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DEVELOPMENT AND EVALUATION OF A MULTI-HERBAL FACE SERUM RICH IN NATURAL ANTIOXIDANTS

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ABSTRACT

Face serums provide targeted benefits such as hydration, anti-aging, skin brightening, acne control, and protection against environmental damage. The herbal ingredients in this formulation include mango leaves, guava leaves, rosemary leaves, and grape seed extract, all rich in antioxidants like mangiferin, flavonoids, polyphenols, and proanthocyanidins that support skin health and delay aging. Mangiferin is a strong polyphenolic antioxidant that neutralizes reactive oxygen species generated by ultra violet radiation and pollution, thereby protecting epidermal and dermal cells from oxidative damage, inflammation, and photoaging. Flavonoids neutralizes free radicals while polyphenols and proanthocyanidins reduces signs of aging and inflammation. This study focuses on the extraction, formulation and evaluation of face serum containing these antioxidant constituents. The serum is prepared using key ingredients like Carbopol 940, glycerin, tween 80, orange oil, vitamin E, sodium benzoate and triethanolamine. The polymer was allowed to swell overnight to which all the ingredients are added one by one with continuous stirring to obtain a homogenous mass. Evaluation revealed a milky white, translucent, homogenous serum with a pH of 5.16 suitable for skin. Spreadability was measured at 0.51gcm/sec while globule size determination was also carried out. 2,2-diphenyl-1-picrylhydrazyl scavenging activity was found to be 40% and stability studies were conducted at various conditions showed no signs of phase separation. Results indicate that the formulations were skin-friendly, stable and effective providing antioxidant protection, supporting the potential of polyherbal face serums in modern skincare.

KEYWORDS: face serum, antioxidant, mango, guava, rosemary, grape seed, DPPH, cosmetic.

INTRODUCTION

The examination of human skin is a crucial area of research in dermatology, toxicology, pharmacology, and cosmetology, as it enables the assessment of how external substances interact with the skin, how they are absorbed, and their potential effects and toxicity on different skin components. From prehistoric eras onward, humans have acknowledged the importance of beautification, with the pursuit of a healthy and attractive appearance continually developing throughout societal evolution.[1] Aging is a complex, multifaceted process arising from both intrinsic and extrinsic mechanisms, which together lead to the progressive deterioration of skin structure and physiological function.[2] Antioxidants play a crucial role in skin aging as they act as natural free radical scavengers. They are highly effective in preventing skin aging and in improving damages caused by aging and UV exposure.[3] A facial serum is a skincare product in the form of a light, moisturizing gel or lotion that contains active ingredients that can penetrate deeper into the skin. Serums often have a light and absorbent texture, created to deliver targeted benefits such as intensive hydration, skin rejuvenation or antioxidant protection. Typically, serums are used as an additional step in the skincare routine after cleansing and before moisturizer application.[4] As a cosmetic formulation, serums provide the advantage of delivering a high concentration of active ingredients. Plant-derived active compounds, such as flavonoids, are frequently used in topical applications because of their strong antioxidant

properties.[5] Mangiferin's xanthonoid framework, characterized by a C-glycosyl bond and multiple hydroxyl groups, underlies its strong free radical–scavenging capacity, resulting in potent antioxidant activity and a wide range of biological effects.[6] Proanthocyanidins (PCs) scavenge for free radicals and prevent the production of reactive oxygen species (ROS). Unlike retinoids, peptides, and vitamin C, PCs offer distinctive anti-aging benefits by enhancing glycolipid metabolism and improving microvascular circulation, which they achieve by inhibiting fat cell formation and supporting mitochondrial function.[7] Gordan said that when reacting with lipid and hydroxyl radicals to create stable molecules, the phenolic compounds found in commercial preparations of rosemary (extract) serve as the principal antioxidants.[8] The DPPH assay is a simple spectrophotometric method used to evaluate antioxidant activity by measuring the reduction in absorbance at 517 nm as antioxidants scavenge the stable DPPH free radical.[9]

MATERIALS AND METHODS

Plant Profile

Mangifera indica

Fig.1 Mango leaves.

