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Research Article

Formulation and Evaluation of Anti-Aging Serum using Ferulic Acid from Matsyakshi Plant

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ABSTRACT

Skin aging is a multifactorial biological process influenced by intrinsic factors such as chronological aging and extrinsic factors such as ultraviolet radiation, pollution, and oxidative stress. These processes lead to collagen degradation, reduced elasticity, dryness, fine lines, and wrinkles. The present study aimed to formulate and evaluate a stable, effective, and cosmetically acceptable anti-aging serum incorporating natural and synthetic antioxidants with proven dermatological benefits. Ferulic acid was extracted, isolated, and identified from Alternanthera sessilis and used as the primary antioxidant agent in combination with L-ascorbic acid, humectants, emollients, and suitable excipients to enhance skin penetration and hydration. The serum was prepared using Carbopol-940 as gelling agents and evaluated for physicochemical parameters including color, odor, texture, homogeneity, pH, viscosity, stability, antioxidant activity, and skin irritation. UV spectrophotometric analysis confirmed the presence and concentration of ferulic acid. The formulation showed significant antioxidant activity with a maximum inhibition of 60% and an IC_{50} value of 12.5 µg/ml. Stability studies under room and refrigerated conditions demonstrated acceptable stability, and no skin irritation was observed after 24 hours of application. The optimized formulation exhibited desirable pH, good spreadability, homogeneity, and cosmetic elegance. The study concludes that the formulated anti-aging serum possesses effective antioxidant potential, good stability, and is safe for topical application, making it a promising formulation for reducing signs of skin aging.

INTRODUCTION

The skin serves as an essential protective layer that guards the body's internal organs from harmful outside elements. It is made up of three primary layers. The outermost layer, called the epidermis, is composed of a type of tissue known as stratified squamous epithelium, which mainly consists of

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keratinocytes and other specialized cells such as dendritic cells, including Merkel cells, Langerhans cells, and melanocytes. The middle layer, known as the dermis, contains blood vessels, nerve endings, and hair follicles. The innermost layer, called the subcutaneous tissue, is made up of fat cells and connective tissue. The skin plays a vital role in maintaining the body's general health and proper functioning. The process of skin aging is complex and is affected by both internal and external factors, which lead to a slow decline in the skin's structure and function. Intrinsic skin aging is marked by features such as smooth, pale, and dry skin, reduced elasticity, and the appearance of fine wrinkles, which are usually noticeable as a person gets older [1]. Antioxidants play a crucial role in combating skin aging by neutralizing free radicals that damage skin cells. They're naturally occurring compounds that help prevent oxidative stress, reduce signs of aging (fine lines, wrinkles), and improve UV damaged skin. Plant-based antioxidants like flavonoids are commonly used in topical preparations due to their potent antioxidant properties. Serum formulations are particularly effective due to their high concentration of active ingredients. Some benefits of antioxidants in skincare include: Protecting skin from environmental stressors, Inhancing skin elasticity, Reducing inflammation The synthesis of anti-aging serum from plant extract has been studied in the past. Thus This review offers an update on skin. Anatomy, a brief synopsis of skin aging that covers both intrinsic and extrinsic aging, and a list of various anti-aging herbs. It also explores an overview of serum, different kinds of serums used in skincare routines to prevent ageing, and essential components found in antiaging face serum.[2]

ANATOMY OF SKIN: STRUCTURE OF SKIN :

1. Epidermis: The epidermis, the outermost skin layer, has varying thickness:0.5 mm (eyelid), 1.5 mm (palms and soles) It's primarily composed of: Melanocytes (produce melanin), Keratinocytes (produce keratin). These cells form a stratified squamous epithelial layer, providing a protective barrier against external factors. [3]

2. Dermis: The dermis is a crucial layer of skin, comprising: Elastic and fibrous tissue, Located between epidermis and subcutaneous tissue composed of Ground substance, Fibers (collagen, elastin, Cells (fibroblasts). Fibroblasts produce collagen and elastin, essential for skin elasticity and strength. Collagen makes Up to 30% of dermal volume, 75% of dry weight. The dermis plays a vital role in skin structure and function.[4]

3. Hypodermis: The hypodermis, also known as the subcutaneous layer, is the innermost layer of skin. Its key functions include: Providing insulation and protection, Serving as an energy reserve (through adipocytes). Allowing skin mobility by gliding over underlying structures. The hypodermis is primarily composed of Adipocytes (fat cells), Fibrous connective tissue Lobules of adipocyte.

