anthraquinone Glycosids:

- Brontager's Test: Pink/red color in ammonical layer indicates phenolic compounds.

Carbohydrates:

- Molisch's Test: Violet ring with α -naphthol and sulfuric acid indicates carbohydrates.
- Fehling's Test: Brick-red precipitate indicates reducing sugars.
- Benedict's Test: Green, yellow, or red color indicates reducing sugars.

Saponins:

- Foam Test: Foam formation after shaking with water indicates saponins.
- Frothing Test: Honeycomb frothing with sodium bicarbonate indicates saponins.

Flavonoids:

- Ferric Chloride Test: Green color indicates phenolic compounds.
- Alkaline Reagent Test: Color change indicates phenolic compounds.
- Shinoda Test: Pink color with alcohol, hydrochloric acid, and magnesium indicates flavonoids.

Cardiac Glycosides:

- Tollen's Test: Silver mirror formation indicates aldehydes/reducing sugars.
- Raymond's Test: Violet to blue color change indicates phenolic compounds.
- Keller-Killani Test: Reddish-brown color and bluish-green upper layer indicate cardiac glycosides.

Proteins and Amino Acids:

- Millon's Test: Red precipitate indicates tyrosine.
- Xanthoprotein Test: Yellow color indicates nitration of aromatic rings in proteins.
- Biuret Test: Red/violet color indicates proteins.

Sterols:

- Liebermann-Burchard's Test: Red to green color change indicates steroids/triterpenoids.
- Salkowski Reaction: Red color and greenish fluorescence indicate sterols/triterpenoids.

Tannins:

- Matchstick Test: Magenta/purple color with hydrochloric acid indicates phenolic compounds.
- Vanillin-HCl Test: Pink/red color indicates flavonoids/phenolic compounds.
- Acetic Acid Test: Red color indicates anthraquinones.

Phenolic Compounds:

- Ferric Chloride Test: Blue-black color indicates phenolic compounds.
- Lead Acetate Test: White precipitate indicates sulfides or phenolic compounds.

Acidic Compounds:

- Effervescence Test: Gas bubbles with sodium bicarbonate indicate carboxylic acids.
- Litmus Test: Red color with litmus paper indicates acidity.

Resins:

- Solubility Test: Insolubility in water and petroleum ether indicates resin.
- Hydrochloric Test: Pink color with acetone and hydrochloric acid indicates specific compounds.

Organoleptic evaluation

Organoleptic evaluation of the product was conducted through sensory and visual inspections to assess its color, taste, and texture. This evaluation involved comparing the product's attributes with those of marketed patches.

- **Color:** The color of the product was observed and compared to ensure it matched the desired or expected shade. This step helped in determining if the product's appearance was consistent with standards or if it deviated significantly from what is typically available in the market.
- **Taste:** The taste of the product was assessed, which is particularly relevant if the product is intended for oral application or ingestion. This sensory evaluation was used to confirm that the product's taste was acceptable and in line with consumer expectations or market standards.



- **Texture:** The texture of the product was examined to evaluate its feel and consistency. This included assessing whether the texture was smooth, gritty, or otherwise, and whether it was comparable to the texture of similar products available in the market.

Overall, this organoleptic evaluation was aimed at ensuring that the product met quality standards and was comparable to or better than commercially available patches in terms of its sensory attributes.

Drug- Excipients Compatibility study by Differential scanning calorimetry

Differential Scanning Calorimetry (DSC), conducted using the JADE DSC from Perkin Elmer, USA, was employed to investigate the thermal properties and compatibility between the drug and excipients. To perform this analysis, mixtures of the extract and excipients were prepared in a 1:1 mass/mass ratio. The DSC analysis involved scanning these drug-excipient mixtures over a temperature range of 50-220°C while maintaining an inert nitrogen atmosphere. The heating rate was set to 20°C per minute. The resulting thermograms were carefully examined for any interactions between the drug and excipients. This analysis aimed to detect changes in thermal behavior, such as shifts in melting points or changes in heat flow, which could indicate potential interactions or incompatibilities between the components (Singh *et al.*, 2011)

pH

The pH of the product was determined by first dissolving 1 gram of the product in 9 milliliters of water, followed by vigorous shaking to ensure thorough mixing. The resulting aqueous solution was then tested for pH using a pH meter.