Table 1: Plant profile of *Mangifera indica.*

Scientific name	***Mangifera indica***
Family	Anacardiaceae
Division	Mangoliophyta
Class	Magnoliopsida
Order	Sapindales
Maximum height	A full size tree can grow over 100 feet

Morphology

Young leaves exhibited light green coloration or light green with a brownish tinge, while mature leaves ranged from pale green to green and dark green. Leaf blade shapes included elliptic, oblong, ovate, obovate, lanceolate, and oblanceolate. The leaf apex was observed to be obtuse, acute, or acuminate, and the leaf base varied from acute to obtuse or rounded. Leaf margins were either entire or wavy, and the angle between the midrib and secondary veins was classified as narrow less than 45 °, medium 45 ° to 60 °, or wide greater than 60 °. Leaf textures were recorded as coriaceous, chartaceous, or membranous.[10]

Distribution

It is cultivated extensively across tropical and subtropical regions around the world and is considered a key agricultural crop in South and Southeast Asia. Leading producer nations include Pakistan, India, Bangladesh, Thailand, China, Indonesia, the Philippines, Mexico, and Nigeria.[11]

Phytoconstituents

Mangiferin is the most biologically active compound found in mango leaves, along with phenolic acids, benzophenones, and other antioxidant constituents such as flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols.[12]

Uses

- Mango leaf extracts are rich in phenolic compounds such as mangiferin, gallic acid, and quercetin glycosides, which exhibit strong antioxidant activity and inhibit elastase, an enzyme linked to skin aging.
- The phenolic components have also been shown to suppress tyrosinase activity, which plays a central role in melanin production.
- Due to their antioxidant and enzyme-inhibiting properties, mango leaf phenolics offer natural bioactivity comparable to conventional cosmetic ingredients like tocopherol for anti-aging and ascorbic acid for skin brightening, highlighting their potential as safer, plant-derived alternatives in cosmetic formulations.[13]

Psidium guajava

Fig.2: Guava leaves.

Table 2: Plant profile of *Psidium guajava.*

Scientific name	Psidium guajava
Family	Myrtaceae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Maximum growth	A full size tree can grow around 20 feet

Morphology

Guava leaves exhibit an opposite leaf arrangement, a leathery consistency, and an elliptical to oval form. They usually measure 7 to 15 cm in length and 3 to 5 cm in width. The leaves are marked by distinct, parallel veins that arise from the central midrib and spread toward the margins.[14]

Distribution
Psidium guajava (L.) is classified under the family Myrtaceae and is extensively grown in tropical areas, including India, Indonesia, Pakistan, Bangladesh, and regions of South America.[14]

Phytoconstituents
Guava leaves (GLs) contain health-enhancing micro- and macronutrients, along with various bioactive compounds. Their composition consists of about 82.47% moisture, 3.64% ash, 0.62% fat, 18.53% protein, 12.74% carbohydrates, 103 mg of ascorbic acid, and a total phenolic content of 1717 mg gallic acid equivalents per gram. Extensive research has highlighted the health-promoting properties of guava leaves, which are largely contributing to their diverse phytochemical profile. These compounds include quercetin, apigenin, kaempferol, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid.[14]

Uses
- Guava leaf extracts are abundant in polyphenols and flavonoids, which demonstrate potent antioxidant activity and the ability to inhibit enzymes such as collagenase that are responsible for collagen degradation in the skin.[15]
- Guava leaf extract has been reported to exhibit anti-inflammatory activity by lowering the production of inflammatory mediators, thereby soothing irritated skin and potentially enhancing skin comfort when used in cosmetic serum formulations.[16]

Rosmarinus officinalis

Fig.3: Rosemary leaves.

