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Formulation, Optimization, and Evaluation of a Polyherbal Anti-Oxidant Lotion Incorporating Psidium guajava and Moringa oleifera Leaf Extracts

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ABSTRACT: The increasing demand for natural skincare products has led to the development of a polyherbal antioxidant lotion incorporating Psidium guajava (guava) and Moringa oleifera (moringa) leaf extracts. Both plants are known for their potent antioxidant properties, due to their high levels of flavonoids, phenolics, and vitamins. The extracts were prepared through cold maceration and tested for antioxidant activity, demonstrating significant free radical scavenging potential.

Different formulations of the lotion were developed using different concentrations of the extracts and evaluated for key physicochemical parameters, including pH, viscosity, spreadability, and texture. The optimized formulation exhibited a pH range of 5.8–6.5, smooth texture, and ideal viscosity for easy application. Stability testing over 30 days under various temperature conditions (4°C, 25°C, and 40°C) showed no signs of phase separation or instability.

The optimized formulation demonstrated high antioxidant activity, confirming the synergistic effects of Psidium guajava and Moringa oleifera extracts. The emulsion was characterized as oil-in-water (O/W), promoting skin hydration without greasiness. Microbial testing confirmed the absence of harmful microorganisms, ensuring the lotion's safety for use. A skin irritation test conducted on human volunteers showed no signs of redness, itching, or irritation, indicating excellent dermatological compatibility.

This polyherbal antioxidant lotion is a promising natural skincare product, offering protection from oxidative stress and contributing to skin health. Further clinical studies are recommended to explore its long-term efficacy and commercial viability.

KEYWORDS: Polyherbal lotion, Psidium guajava, Moringa oleifera, Antioxidant activity, Optimized formulation, Physicochemical parameters, Oil-in-water (O/W)

I. INTRODUCTION:

Herbal and plant-based skincare products. Herbal products are generally considered safer, more skin-friendly, and environmentally sustainable. They contain natural compounds that offer not only antioxidant protection but also anti-inflammatory, antimicrobial, and moisturizing benefits. Among the various forms of herbal products, lotions are widely used due to their smooth texture, ease of application, and ability to deliver active ingredients directly to the skin.

Psidium guajava (guava) and Moringa oleifera (moringa) are two medicinal plants well-known for their health-promoting properties. Guava leaves are rich in flavonoids like quercetin, tannins, carotenoids, and vitamin C, all of which are powerful antioxidants. These compounds help protect the skin from damage, reduce inflammation, and support skin healing. Moringa leaves, on the other hand, are packed with nutrients and bioactive compounds such as ascorbic acid, polyphenols, chlorogenic acid, and minerals. Moringa is known for its antioxidant, antibacterial, moisturizing, and anti-aging properties, making it a valuable ingredient in cosmetic formulations.

Both plants have been studied individually for their skincare benefits, but limited research has focused on combining them into a single topical product. Using both extracts together in a polyherbal lotion may produce better results due to the combined action of multiple antioxidants and nutrients. This approach, known as synergism, can enhance the overall effectiveness of the product while reducing the need for high concentrations of each ingredient.

Formulating a herbal lotion involves more than mixing plant extracts. It requires careful

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selection of ingredients, proper formulation methods, and thorough testing. A good lotion must be physically and chemically stable, have an appropriate pH for skin, spread easily, and feel pleasant to use. It must also be safe, free from harmful microbes, and maintain its effectiveness over time.

This study focuses on the formulation, optimization, and evaluation of a polyherbal antioxidant lotion containing guava and moringa leaf extracts. The research includes extraction of active compounds from the leaves, preparation of different lotion formulations, and selection of the best formulation based on parameters such as pH, viscosity, spreadability, stability, and emulsion type. The antioxidant activity of the final product is tested using standard methods. In addition, the lotion is evaluated for microbial safety and potential skin irritation to ensure it is safe for topical use.