Moisture Content

To determine the moisture content of the product, “10 grams of the sample were weighed and placed in a porcelain dish. The dish was then dried in an oven at 105°C. After drying, the dish was allowed to cool in a desiccator. The loss in weight was calculated to determine the percentage moisture content using the following formula”:

$$\% \text{ moisture} = \frac{\text{Original sample weight} - \text{dry sample weight}}{\text{Original sample weight}} \times 100$$

Water Soluble Extractive

“Accurately weighed air dried powdered (2 g) material were taken in a glass stoppered flask and macerated with 100 ml of water .then it was shaken frequently for 6 hrs in a shaker and then allowed to stand for 18 hrs. After that 10 ml of filtrate was evaporated to dryness in a tared flat bottomed and transferred to petri dish and dried at 105 c and cool in a dessicator. The percentage of water soluble extractive was calculated with reference to air dried drug.”

Determination of Volatile Matter

The procedure for determining the moisture content of a sample involves several key steps. “First, a Petri dish was dried and accurately weighed. Then, 2 grams of the sample were carefully weighed and placed in the Petri dish. The sample was dried in an oven at 105 ± 2°C for 4 hours or until it reached a constant mass. After drying, the Petri dish with the sample was allowed to cool in a desiccator to prevent moisture absorption from the air. Finally, the cooled dish and sample were weighed again. The difference in weight before and after drying was used to calculate the moisture content of the sample.”

Volatile content (%) = (loss in weight/weight of the sample in g) x 100

Test for Hard and Sharp-Edged Abrasive Particles

To assess the quality of the toothpowder, a sample weighing 5 grams was transferred to a 50 ml beaker. Water was added gradually to the beaker while stirring, until a gel-like consistency was achieved. The prepared gel was then spread onto butter paper. To test for the presence of hard or sharp-edged abrasive particles, the paste was pressed along the length with a finger. Any hard or sharp particles would be easily distinguishable from other components. The toothpowder was considered acceptable if it was free from such abrasive particles.

Viscosity

The viscosity of the formulated gels was measured using a Brookfield Viscometer. The measurement was conducted at a speed of 500 rpm and at a temperature of 25°C.

Spreadability

The spreadability of the gel formulations was assessed by measuring the spreading diameter of 1 gram of gel placed between two horizontal plates. The diameter of the spread gel was recorded to evaluate its spreadability.

Stability Study (Storage Stability)

The patches were stored at 40°C and 75% RH for 90 days. Flavonoid and phenolic contents were assessed at five points, starting from day zero, every 18 days. A 1 ml sample was refluxed with 75 ml distilled water and ethanol for 30 minutes, filtered, centrifuged, and the supernatant was adjusted to volume. This process was repeated for each sample, and the contents were determined (Jain, 2006; U.S. Patent 7083779; Swami *et al.*, 2012).

3. RESULTS AND DISCUSSION

In this chapter, we discuss the evaluation of fresh fruits of blueberries and bananas for their antimicrobial and anti-aging properties. The active constituents from these fruits were isolated, and subsequently, herbal formulations were prepared and assessed. The observations and results from these evaluations are detailed in the following sections.



Extraction, Isolation and Screening of Activity (Blue Berry)
Preliminary Investigation of the Methanolic Extract of *Blue Berry*.

Table 1: Physical characteristics

Extracts	Consistency	Colour	Odour	Taste	% Yield
Methanolic extract	Solid	Dark purple	Pungent	sweet	11.68

Table 2: Different Ash values of berries

S No	Total ash (%w/w)	Acid Insoluble Ash	Water soluble Ash (% w/w)
1	4.64%	2.08%	1.52%

Table 3: Different Extractive values of Blue berry Fruits.