Table 3: Plant profile of *Rosmarinus officinalis.*

Scientific name	*Rosmarinus officinalis*
Family	Lamiaceae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Maximum height	A full-size plant can grow around 2-6 feet

Morphology
Rosemary leaves are simple, evergreen, and slender, with a needle-like shape ranging from linear to lanceolate. They are typically 2 to 4 cm long and 2 to 5 mm wide, and are oppositely arranged on the stem with little or no visible petiole. The leaf edges are slightly rolled downward (revolute). The lower surface bears both glandular trichomes (capitate and peltate) and non-glandular hairs, which serve as sites for essential oil production and are responsible for the plant's distinctive aroma.[17]

Distribution
Rosemary originates from the Mediterranean Basin, especially the coastal and arid rocky areas of southern Europe, North Africa, and western Asia, where it developed naturally before being widely distributed across the world through cultivation.[17]

Phytoconstituents
Rosemary essential oil mainly contains 1,8-cineole (15–20%), camphor (15–25%), borneol (16–20%), bornyl acetate (7%), and α-pinene (about 25%). It also includes smaller amounts of other compounds such as β-pinene, linalool, camphene, limonene, β-cymene, terpinolene, thujene, copaene, terpinen-4-ol, α-terpineol, caryophyllene, methyl chavicol, and thymol.[18]

Uses
- Rosemary-based cleansing creams are suitable for oily skin types. Rosemary oil is widely incorporated into products such as soaps, room fresheners, deodorants, perfumes, and skin lotions, either alone or mixed with other herbal oils.[19]
- Studies have shown that prolonged use can enhance skin elasticity, strengthen the skin barrier, and reduce wrinkle depth and dark spots.
- Extracts of *Rosmarinus officinalis* leaves are commonly used at concentrations of up to 10% in hand and body care products, and up to 3% in formulations such as eyeshadows, soaps, and detergents.

Vitis vinifera

Fig.4: Grape seed.

Table 4: Plant profile of *Vitis vinifera.*

Scientific name	*Vitis vinifera*
Family	Vitaceae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Vitales

Morphology
Grape seeds are ovoid or rounded, tapering to a beak, and featuring a dorsal side with a deep furrow and a

ventral side with two depressions separated by a ridge (raphe).[20]

Distribution

Grapefruit seeds are geographically distributed where humans cultivate grapefruit, mainly in subtropical regions across America, Asia, Africa, Europe, and Australia.`

Phytoconstituents

Grape seed (from *Vitis vinifera*) contains several important phytoconstituents, mainly polyphenols such as oligomeric proanthocyanidins (OPCs), catechin, epicatechin, and gallic acid. It is also rich in flavonoids, phenolic acids, and tannins, which provide strong antioxidant and anti-inflammatory effects. In addition, grape seeds contain fatty acids like linoleic and oleic acid, along with sterols and small amounts of vitamin E.[21]

Uses

- Grape seed extract is rich in polyphenols which protect skin from damage due o free radical and premature aging.
- It helps to reduce wrinkles and fine lines by improving skin elasticity and collagen strength.
- Grape seed oil is lightweight, non-greasy, and deeply hydrates the skin.
- Its antimicrobial and anti-inflammatory activities help in reducing acne and calming irritated skin.
- It also strengthens hair, adds shine, and nourishes the scalp in shampoos and hair oils.[22]

Method of extraction

Mangifera indica

Mango leaves were dried and ground into a powder and soaked in ethanol (at room temperature, using 5 L of ethanol per kg of leaf material) for 24 hours, then filtered. The remaining residue was extracted again with ethanol, and the solvent was subsequently removed by evaporation.[23]

Psidium guajava

The dried guava leaves were ground into a coarse powder and extracted by soaking in 99.8% ethanol (ratio 1:4) for 24 hours at room temperature. The filtrate is then concentrated.[24]

Rosmarinus officinalis

The dried rosemary leaves were soaked in 96% ethanol at a 1:10 ratio for maceration. Once the extraction was complete, the mixture was filtered through Whatman filter paper, and the solvent was evaporated to yield a powdered extract.[25]

Vitis vinifera

A solution containing 40 ml of water and 360 ml of ethanol was added to 100 g of whole grape seeds. The resulting extract was then filtered through filter paper, and then the solvent was evaporated to concentrate the extract.[26]

Formulation of face serum

Preparation of phase A: Distilled water is taken in a beaker and sprinkled with Carbopol 940 with continuous stirring and allow it to hydrate overnight.

Preparation of phase B: In a separate beaker, add glycerin and sodium benzoate (previously dissolved in a small portion of water).