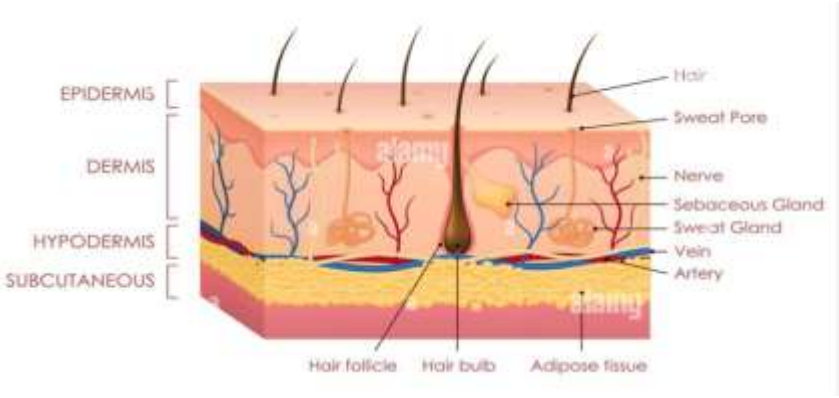

Fig.1 Structure of Skin

OVERVIEW ON SKIN AGING

Aging skin is characterized by various signs, including: Wrinkles, Uneven pigmentation, Darkening, Thinning, Sagging, Roughening. These changes can be attributed to either intrinsic (internal) or extrinsic (external) factors. Theories suggest that DNA damage and repair processes contribute to aging, leading to: Epigenetic changes, Cell senescence, Loss of cell function, Genomic abnormalities. Extrinsic aging is driven by environmental factors like: UV radiation, Pollution, Reactive oxygen species production. Intrinsic aging is linked to: Programmed aging, Cellular senescence, Endogenous oxidative stress, Cellular damage. [5]

Fig.2 Difference between young Skin and Aged Skin

INTRINSIC SKIN AGING

It is a chronologically-driven physiological transformation process. Essentially, intrinsic skin aging is a condition that only appears in old age and is symbolized by smooth, unblemished, pale, dry, less elastic skin that has fine wrinkles. Intrinsic aging is characterized by a decline in dermal fibroblast count, which lowers the synthesis of collagen and elastin, especially types I and III collagen, and results in skin thinning, wrinkles, and loss of elasticity. Skin aging is partly caused by increased expression of matrix metalloproteinases (MMPs), which break down collagen and elastin in the dermal skin layer. Antioxidants such as resveratrol and iso orientin can reduce its expression both intrinsic and extrinsic variables can contribute to skin aging. [6]