S. No.	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)
1	12.20%	6.43%

Table 4: Moisture content of *Berries*

S.No.	Moisture content (% w/w)
1.	40.87%

Table 5: Quantitative chemical tests

Chemical Tests	Blue berries Methanolic extract	Banana Methanolic extract
Alkaloids		
<i>Mayer's reagent test</i>	--	+++
<i>Hager's reagent test</i>	--	++
<i>Wagner's reagent test</i>	--	++
<i>Dragendorff's reagent test</i>	--	+++
General Glycosides (+Ve)		
<i>Baljet test</i>	++	++
<i>Legal's test</i>	+	+
<i>Keller-Kiliani</i>	++	++
Phenols/Tannins		
<i>Ferric chloride</i>	+++	+++
<i>Gelatin Solution</i>	++	++
<i>Lead acetate test</i>	++	++
Flavonoids		



<i>FeCl3 test</i>	++	++
<i>Alkaline reagent test</i>	++	++
<i>Shinoda test</i>	++	++
Saponins		
<i>Foam test</i>	+	--
<i>Hemolytic test</i>	-	-
<i>Lead acetate</i>	++	+
Fixed oil/Fats		
<i>Spot</i>	-	-
<i>Saponification</i>	-	-
Gums & Mucilage		
<i>Water</i>	-	-
Carbohydrates		
<i>Molish test</i>	--	++
<i>Fehling's solution test</i>	++	++
<i>Benedict's test</i>	--	++
Amino acids		
<i>Ninhydrin Test</i>	--	++
<i>Millons Test</i>	-	++
<i>Xantoprotein Test</i>	--	++
Terpenoids		
<i>Lieberman Burchard Test</i>	+	++
<i>Salkowski test</i>	+	+
Steroids		

Drug- Excipient Interaction Studies (By DSC)

The DSC analysis of the methanolic extracts of Blueberries and Bananas, along with their formulations, revealed that both extracts maintained their thermal stability. The Blueberry extract had a melting point of 24.50°C, and after 15 days of accelerated conditions (40°C, 75% RH), the peak shifted slightly to 24.80°C, indicating no significant interaction with the excipients. Similarly, the Banana extract, with a melting point of 22.50°C, showed no change in its peak after exposure to the same conditions, remaining at 22.50°C. These results suggest that the extracts retained their stability and no significant formulation-related interactions occurred under stressed conditions.