By developing a stable and effective polyherbal lotion, this research aims to provide a natural and affordable skincare product that supports healthy skin, prevents oxidative damage, and meets the growing demand for herbal cosmetics.

II. MATERIALS AND METHODS:

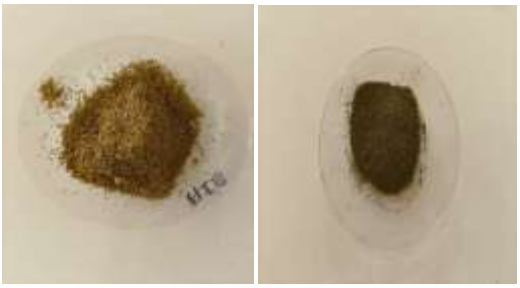
Fresh leaves of **Psidium guajava** (guava) and **Moringa oleifera** (moringa) were collected locally. The following ingredients and excipients were used in the formulation:

- **Guava leaf Extract**
- **Moringa leaf Extract**
- **Liquid paraffin** (emollient)
- **Cetyl alcohol** (emulsifying agent and thickener)
- **Stearic acid** (emulsifier and consistency agent)
- **Lanolin** (moisturizer and emollient)
- **Glycerin** (humectant)
- **Sodium benzoate** (preservative)
- **Rose oil** (fragrance)
- **Beetroot extract** (natural colorant)

All reagents and chemicals were of analytical grade and procured from certified suppliers.

Preparation of Plant Extracts:

Fresh guava and moringa leaves were washed thoroughly with distilled water, shade-dried, and ground to a coarse powder. The powdered material was subjected to cold maceration using methanol using a Soxhlet apparatus:



Guava and Moringa leaf dried powders

- **Guava leaf extract**: 50 g of dried leaf powder was extracted with methanol
- **Moringa leaf extract**: 50 g of dried moringa leaf powder was similarly extracted.

The obtained extracts were filtered, concentrated using a rotary evaporator, and stored at 4°C in amber-colored bottles until further use.

Preparation of herbal extracts by Soxhelet apparatus

Formulation of Polyherbal Lotion

The polyherbal lotion was prepared using the oil-in-water (O/W) emulsion method as follows:

Phase I (Oil Phase)

- Liquid paraffin
- Cetyl alcohol
- Stearic acid
- Lanolin

The oil phase ingredients were weighed and heated together to 70–75°C until completely melted.

Phase II (Aqueous Phase)

- Glycerin
- Sodium benzoate
- Alcoholic extracts of guava and moringa leaves (1:1 ratio)
- Distilled water – q.s. to 100%

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The aqueous phase was heated to the same temperature (70–75°C). The oil phase was then slowly added to the aqueous phase with continuous stirring using a homogenizer at 3000 rpm for 10 minutes to form a uniform emulsion.

Addition of Additives:

Once the lotion was formed and cooled to below 40°C:

- Rose oil – was added as a fragrance.
- Beetroot extract – was added as a natural colorant.

The final product was filled into clean, opaque containers and stored at room temperature for further evaluation.

Formulation of Polyherbal Lotion with leaf extracts

Formulated Lotion with natural colorant

Formulation of Polyherbal Lotion

Ingredients	F1	F2	F3	F4	F5
Guava leaf extract(gm)	0.4	0.3	0.1	0.15	0.15
Moringa leaf Extract(gm)	0.5	0.4	0.2	0.2	0.2
Liquid paraffin(ml)	2.5	2	2.5	2.3	2.4
Cetyl alcohol(gm)	1	1	1.2	1.2	1.2
Stearic Acid(gm)	1	0.75	0.9	1	1.2
Lanolin(gm)	1.75	1.5	1.6	1.7	1.4
Glycerin(ml)	1	1	1	1.5	2
Distilled Water	50	50	50	50	50
Sodium Benzoate (%)	0.2	0.2	0.2	0.2	0.2
Rose oil (%)	0.4	0.4	0.4	0.4	0.4
Beetroot extract (%)	0.3	0.3	0.3	0.3	0.3

Of the above formulations F1 & F3 are considered as the optimized formula showing good evaluation parameters.