Preparation of emulsion: Add this entire Phase B into Phase A. Add the extracts and gently mix. Mix orange oil with tween 80 in a small beaker, then add vitamin E with gentle stirring. Add this oil solubilized mixture into the main batch. Adjust pH using triethanolamine. Next, gradually add the remaining distilled water while gently stirring until the total volume reaches 25 ml.

Table 5: Formulation of face serum.

Ingredients	F1	F2	F3	Category
Mango leaves extract	1.25 ml	1.25 ml	1.25 ml	Plant extract
Guava leaves extract	1.25 ml	1.25 ml	1.25 ml	Plant extract
Rosemary leaves extract	1.25 ml	1.25 ml	1.25 ml	Plant extract
Grape seed extract	1.25 ml	1.25 ml	1.25 ml	Plant extract
Carbopol 940	0.1 g	0.2 g	0.3 g	Gelling agent
Triethanolamine	0.3 ml	0.3 ml	0.3 ml	pH adjuster
Glycerin	7 ml	7 ml	7 ml	Humectant
Tween 80	2 ml	2 ml	2 ml	Emulsifier
Orange oil	0.3 ml	0.3 ml	0.3 ml	Perfume
Vitamin E	1 ml	1 ml	1 ml	Antioxidant
Sodium benzoate	0.1 g	0.1 g	0.1 g	Preservative
Distilled water	QS	QS	QS	Vehicle

Fig 5: Formulated face serums.

Evaluation of face serum

Physical Evaluation

The physical characteristics of the prepared face serum such as colour, odour, texture, and clarity were visually inspected. After the face serum formulation have been set in the beaker, the serum was evaluated for homogeneity by visual observation, assessing its appearance and checking for the presence of lumps, flocculates, or aggregates. Approximately 3ml of serum was taken in a beaker & tested for transparency by visual inspection.

Measurement of pH

The pH meter was initially calibrated using a standard buffer solution. About 1 ml of the face serum was accurately measured and dissolved in 50 ml of distilled water, and its pH was then recorded. As the skin maintains an acidic pH, the serum's pH should ideally be between 4.1 and 6.7.

Washability

The washability test was carried out by applying a small quantity of the formulation to the hand, followed by rinsing with tap water and then with soapy water.

Spreadability

A 0.5 g sample of the serum was sandwiched between two glass slides and subjected to a 100 g load for 10 minutes. After removing the weight, the diameter of the spread circle was measured at different points. Spreadability is calculated using the formula.

$S=(M*L)/T$

where:

S = Spreadability

M = Weight applied on the slide

L = Diameter of the circle in cm

T = Time in seconds

Phase separation

The formulation was stored in a sealed container at room temperature and protected from light. After 24 hours, it was examined for any phase separation and any changes were noted.

Optical Microscopy

The appearance of face serum was observed under a microscope, and the globule size was determined.

DPPH free radical scavenging activity(Determination of antioxidant activity)

This method was used to assess free radical scavenging activity. The extract was prepared in methanol at different concentrations, and each sample was combined with 1.5 mL of DPPH solution. After incubating at room temperature for 30 minutes, the absorbance was measured at 517 nm. Negative and positive controls were included alongside the test samples. The scavenging activity was determined using the following formula: % Scavenging = $[(A_{517}$ control $- A_{517}$ sample) / A_{517} control$] \times 100$

Stability Studies

The formulation and development of a pharmaceutical product are incomplete without stability testing to assess its physical and chemical stability and ensure its safety. Accordingly, a stability study was performed on the prepared formulation for one month, with samples kept at room temperature.

RESULTS AND DISCUSSION

Physical Evaluation

Physical appearance of the formulations was performed by determining the colour, odour & smoothness respectively.

Table 6: Physical evaluation of face serum.

Parameters	F1	F2	F3
COLOUR	Milky white	Milky white	Milky white
ODOUR	Pleasant	Pleasant	Pleasant
SMOOTHNESS	Good	Excellent	Average

Fig 6: Physical appearance of face serum.

The result of organoleptic parameters like colour & odour, revealed that the formulations F1, F2& F3 was milky white, fruity and pleasant. Hence according to smoothness, the F1 was the good formulation.

Homogeneity

All three developed face serums were evaluated for homogeneity by visually inspecting their appearance and checking for any lumps, aggregates, or flocculates.