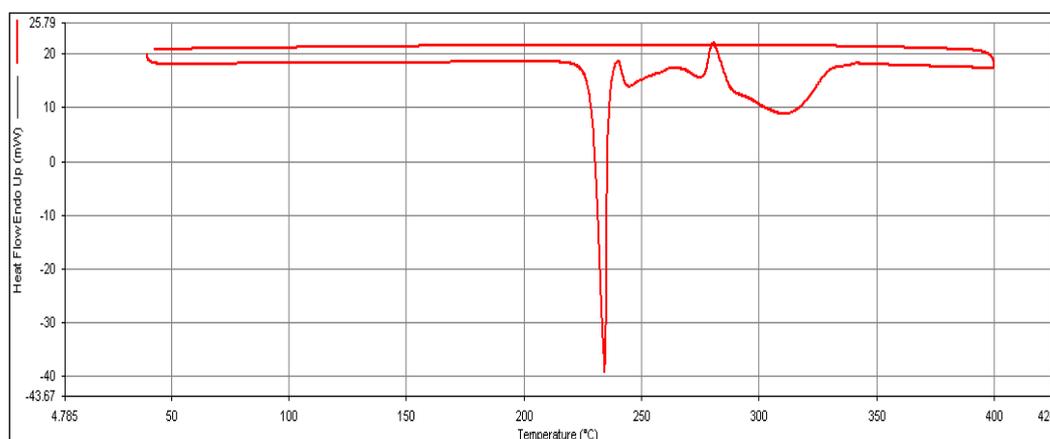


Fig 1: DSC of Methanolic Extract of Blue berry

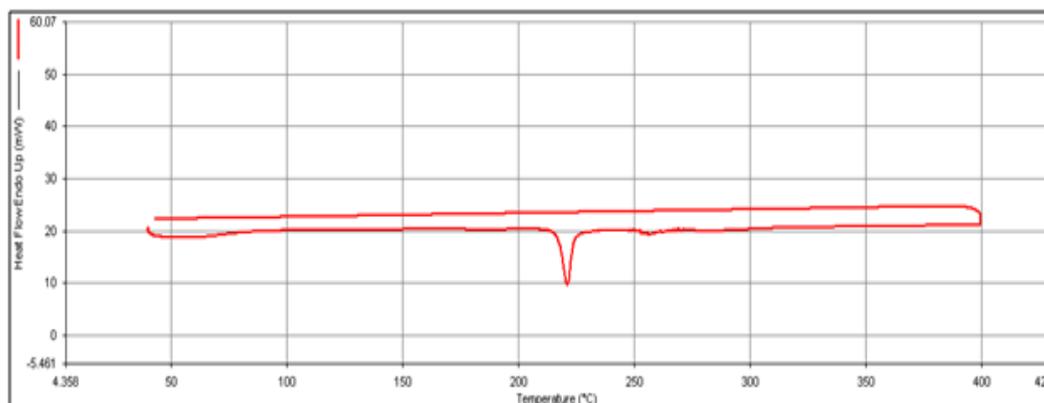


Fig 2: DSC of Methanolic Extract of Banana

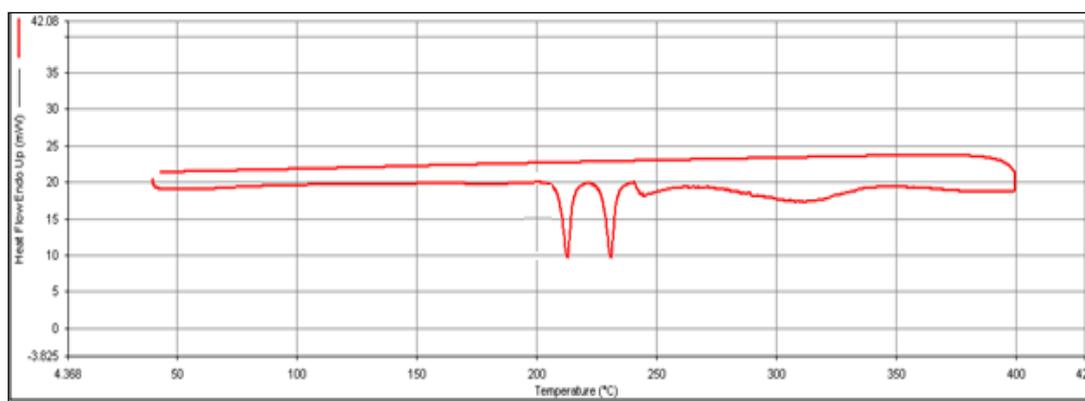


Fig 3: DSC of all excipient and Extracts (Formulation)

Development of Poly Herbal Gel

Table 6: Formulation composition

Ingredient /Formulations	F1	F2	F3	F4	F5	F6	F7	F8
Carbapol (g)	1	0.25	0.5	0.75	0.4	0.6	1	0.5
Propylene glycol (ml)	5	5	5	5	5	5	5	5
Potassium sorbate (ml)	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25
Isopropyle myristate (ml)	5	5	5	5	5	5	5	5
Blueberry extract fraction (g)	2	2	2	2	2	2	2	2
Banana extract active fraction (g)	3	3	3	3	3	3	3	3
Alcohol (ml)	25	25	25	25	25	25	5	25
Cremophor (g)	2	2	2	2	2	2	2	2
Water Q.S (ml)	100	100	100	100	100	100	100	100

The evaluation of the physical characteristics of the herbal gel formulations revealed that all formulations (F1 to F5) were semi-solid, light yellow in color, and had a mood-elevating odor. The reference formulation (F6) was also semi-solid but white in color, while still maintaining the same mood-elevating odor. Overall, the formulations shared similar physical characteristics, with the main difference being the color, indicating uniformity in the gel's base properties.