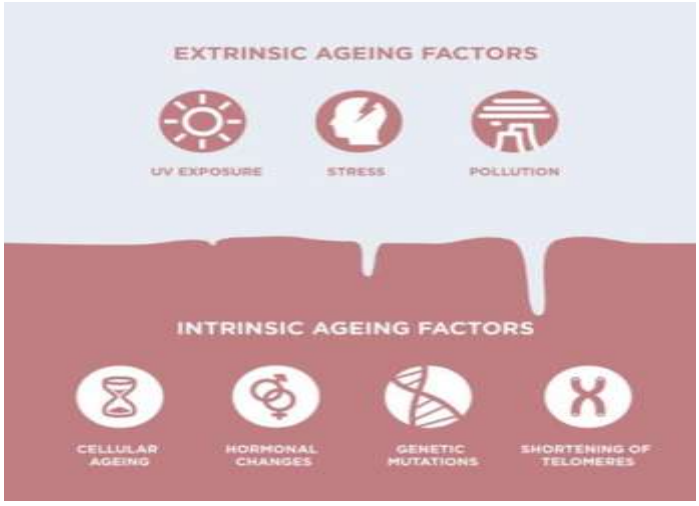

Fig.3 Intrinsic factor responsible for skin aging

EXTRINSIC SKIN AGING

Low-grade chronic UVR exposure is Responsible for over 80% of the aging process of Facial skin, while exposure can also result in Sunburn, tanning, inflammation, and damage to Dermal connective tissue. Extrinsically aged skin, Primarily from UVR rays, has sallow complexion with uneven pigmentation, rough texture, coarse Wrinkles, and decreased skin elasticity. Accelerated skin aging can be caused independently by long-term exposure to UV Radiation and cigarette smoking. ROS produced by UV exposure lead to harmful oxidative stress. Because 02 can take electrons, it produces extremely reactive chemical compounds known as ROS. Reactive oxygen species (ROS) are produced when the skin is exposed to photoaging stimuli. These ROS subsequently stimulate dermal enzymes Such as collagenase and elastase. These enzymes Speed up premature skin aging by breaking down Elastin and collagen, respectively. Signs of this Process include deep furrows or severe atrophy, Wrinkles, freckles, sallowness, laxity, and a leathery Appearance.

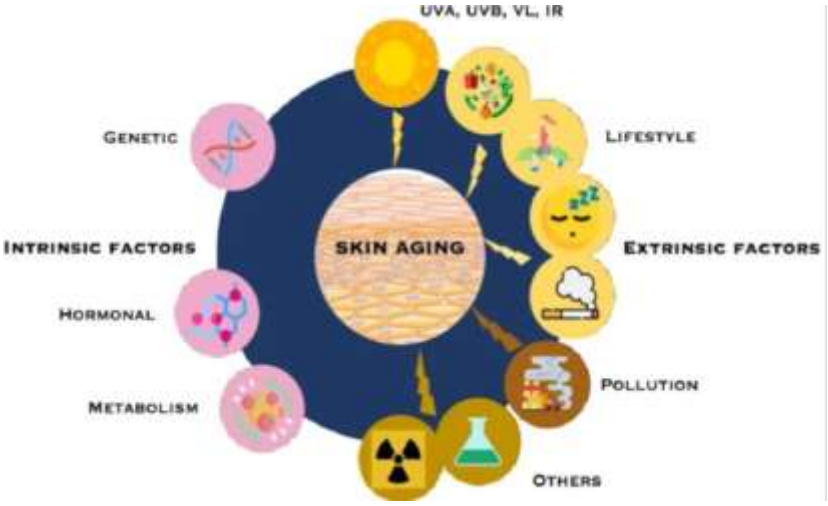

Fig.4 Extrinsic factor responsible for skin aging

FUNCTION OF SKIN :

1. Provide a barrier of defence against harmful substances, mechanical, thermal and physical harm.

2. Prevents moisture loss.
3. Minimizes the negative effects of UV light.
4. Aids in temperature regulation.
5. Serves as a sense organ .[9]

FACE SERUM :

Face serums are formulated to deliver a high concentration of active ingredients without unnecessary additives. Their thinner consistency and absence of heavy oils enhance absorption and penetration, making them effective for nourishing and layering on the skin. The focus on essential ingredients without emulsifiers ensures optimal delivery of active agents, such as vitamins or botanical extracts.

ADVANTAGES OF ANTI-AGING SERUM:

1. Keep skin moisturized

2. Help remove skin blemishes

3. Anti-aging benefits and protect against wrinkles [11]

AIM OF PROJECT:

To formulate and evaluate a stable, effective, and cosmetically acceptable anti-aging serum containing selected active ingredients that reduce signs of skin aging such as wrinkles, fine lines, dryness, and loss of elasticity.

NEED OF THE PROJECT:

Degradation of collagen and elastin Increased oxidative stress due to free radicals Reduced skin hydration Loss of skin firmness and glow Formation of wrinkles and fine lines.