Table 7: Homogeneity of face serum.

Formulation	Homogeneity
F1	Good
F2	Excellent
F3	Average

The homogeneity was found to be excellent for F2.

Transparency

Transparency for all the face serum formulations (F1,F2 &F3) were performed.

Table 8: Transparency of face serum.

Formulation	Results
F1	Translucent
F2	Translucent
F3	Translucent

Measurement of pH

The determination of pH was conducted for the three formulations (F1, F2& F3)

Table 9: Measurement of pH.

Formulations	pH
F1	4.90
F2	5.16
F3	5.96

Fig 7: pH meter.

The results showed that the pH values of all three formulations (F1, F2, and F3) were close to the natural pH of the skin, indicating that they can be safely applied.

Washability

The formulation was applied to the skin, and the ease and completeness of its removal with water were manually assessed.

Table 10: Washability of face serum.

Washability	F1	F2	F3
With soap	Medium	More	Less
Without soap	Medium	More	Less

Washability tests were carried out for all formulations (F1, F2, and F3). Among them, F2 demonstrated the highest ease of removal, both with and without soap.

Spreadability

The spreadability of the formulated face serum was evaluated to determine its ability to uniformly cover the skin or affected area, an essential parameter for ensuring ease of application, consumer satisfaction, and effective delivery of active ingredients.

Table 11: Spreadability of face serum.

Formulations	Spreadability (gcm/sec)
F1	0.33
F2	0.51
F3	0.70

Fig 8: Spreadability of face serum.

From this the formulation F2 shows more spreadability than that of other two formulations F2 & F3.

Phase separation

No signs of phase separation were observed in any of the three formulations, which were stored in sealed containers away from light.

Fig 9: Phase separation of F2 formulation.

There were no signs of phase separation in the three formulations.

Optical microscopy

The globule size of the emulsion was measured and observed under microscope.

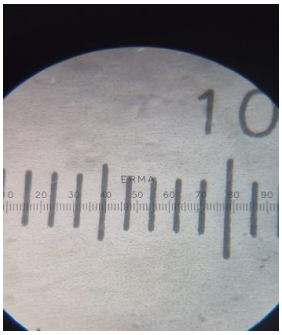

Fig 10: Microscopic view of globules of F2 formulation.

DPPH free radical scavenging activity (Determination of antioxidant activity)

The antioxidant activity of face serum was found to be 40 ppm.

Table 12: Antioxidant activity category.

IC50 value (ppm)	Category Antioxidant activity
<50	Very strong
50-100	Strong
100-150	Moderate
150-200	Weak

Result: The antioxidant activity of the prepared face serum was found to be very strong.

Stability studies

The formulation underwent stability testing to assess physical and chemical changes, and no significant variations in its properties were observed.

CONCLUSION

Face serums offer a concentrated blend of active ingredients tailored to specific skincare needs. Their lightweight texture penetrates deeply into the skin, delivering potent benefits such as hydration, brightening, and anti-aging effects. This study successfully developed and evaluated a polyherbal face serum enriched with antioxidant-rich extracts of mango leaves, guava leaves, rosemary leaves, and grape seed. Mangiferin in mango leaves provides potent antioxidant activity, as it neutralizes free radicals, protects skin cells from UV-induced damage, and helps reduce signs of premature aging. Flavonoids neutralizes free radicals while polyphenols and proanthocyanidins reduces signs of aging and inflammation. The formulation was designed to deliver targeted skincare benefits such as antioxidant protection, anti-aging effects, and skin nourishment. The serum was prepared using suitable excipients to ensure optimal texture, stability, and skin compatibility. Evaluation studies demonstrated that the formulations were milky white, translucent, homogeneous, easily washable, and exhibited good spreadability with pH values close to that of normal skin, indicating safety for topical application. Optical microscopy confirmed uniform globule size, while stability studies revealed no signs of phase separation or significant physical changes during storage. The DPPH free radical scavenging assay confirmed notable antioxidant activity, supporting the role of herbal constituents in protecting the skin from oxidative stress and premature aging. Overall, the findings suggest that the formulated polyherbal face serum is stable, skin-friendly, and effective, highlighting its potential application in modern cosmetic and dermocosmetic products.

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