Table 7: Evaluation of physical characteristics of herbal gel

Formulation	Consistency	Colour	Odour
F1	Semi solid	Light yellow	Mood elevating
F2	Semi- solid	Light yellow	Mood elevating
F3	Semi- solid	Light yellow	Mood elevating
F4	Semi- solid	Light yellow	Mood elevating
F5	Semi- solid	Light yellow	Mood elevating
Reference F6	Semi- solid	White	Mood elevating

pH

The pH values of all formulations are consistent and close to neutral, which is ideal for most topical applications. The slight variations observed between the formulations are within a range that should not significantly impact their performance or stability

Table 8: pH of the gel

S. no.	Formulation Code	pH
1	F1	7.0
2	F2	7.1
3	F3	7.0
4	F4	7.0
5	F5	7.1
6	Reference F6	7.0

Evaluation for Fragrance

All formulations, including the reference, were evaluated positively for fragrance, indicating that the gels have a desirable and consistent scent quality.

Table 9: Evaluation of Herbal Gel on Different Parameters

S. no.	Evaluation Parameter	Grades on the basis of evaluation criteria					Reference grade
		F1	F2	F3	F4	F5	
1	Fragrance	A	A	A	A	A	A

Moisture Content / Volatile Content

For the herbal gel formulations, the percentage of moisture content varied slightly across different samples. Formulation F1 had a moisture content of 25%, which is consistent with the reference grade. Formulation F2 exhibited a slightly higher moisture level at 28%. Formulation F3 also had a moisture content of 25%, similar to F1. Formulation F4 showed a moisture content of 26%, while F5 had the highest moisture content at 29%. The reference grade maintained a moisture content of 25%, aligning with Formulations F1 and F3. Overall, the moisture content across the different formulations is comparable, with slight variations observed.



Table 10: Moisture content in herbal gel

Formulation Code	F1	F2	F3	F4	F5	Reference grade
% moisture content	25	28	25	26	29	25

Physical Evaluation

The physical evaluation of Formulation F3 showed the following results: The total ash value was 5.50%, matching the reference grade, indicating the total ash content is within an acceptable range. The acid-insoluble ash was 1.25%, within the specified range of 1.23% to 1.25%. The water-soluble ash was 3.7%, falling within the acceptable range of 3.5% to 3.7%, reflecting effective water solubility. Chloroform soluble extractives were 4.25%, matching the reference grade range of 4.22% to 4.25%, showing consistent solubility in chloroform. Ethanol soluble extractives were 10%, within the reference range of 9% to 10%, indicating good solubility in ethanol. Petroleum soluble extractives were 1.45%, within the reference range of 0.5% to 1.5%, indicating appropriate solubility in petroleum. The water-soluble extractives were 11.5%, within the reference range of 10% to 12%, demonstrating effective extraction properties. Overall, the physical characteristics of F3 are consistent with the reference grade, meeting the required specifications.

Results of Physical Evaluation of F3

Formulation F3 meets the evaluation criteria by being free from hard and sharp-edged abrasive particles, ensuring safety and comfort during use. It also passes the fineness test, indicating an appropriate particle size distribution that contributes to its smooth texture and consistency. Overall, Formulation F3 satisfies the standards for both the absence of abrasive particles and fineness, aligning with the reference grade.

Table 11: Results of physical evaluation of F3

S.No.	Physical parameters	%w/w	Reference grade
1.	Total ash value	5.50	5.50
2.	Acid insoluble ash	1.25	1.23-1.25
3.	Water soluble ash	3.7	3.5-3.7
4.	Choloroform soluble extractives	4.25	4.22-4.25
5.	Ethanol soluble extractives	10	9-10
6.	Petroleum soluble extractives	1.45	0.5-1.5
7	Water soluble extractives	11.5	10-12

Table 12: Results of evaluation of F3

S. No.	Hard and sharp edged abrasive particles	Fineness
1	Absent	Passes
2	Absent	Passes
3	Absent	Passes
4	Absent	Passes
5	Absent	Passes
Reference grade	Absent	Passes