OBJECTIVE OF PROJECT :

1. To formulate a stable and cosmetically acceptable anti-aging serum using suitable active ingredients and excipients.
2. To select and incorporate anti-aging agents (e.g., antioxidants, vitamins, humectants, peptides) based on their proven skin benefits.
3. To optimize the formulation for desirable properties such as pH, viscosity, spreadability, and skin feel.
4. To evaluate the prepared serum for physicochemical parameters including appearance, pH, viscosity, homogeneity, and stability.
5. To assess the antioxidant activity of the formulation by suitable in-vitro methods.
6. To study the compatibility of drug and excipients used in the formulation.
7. To perform stability studies of the serum as per ICH guidelines.
8. To evaluate the safety of the formulation by conducting skin irritation tests.
9. To assess the moisturizing and skin-hydration effect of the serum.
10. To compare the prepared formulation with a marketed anti-aging serum.

DRUG PROFILE :

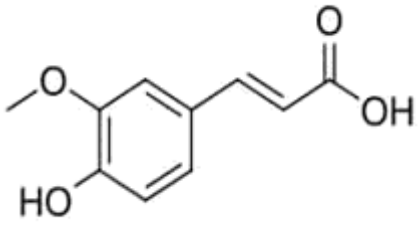

Fig.5 Ferulic acid

Fig. 6 *Alternanthera sessilis*

Table.1 Drug profile [12]

SOURCE OF FERULIC ACID	*Alternenthra sessilis* plant
SYNONYMS	In marathi = Matsyakshi , In tamil= poonanganni
CHHEMICAL CONSTITUENTS	• Alkaloids: Indole-type alkaloids (contributing to antimicrobial activity) • Flavonoids: Quercetin, kaempferol, rutin • Saponins: Known for immunomodulatory and cholesterol-lowering effects • Tannins: Provide astringent and antioxidant properties Steroids & Triterpenoids: β-sitosterol, stigmasterol, lupeol Phenolic • Compounds: Gallic acid, caffeic acid, ferulic acid
GEOGRAPHICAL SOURCE	Asia, Australia, and the Americas. In India (south india- tamil nadu, Andhra Pradhesh, kerala) including Maharashtra.
USES	Antioxidant, anti-inflammatory, hepatoprotective, and nutritional properties.

LITERATURE REVIEW :

1. (Graf, 1992) Antioxidant Activity: Ferulic acid is a naturally occurring phenolic compound that exhibits strong antioxidant activity by scavenging reactive oxygen species (ROS) and preventing oxidative damage to lipids, proteins, and DNA. This property makes it highly useful in topical formulations aimed at protecting the skin from environmental stress.

2. (Lin et al., 2005) Synergistic Effect with Vitamin C: Ferulic acid enhances the antioxidant capacity of Vitamin C and Vitamin E when used in combination. This synergistic effect significantly increases the photoprotective potential of the formulation compared to individual antioxidants.

3. (Pinnell et al., 2001): Stabilization of Vitamin C: L-ascorbic acid is highly unstable and degrades rapidly when exposed to light, heat, and oxygen. Ferulic acid stabilizes Vitamin C by reducing its oxidation, thereby improving formulation stability and shelf life.

4. (Murray et al., 2008): Photoprotective Effect: Topical application of ferulic acid combined with Vitamin C reduces UV-induced erythema, inflammation, and cellular damage. This combination has been shown to significantly improve protection against UV radiation.

5. (Briganti and Picardo, 2003): Anti-aging Properties: Oxidative stress contributes to premature skin aging, including wrinkles and loss of elasticity. Antioxidants such as ferulic acid and Vitamin C reduce these effects by protecting skin structures and improving skin appearance.

6. (Pullar et al., 2017): Collagen Synthesis: Vitamin C plays a key role in collagen synthesis by acting as a cofactor in hydroxylation reactions, while ferulic acid protects collagen fibers from oxidative degradation.

7. (Cavalcanti et al., 2020): Anti-inflammatory Activity: Ferulic acid exhibits anti-inflammatory effects by inhibiting inflammatory mediators, reducing redness and irritation in the skin.