EVALUATION OF HERBAL LOTION:

1. **Organoleptic properties:** The formulations were evaluated for their visual appearance, colour odor and homogeneity.
2. **Determination of pH:** The pH of the prepared herbal lotions was evaluated utilizing a pH analyzer at room temperature. The pH should be neutral.

pH meter

3**. Skin irritancy test:** The lotion was applied on the left-hand dorsal skin surface of 2sq.cm and observed in equal intervals up to 24 hrs for irritancy, sensitivity, and edema.

4. **Spreadability:** It was tested to determine how easily the lotion can be applied to the skin. About 1 gram of lotion was placed between two glass slides, and a 500 g weight was placed on top for 5 minutes. Then, a 30 g weight was used to pull the top slide, and the time taken for the slide to move was recorded.

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Spreadability was calculated using the formula:
S = (M × L) / T, where M is the applied weight (30 g), L is the length the slide moved (cm), and T is the time taken in seconds. A higher value indicates better spreadability.

Spreadability Test

5. **Removal/ Washability:** The removal test was carried out to assess how easily the lotion could be washed off from the skin surface. A small amount of the lotion was applied evenly on the back of the hand and allowed to remain for 10 minutes. After that, the area was rinsed with lukewarm water without using soap, and the ease of removal was observed visually and by touch. The formulation was considered easily removable if no greasiness or residue remained after washing, indicating good cosmetic acceptability.

6. **Sensitivity test:** The sensitivity test was performed to check for any allergic or irritant reactions caused by the lotion. A small amount of the lotion was applied to a clean area on the inner forearm of healthy human volunteers and left undisturbed for 24 hours. The area was then observed for signs of redness, itching, swelling, or irritation. Absence of any visible reaction indicated that the lotion was non-irritant and safe for topical use.

Sensitivity Test

7. **Viscosity:** It was measured to determine lotion thickness and flow properties. A Brookfield viscometer was used with spindle number 64 at 50 rpm at room temperature ($25 \pm 2°C$). The lotion sample was placed in a clean container, and the viscosity reading was recorded in centipoise (cP). An appropriate viscosity ensures the lotion is easy to apply and stays on the skin without running off.

8. **Accelerated stability studies**: It was conducted to evaluate the physical and chemical stability of the lotion over time under stress conditions. The formulated lotion was stored in tightly closed containers at three different temperature conditions: room temperature ($25 \pm 2°C$), refrigerated temperature ($4 \pm 2°C$), and elevated temperature ($40 \pm 2°C$) for a period of 30 days. Samples were observed at regular intervals (0, 15, and 30 days) for any changes in color, odor, pH, consistency, phase separation, and spreadability. The formulation was considered stable if no significant changes were observed during the study period.

9. **Skin permeation test:** It was carried out to evaluate the ability of the lotion to allow active herbal components to pass through the skin. An egg membrane was mounted on a Franz diffusion cell, with one side of the membrane facing the donor compartment. A fixed amount of the lotion was applied to the membrane surface, and the receptor compartment was filled with phosphate buffer (pH 7.4), maintained at $37 \pm 0.5°C$ and stirred continuously. At specific time intervals (e.g., 1, 2, 4, 6, and 8 hours), samples were withdrawn from the receptor compartment and analyzed using UV spectrophotometry to determine the amount of permeated active ingredients. The test helps assess how effectively the herbal actives (from guava and moringa extracts) penetrate the skin.

Franz diffusion cell

10. **Anti-oxidant activity:** The antioxidant activity of the combined guava and moringa leaf extracts was tested using the DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay method. Different concentrations of the extract were mixed with DPPH solution and kept in the dark for 30 minutes. The absorbance was measured at 517 nm, and the percentage inhibition of DPPH was calculated to evaluate the anti-oxidant activity of herbal extracts.