Storage Stability of Optimized Formulation

The storage stability of the formulations was evaluated under different conditions and compared to a reference grade. At 40°C for 45 days, formulations F1, F2, F4, and F5 were graded B, indicating significant changes or degradation, while F3 and the reference grade maintained an A grade, showing better stability at high



temperatures. At room temperature for 45 days, all formulations (F1 to F5) and the reference grade received an A grade, indicating stability under standard conditions. At 5°C for 45 days, all formulations and the reference grade also achieved an A grade, demonstrating excellent stability in cooler conditions. Overall, most formulations were stable at room and cooler temperatures but showed varying instability at higher temperatures, with F3 and the reference grade performing the best in maintaining stability.

Table 13: Evaluation of storage stability

S. no.	Storage stability	Grades on the basis of evaluation criteria					Reference grade
1	45 days at 40°C	B	B	A	B	B	A
2	45 days at room tem.	A	A	A	A	A	A
3	45 days at 5°C	A	A	A	A	A	A

Table 14: *In vitro* drug release study of polyherbal Formulation

Time (min.)	Cumulative % of Drug Release					
	F1	F2	F3	F4	F5	F6
5	26.35	24.65	21.26	15.26	12.23	21.26
10	33.63	29.36	26.36	20.36	18.56	26.36
15	42.26	35.26	33.26	29.69	38.23	33.26
20	45.56	42.56	40.25	35.65	45.56	40.25
25	50.26	48.89	55.56	43.48	50.26	55.56
30	55.45	52.15	65.36	48.87	56.25	65.36

Release Kinetics of Polyherbal Gel

Formulation F3 shows the highest cumulative drug release, reaching 65.36% at 30 minutes. F6 also exhibits high drug release, similar to F3, at 30 minutes. F5 demonstrates a relatively high release at 30 minutes, with 56.25%. F1 and F2 show moderate drug release profiles compared to F3 and F6. F4 has the lowest drug release overall, except at 25 minutes, where it shows a higher release than at other times. Overall, F3 and F6 exhibit superior drug release characteristics, suggesting they may be more effective in drug delivery.

Table 15: Zero order release kinetics data of Formulation F1-F5

S.No.	Time in minutes	% Cum. drug release					
		F1	F2	F3	F4	F5	F6
1.	0	0	0	0	0	0	0
2.	5	26.35	24.65	21.26	15.26	12.23	21.26
3.	10	33.63	29.36	26.36	20.36	18.56	26.36
4.	15	42.26	35.26	33.26	29.69	38.23	33.26
5.	20	45.56	42.56	40.25	35.65	45.56	40.25
6.	25	50.26	48.89	55.56	43.48	50.26	55.56
7.	30	55.45	52.15	65.36	48.87	56.25	65.36