8. (Saija et al., 1999): Skin Brightening Effect: Ferulic acid inhibits tyrosinase activity, which reduces melanin synthesis and leads to improved skin tone and reduction in hyperpigmentation.

9. (Das and Wong, 2020): Formulation Challenges: Ferulic acid has limited solubility and stability in aqueous systems. Advanced formulation strategies such as nanoencapsulation and emulsions improve its stability and skin delivery.

10. (Telang, 2013): Evaluation Parameter pH: The pH of Vitamin C serum plays a critical role in stability and penetration. Optimal pH (2.5–3.5) enhances absorption and effectiveness.

11. (Patil et al., 2020): Evaluation Parameter: Drug Content: Quantitative analysis of ferulic acid in formulations is commonly performed using RP-HPLC methods to ensure accuracy, precision, and consistency.

12. (Mancuso et al., 2021): Evaluation Parameter; In-vitro Release: In-vitro diffusion studies demonstrate controlled release of ferulic acid from optimized formulations, improving bioavailability.

13. (Pinnell et al., 2001): Stability Studies: Accelerated stability studies show that ferulic acid improves the stability of Vitamin C formulations under various storage conditions.

14. (Liu et al., 2026): Clinical Evaluation: Clinical studies report improvements in skin hydration, reduction in erythema, and enhanced skin texture following the use of ferulic acid and Vitamin C formulations.

15. (Marcato et al., 2020): Safety and Efficacy: Ferulic acid-based serums are generally safe, non-irritating, and suitable for long-term use in dermatological applications .

PLAN OF WORK :

Aim, need and objective

Selection of ingredients and their roles Extraction, isolation, Purification and Identification of *Alternenthra sessilis plant*

Formulation and development

Evaluation

Result and Conclusion

MATERIAL AND METHODOLOGY :

Table 2. Master Formula

SR.NO	INGREDIENTS	F1	F2	F3	F4	F5	ROLES
1	Ferulic acid	0.12g	0.12g	0.12g	0.12g	0.12g	Antioxidant
2	L-ascorbic acid	3.0g	3.6g	4.5g	3.6g	3.0g	Antioxidant
3	Propylene glycol	4ml	5ml	6ml	5ml	4ml	Solvent
4	Glycerin	3ml	2.5ml	2ml	3.5ml	4ml	Humectant
5	Sorbitol	1g	1g	1.5g	1g	0.5g	Moisturizer
6	Almond oil	0.5ml	0.5ml	0.7ml	0.3ml	0.5ml	Emollient
7	Tween 20	0.6ml	0.7ml	0.8ml	0.5ml	0.6ml	Emulsifier
8	Carbopol 940	0.15g	0.20g	0.25g	0.10g	0.15g	Polymer
9	Triethanolamine	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	PH adjust

10	Sodium Benzoate	0.15g	0.15g	0.15g	0.15g	0.15g	Preservative
11	Disodium EDTA	0.03g	0.03g	0.03g	0.03g	0.03g	Chelating Agent
12	Purified Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Vehicle

Preparation of Alternenthra sessilis plant leaf extract :

Method of extraction: Maceration

Take 100g of Matsyakshi leaf powder

↓

after 2 to 3 hour we get powder extract

↓

Transfer the drug to the round bottom flask

↓

extract to the 80% of the ethanol for 48 hours

↓

Filter and Concentrate using rotary evaporator

↓

After 2 to 3 hours we get powder extract

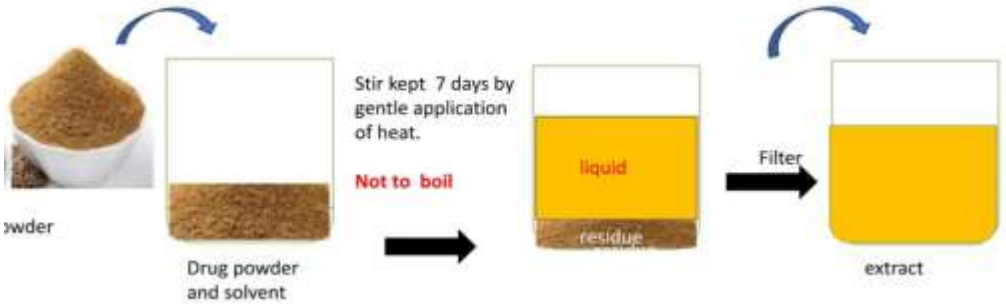

Fig.7 Extraction of ferulic acid from *Alternenthra sessilis* plant by Maceration