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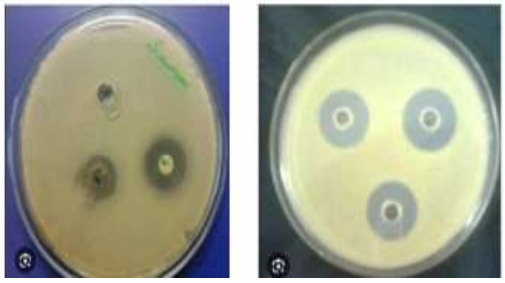
11. **Antimicrobial test:** The antimicrobial test for the formulated herbal lotion was carried out using the sterile agar plate method where the agar medium is uniformly spread in the plates and the inoculum was introduced onto the wells on the agar medium. Now the polyherbal formulation of varying concentrations was introduced into the bells along with the control. Now incubate the plates at 37^0C for above 24 hours. Further, the zone of inhibition (MIC) was determined for the specific concentration of the lotion and the area of the zone of inhibition was measured to calculate the anti-microbial activity.



Anti-microbial Activity

III. RESULTS AND DISCUSSION:

The formulations and evaluation of herbal lotion involves a meticulous process of selecting, combining and testing natural ingredients to create a product that is effective and safe for skin care. The chosen ingredients such as guava and moringa leaf extract, vitamin E, rose oil, and glycerin have numerous benefits such as moisturizing, anti-bacterial, and emollient effect. These ingredients are widely recognized in traditional and modern skin care routines.

During the formulation, the appropriate ratios of these ingredients are determined through optimization trials. This ensures the lotion achieved the desired consistency, spreadability and absorption without compromising the stability of the herbal extracts. The use of natural colorants such as beetroot extract enhances the safety profile of the lotion while maintaining the product's shelf life.

Phytochemical Screening of Herbal extracts

Phytochemical Test	Guava Extract	Moringa Extract
Alkaloids (Mayer's test)	+	+
Flavonoids (Alkaline test)	+	+
Tannins (Ferric chloride)	+	+
Saponins (Foam test)	+	+
Phenols (Ferric chloride)	+	+
Terpenoids (Salkowski test)	+	+
Glycosides (Keller-Kiliani)	+	+
Carbohydrates (Molisch's test)	+	+

Results for Anti-oxidant activity of Herbal Extracts

Concentration (µg/ml) of herbal extracts	Absorbance	%Inhibition
25	0.612	32.4
50	0.495	45.1
75	0.382	57.6
100	0.295	66.9
125	0.210	75.1
150	0.162	80.0

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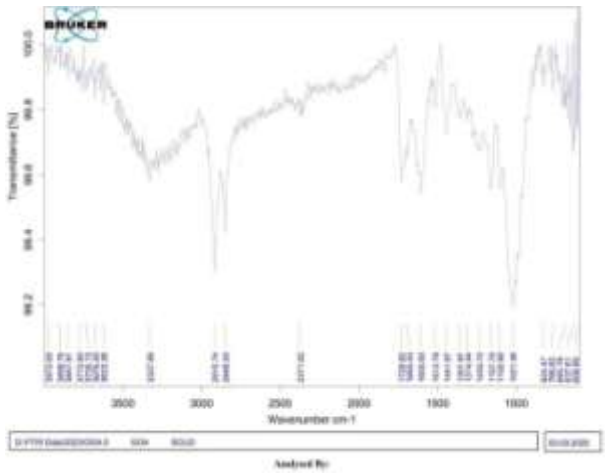
Evaluation test results for optimized formulations

Parameter	F1	F3
Homogeneity	Uniform with no phase separation	Uniform with no phase separation
Appearance	Pale pink with smooth texture	Pale pink with smooth texture
Odor	Pleasant	Pleasant
pH	5.97	6.17
Irritancy	No redness or itching	No redness or itching
Washability	Easily removed without greasiness	Easily removed without greasiness
Spreadability	4.7cm	5.3cm
Viscosity	3000cps	3500cps
Moisture content	65%	60%
Skin permeation	94%	90%