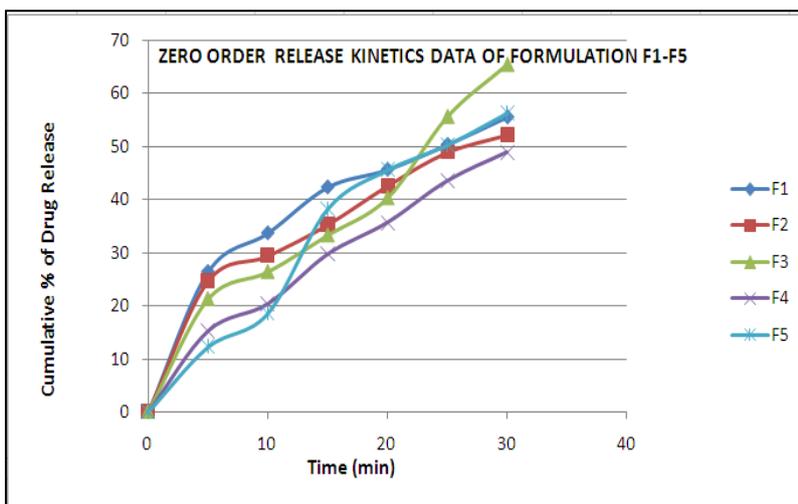


Fig 4: Zero order release kinetics data of Formulation F1-F5

Table 16: First order release kinetics data Formulation F1-F5

S. No.	Time in minutes	Log % Cum. drug remain to be release					
		F1	F2	F3	F4	F5	F6 Nicco gum
1.	0	0	0	0	0	0	0
2.	5	1.867173	1.877083	1.896195	1.928088	1.943346	1.896195
3.	10	1.821972	1.849051	1.867114	1.901131	1.910838	1.867114
4.	15	1.761477	1.811173	1.824386	1.847017	1.790778	1.824386
5.	20	1.735918	1.759214	1.776338	1.808549	1.735918	1.776338
6.	25	1.696706	1.708506	1.647774	1.752202	1.696706	1.647774
7.	30	1.648848	1.679882	1.539578	1.708676	1.640978	1.539578

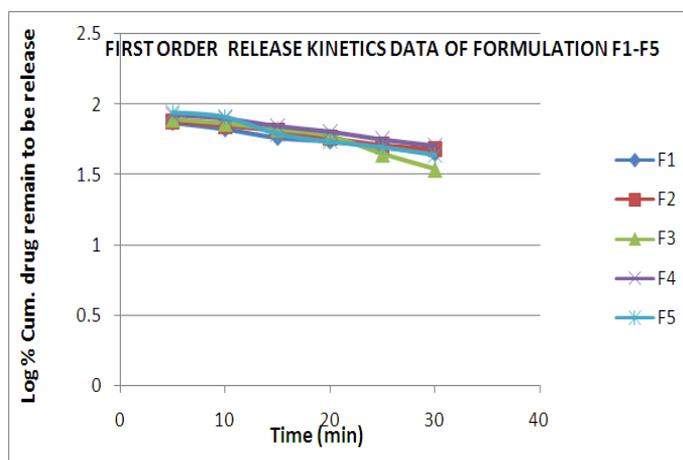


Fig 5: First order release kinetics data of Formulation F1-F5

Table 17: Higuchi release kinetics data of Formulation F1-F5

S.No.	Root Time	% Cum. drug remain to be release					
		F1	F2	F3	F4	F5	F6 Nicco gum
1.	0	0	0	0	0	0	0
2.	2.236068	73.65	75.35	78.74	84.74	87.77	78.74
3.	3.162278	66.37	70.64	73.64	79.64	81.44	73.64
4.	3.872983	57.74	64.74	66.74	70.31	61.77	66.74
5.	4.472136	54.44	57.44	59.75	64.35	54.44	59.75
6.	5	49.74	51.11	44.44	56.52	49.74	44.44
7.	5.477226	44.55	47.85	34.64	51.13	43.75	34.64