Isolation and purification of ferulic acid :

Procedure :

Dissolve extract in distilled water

Add 2N NaOH

Heat at 60–80°C for 1–2 hours

↓

Cool solution

Add HCl dropwise until pH = 2–3

Result: Ferulic acid precipitates out

↓

Transfer mixture to separatory funnel

Extract with ethyl acetate (3×)

Collect organic layer

Contains: Ferulic acid

↓

Evaporate ethyl acetate using rotary evaporator

Obtain semi-solid crude ferulic acid

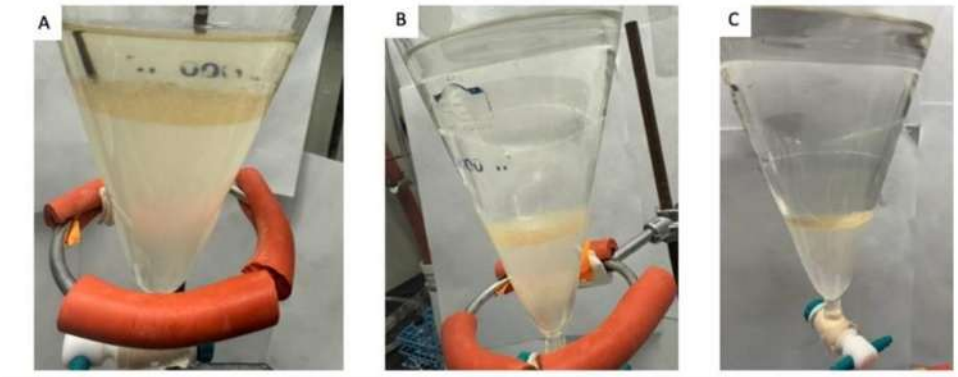

Fig.8 Isolation of ferulic acid

Purification:

Method A: Recrystallization

Dissolve in hot ethanol

Cool slowly → yellow crystals form

↓

Method B: Column Chromatography

Stationary phase: Silica gel

Mobile phase: Hexane: Ethyl acetate (gradient) [16]

Identification :

1. **TLC Analysis :**

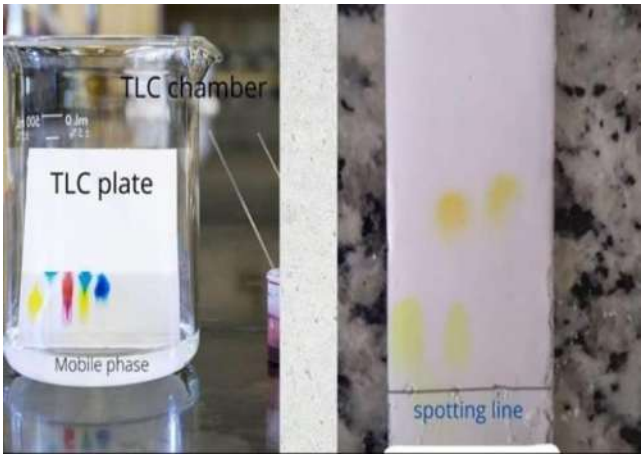

Fig.9 TLC Analysis

RF Value = 0.42

Procedure for Anti-aging Serum :

Preparation of gel phase (A)

Disperse Carbopol 940 in purified water and allow hydration for 20-30 minutes

↓

Preparation of antioxidant phase (B)

Dissolve a gel phase in water

Dissolve ascorbic acid in a small amount of water

↓

Preparation of humectant phase (C)

Propylene glycol, glycerin, and sorbitol were stirred.