Anti-microbial activity results

Formulation	Zone of Inhibition(mm)		
	E.Coli	Pseudomonas	Staphylococcus
F1	15	13	17
F3	13	9	12

FTIR spectrum of combined leaf extract:



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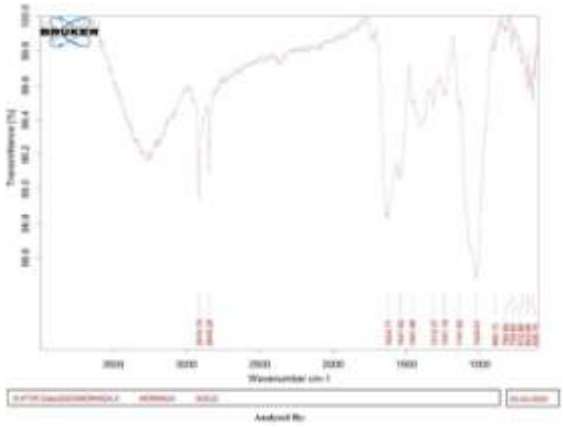
FTIR spectrum of Optimized formulation



FTIR spectral studies were conducted to identify the functional groups present in the herbal drugs Guava and moringa and to evaluate the compatibility of these constituents in the optimized polyherbal formulation. The herbal drug extracts showed major peaks at 3330, 2915, 1728, 1605, 1021cm^{-1}which may be due to functional groups such as phenols, alcohols, carboxylic acids and other aromatic rings in the chemical constituents such as alkaloids, glycosides terpinoids etc.The presence of all major functional group peaks in the optimized formulation suggests that there were no significant chemical interactions between the individual components. No major shifting, disappearance or formation of new peaks was observed indicating that the herbal ingredients are compatible with the excipients used in the formulation supporting the stability of the polyherbal formulation.

IV. SUMMARY AND CONCLUSION:

The present study focuses on the formulation and evaluation of a polyherbal preparation incorporating Guava (Psidium guajava) and Moringa (Moringa oleifera) leaf extracts. These plants are known for their potent pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. The objective of this project was to develop a stable polyherbal formulation and assess its physicochemical, phytochemical, and biological properties to determine its therapeutic potential.

The formulation was prepared using standardized extracts of guava and moringa leaves, blended with suitable excipients to enhance stability and bioavailability. The choice of formulation was optimized based on solubility, stability, and ease of administration.

The formulated poly herbal lotion showed a smooth texture and uniform consistency with no phase separation, indicating good compatibility of the herbal ingredients with the bases used in the formulation. The pH of the formulation is about 5.97, ensuring skin compatibility and no irritation. A patch test done on the formulation signifies no skin irritancy. Anti-microbial studies of the polyherbal lotion confirmed the anti-bacterial activity of guava and moringa leaf extracts against skin disease conditions such as acne and skin inflammations. Stability studies of the lotion reveal no significant changes in physical properties over 4 weeks of observation, highlighting the formulation's robustness. Spreadability test demonstrated easy application on the skin without leaving a greasy residue. Skin permeation studies were performed using a Franz diffusion cell using the synthetic membrane reveals good permeation of active ingredients into the skin. FTIR analysis confirmed no undesirable interactions between the herbal ingredients and the bases used in the formulations, suggesting the stability of the lotion. Viscosity was measured using a Brookfield viscometer at varying shear rates to assess the rheological behavior. Physical properties of the lotion such as color, odor, and texture are uniformly stable throughout the storage, ensuring the stability of the polyherbal lotion. The formulated herbal lotion can easily be washed off from the skin with water, leaving no greasiness. The moisture content of the formulated lotion was found to be 65%, which indicates that the herbal lotion is w/o type of emulsion, which can be used for a hydration and emollient effect on the skin.

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