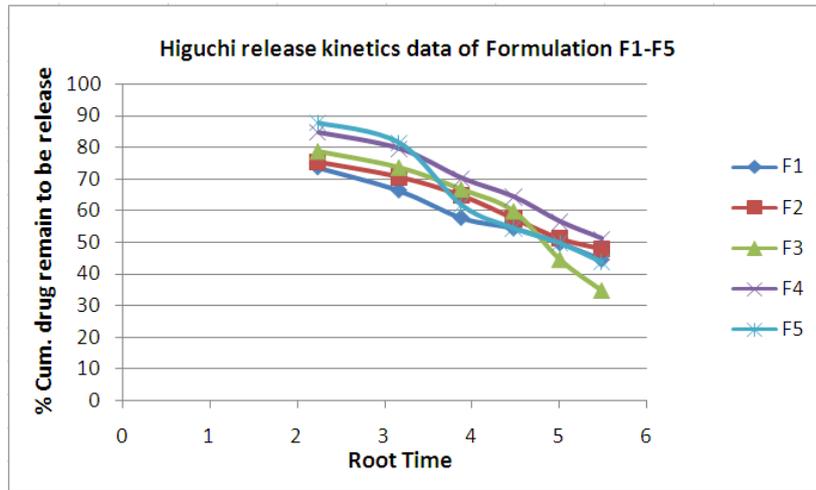


Fig 6: Higuchi release kinetics data of Formulation F1-F5

Table 18: Korsmayer Papas release kinetics data of Formulation F1-F5

S.No.	log Time	Log cum % drug release					
		F1	F2	F3	F4	F5	F6 Nicco gum
1.	0	0	0	0	0	0	0
2.	0.271185	1.867173	1.877083	1.896195	1.928088	1.943346	1.896195
3.	0.260542	1.821972	1.849051	1.867114	1.901131	1.910838	1.867114
4.	0.245877	1.761477	1.811173	1.824386	1.847017	1.790778	1.824386
5.	0.239529	1.735918	1.759214	1.776338	1.808549	1.735918	1.776338
6.	0.229607	1.696706	1.708506	1.647774	1.752202	1.696706	1.647774
7.	0.217181	1.648848	1.679882	1.539578	1.708676	1.640978	1.539578

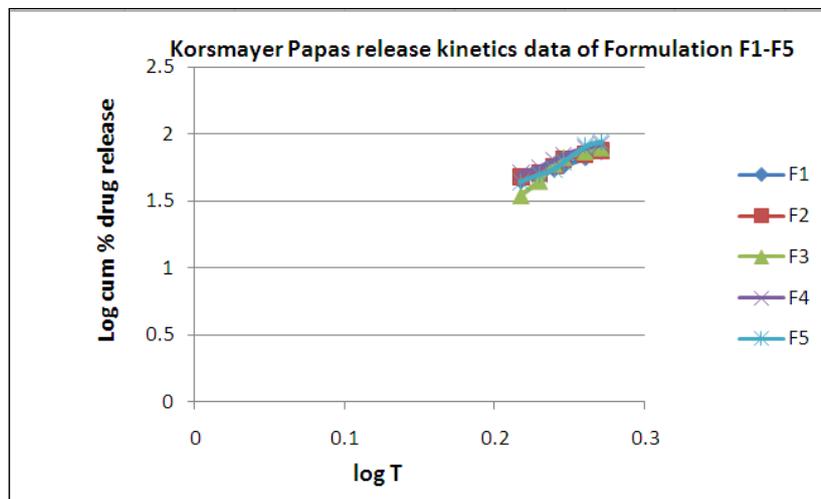


Fig 7: Korsmayer Papas release kinetics data of Formulation F1-F5

**Table 19:** Kinetic data of polyherbal formulation in comparison with all formulations

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMAYER PAPAS
F1	0.866	0.990	0.993	0.999
F2	0.900	0.990	0.977	0.969
F3	0.968	0.925	0.913	0.907
F4	0.978	0.993	0.981	0.985
F5	0.963	0.968	0.964	0.978
F6 Nicco gum	0.968	0.925	0.913	0.907

Summary and Conclusion

This study explored the anti-aging and antimicrobial properties of fresh blueberry and banana fruits. It began with phytochemical screening and identification of compounds in the extracts, forming the basis for understanding their therapeutic effects. The physical properties of the extracts were assessed, followed by screening for antioxidant and anti-aging activities. This led to the development of an anti-aging herbal transdermal patch, which was rigorously standardized and evaluated for physicochemical characteristics, pH, antioxidant properties, and in-vitro drug diffusion. A drug release kinetic study was also conducted to determine the release profile of the active compounds. The findings confirmed the significant anti-aging and antimicrobial potential of blueberry and banana extracts, culminating in an effective herbal patch formulation. Future research will focus on optimizing the anti-aging and antimicrobial potential of these extracts. Further studies could isolate and characterize specific bioactive compounds responsible for these effects, enabling more targeted formulations. Clinical trials could evaluate the efficacy and safety of the transdermal patches in humans. Expanding the research to include other complementary natural ingredients could enhance formulation effectiveness, paving the way for a broader range of cosmeceutical and pharmaceutical products based on these fruit extracts.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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