↓

Preparation of oil phase(D)

mixed almond oil with tween20

↓

Combine phases:

Add phase C to phase A

Add phase B to phase slowly

Add phase D with continuous stirring

↓

Add preservative:

Dissolve sodium benzoate and EDTA, mix well, and then

↓

Add pH adjuster:

Measure the pH

Add triethanolamine dropwise until pH 5-6

↓

Make the final volume:

Add purified water and mix thoroughly [16]

Anti-aging serum :

Fig.10 After mixing all the phases

Fig.11 Anti-aging serum

Formulation Trials :

FORMULATION	PROCEDURE	RESULTS
F1	Same Procedure with ethanol	Unstable
F2	Same Procedure but replace ethanol	Stable
F3	Same as F2	Stable
F4	Same as F2	Stable
F5	Same as F2	Stable
FORMULATION	**PROCEDURE**	**RESULTS**
F1	Used Mechanical stirrer	Visible fine particle
F2	Used electric Stirrer	Uniform Mixture
F3	Same as F2	Uniform Mixture
F4	Same as F2	Uniform Mixture

F5	Same as F2	Uniform Mixture

F1: Rejected due to unstable in room temperature

F2: Reject due to acidic pH

F3: Reject due to improper mixing

F4: Reject because, it was causing irritation

F5: Stability, pH, homogeneity, no irritation

UV spectroscopy test ferulic acid :

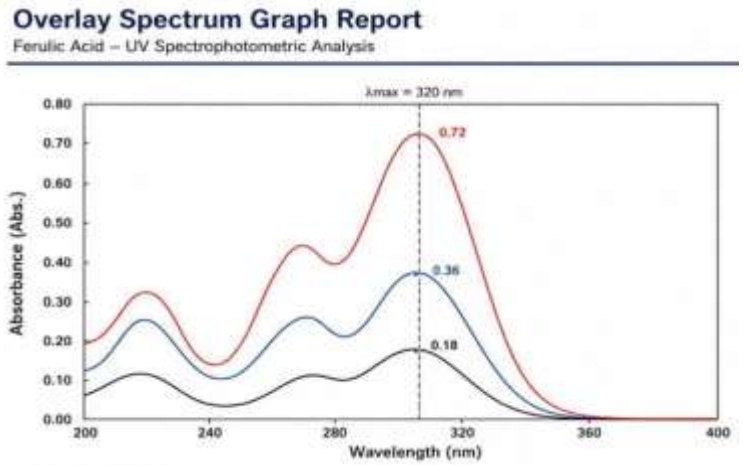

Fig. Test ferulic Acid UV Graph

Observation table :

Table.4 Test ferulic acid UV observation

SR.NO	CONC. (ug/ml)	ABSORBANCE (320nm)
1	5	0.18
2	10	0.36
3	15	0.54
4	20	0.72

Actual conc. Test ferulic acid = 14ug/ml

Take 0.12g ferulic acid

EVALUATION PARAMETER FOR PREPARED ANTI-AGING SERUM

1. **Physical evaluation for serum :**

PARAMETER	F1	F2	F3	F4	F5
Colour	Brown	Dark Yellow	Yellow	Yellow	Pale Yellow
Odour	Rancid	Rancid	Pungent	Floral	Floral
Ph	5	4	5	4	5
Consistency	Hard	oily	Runny	Thick	Smooth
Appearance	Dull	Dull	Faded	Glossy	Lustrous
Spreadability	Moderate	Moderate	Good	Good	Excellent
Homogenity	Non-Homogenous	Non-Homogenous	Homogenous	Homogenous	Homogenous
Irritation	Irritation	Irritation	No Irritation	No Irritation	No Irritation

2. **Anti-Oxidant Test :**

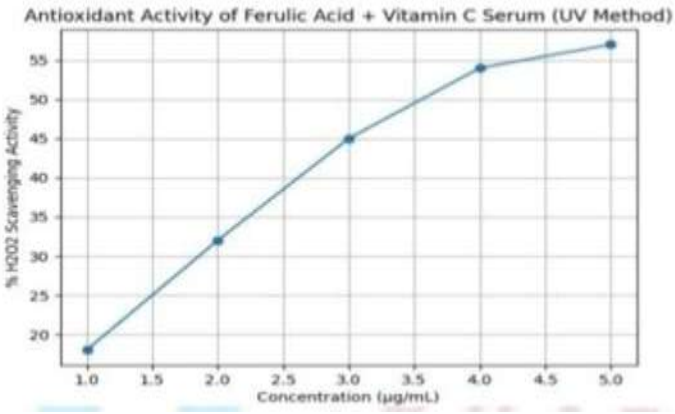

Fig.13 Graph of anti-oxidant activity

Observation table:

SR.NO	CONCENTRATION (ug/ml)	ABSORBANCE (230nm)	% OF SCAVENGING
1	8	0.400	38%
2	10	0.355	45%
3	12	0.325	50%
4	14	0.292	55%
5	16	0.275	58%
6	18	0.260	60%

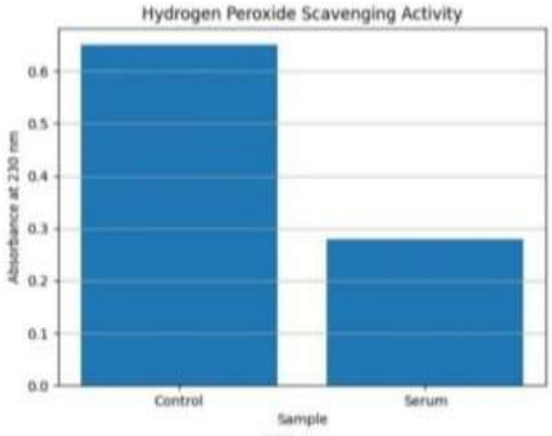

Fig.14 Scavenging activity graph

Observation Table :

SAMPLE	ABSORBANCE AT (230nm)
Control H2O2	0.650
Serum = H2O2	0.292

Result : Maximum anti-oxidant activity = 60%

3. PH Test :

SR.NO.	FORMULATION	PH
1	F1	5
2	F2	4
3	F3	5
4	F4	4
5	F5	5

4. Cyclical temperature study :

FORMULATION	ROOM TEMPERATURE	COOL TEMPERATURE
F1	Unstable	Stable 1-7°F

F2	Stable	Stable 1-7°F
F3	Stable	Stable 1-7°F
F4	Stable	Stable 1-7°F
F5	Stable	Stable 1-7°F

RESULT :

EVALUATION PARAMETER	F1	F2	F3	F4	F5 (Selected Batch)
Colour	Brown	Dark Yellow	Yellow	Yellow	Pale Yellow
Odour	Rancid	Rancid	Pungent	Floral	Floral
PH	5	4	5	4	5
Consistency	Hard	oily	Runny	Thick	Smooth
Appearance	Dull	Dull	Faded	Glossy	Lustrous
Spreadability	Moderate	Moderate	Good	Good	Excellent
Homogenicity	Non-Homogenous	Non-Homogenous	Homogenous	Homogenous	Homogenous
Skin Irritation	Irritation	Irritation	No Irritation	No Irritation	No Irritation
Stability	Unstable	Acidic PH	Improper Mixing	Caused irritation	Stable
Antioxidant activity (Max inhibition)	-----	------	----	----	----
IC 50 value	----	-----	-----	----	----
Overall Acceptability	Rejected	Rejected	Rejected	Rejected	Accepted

CONCLUSION

The present study successfully formulated and evaluated an anti-aging serum incorporating ferulic acid extracted from Alternanthera sessilis with L-ascorbic acid and suitable excipients. Among all the trial batches, F5 was found to be the optimized formulation exhibiting desirable physicochemical properties, excellent spreadability, good homogeneity, suitable pH, stability, and absence of skin irritation. The formulation demonstrated significant antioxidant activity with 60% inhibition and an IC_{50} value of 12.5 µg/ml, confirming the effectiveness of the antioxidant combination. Stability studies under different conditions showed acceptable stability of the serum. Thus, the developed anti-aging serum is stable, safe, cosmetically elegant, and effective for topical application and shows promising potential in reducing signs of skin aging.

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