Formulation And Evaluation of An Herbal Face Serum Containing Withania Somnifera and Citrus Sinensis: A Comprehensive Pharmaceutical Research

Pharmacognosy And Dermatological Significance of Herbal Cosmeceuticals

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***Abstract*—Herbal cosmetics are becoming increasingly popular owing to their natural composition, fewer adverse effects, and diverse skin benefits. The present study focused on developing and accessing a potent herbal face serum formulated with extracts of Ashwagandha (Withania somnifera) and orange peel (Citrus sinensis). The stable serum was successfully prepared by blending Aloe Vera gel, glycerin, jojoba oil, rose water, vitamin E, and distilled water with an appropriate emulsifier and preservative using a straightforward mixing and emulsification technique. The final product appeared whitish with a refreshing herbal-citrus fragrance. It exhibited a smooth, lightweight consistency with excellent spreadability, skin-compatible pH, and no signs of irritation. The serum demonstrated good physical stability and imparted a feeling of deep hydration, softness, freshness, and radiant glow after application. This formulation harnesses the synergistic antioxidant, anti-inflammatory, brightening, and moisturizing benefits of Ashwagandha and Orange peel extracts, making it a safe and effective natural option for daily skincare use.**

***Index Terms*—Herbal face serum, Ashwagandha extract, orange peel extract, Withania somnifera, Citrus sinensis,**

I. INTRODUCTION

Modern cosmetic market is experiencing a patterned shift, changing the synthetic cosmetic formulations that contain chemical elements to the bioactive, plant-based products. This is due to an advanced consumer market that is more focused on sustainability, safety, and long-run skin health. In the pharmacy practice, this can be seen as a convergence of Ayurvedic traditional values and contemporary pharmaceutical technologies.[1] The commercial and therapeutic potential of these concentrated delivery systems is demonstrated by the global facial serum market that is estimated at high and projected to reach USD 8.58 billion in 2030 due to the high concentration of active constituents of these delivery systems enabling rapid absorption and diffusion into the deeper epidermis and dermis layers.[2]

The justification of developing a serum containing *Withania somnifera* (Ashwagandha) and Citrus sinensis (Sweet Orange) is the complementing phytochemical profiles of the two ingredients. Ashwagandha, a so-called Rasayana over three millennia, is an adaptogenic supplement with anti-inflammatory effects that stabilize the physiological condition of the skin under stress through the provision of D-limonene, flavonoids, and Vitamin C, which are antioxidants and enzyme inhibitors with high potency and at the same time, Citrus sinensis. [3, 5]

Fig 01: Herbal ingredients employed in serum preparation

This complementary blend of botanicals is not merely about the eradication of visible signs of ageing, but also about boosting the skin's innate immunity to today's environmental and lifestyle challenges—and providing a forward-looking approach that turns skin care into intelligent and proactive skin resilience. This blend combines a concept of "stress-to-radiance" with the potent stress-relieving adaptogenic benefits of Ashwagandha to help defend the skin against stress-induced damage at the cellular level, and with the powerful flavonoids and natural vitamin C from orange peel, to energise and illuminate the complexion.

S. No	Ingredients	Quantity	Purpose
1.	Distilled water	6.9ml	Acts as a base and hydrates the skin
2.	Aloe Vera gel	3.0ml	Soothing, cooling
3.	Rose water	0.7ml	Tones the skin
4.	Glycerin	1.5ml	Humectant
5.	Ashwagandha extract	0.5ml	Antioxidant and adaptogen
6.	Jojoba oil	1.0ml	Balances skin oil
7.	Emulsifier	1.0ml	Uniform blending
8.	Vitamin E	0.15ml	Antioxidant, skin healing
9.	Orange peel extract	0.8ml	Rich in vitamin C and flavonoids; brightens skin, reduces pigmentation
10.	Natural preservative	0.15g	Shelf life and safety

Phytochemical Profile and Taxonomy of Withania somnifera

Withania somnifera (L.) Dunal, belonging to the Solanaceae family, is a perennial woody shrub found abundantly in sub-tropical regions. Its long tuberous roots, ovate leaves, and greenish-yellow flowers that eventually develop into globose berries characterize the plant. In pharmacy practice, the roots are of primary therapeutic interest due to their dense concentration of steroidal lactones, specifically withanolides. [7, 11, 14]

Table 1: Taxonomical Classification of Withania somnifera [7, 9]

Rank	Classification
Kingdom	Plantae
Division	Angiospermae
Class	Dicotyledons
Order	Tubiflorae
Family	Solanaceae
Genus	*Withania*
Species	*somnifera*

The biological activity of Ashwagandha It is largely credited to withanolides, a group of naturally occurring polyoxygenated steroidal lactones with an ergostane skeleton. These compounds, including Withaferin A and Withanolide D, exhibit structural features such as an unsaturated ketone in ring A and an epoxide in ring B, which are critical for their anti-inflammatory and anti-proliferative actions. Beyond withanolides, the root contains a variety of alkaloids like withanine, somniferine, and cuscohygrine, alongside sitoindosides (withanolides with an additional glucose molecule). [13, 14]

Phytochemical Composition of Citrus sinensis Peel

Sweet orange or *Citrus sinensis* is a plant whose peel comprises almost half of the entire fruit biomass and is frequently underused despite its pharmacological properties. The peel is made up of two layers; the flavedo (colored outer layer), and the albedo (white spongy inner layer). Essential oils are especially abundant in the flavedo, being dominated by the monoterpene D-limonene, which makes 90 to 95 percent of the volatile fraction. [10, 15, 16]

Besides volatile terpenes, orange peel is also a major source of flavonoids, in particular, hesperidin and naringin and polymethoxylated flavones. The compounds, in combination with high content of ascorbic acid (Vitamin C), have a complex defense against oxidative stress, scavenging free radicals, and stopping lipid peroxidation, commonly assessed as a decrease. [33, 36, 37]

Fig 02: Clevenger apparatus setup for the hydrodistillation of essential oil from orange peel.

Table 2: Chemical Constituents of Orange Peel Essential Oil (GC-MS Analysis) [17, 20, 22, 25]

Compound Name	Average Concentration (%)	Biological Role
d-Limonene	90.33 – 95.19	Antimicrobial, Penetration Enhancer
β-Myrcene	1.09 – 1.16	Anti-inflammatory
Linalool	1.96 – 5.13	Fragrance, Soothing agent
α-Pinene	1.17 – 1.59	Antioxidant, Anti-acne
Decanal	2.92	Fragrance, Stability
Vitamin C	Trace (Extract)	Collagen synthesis, Brightening

II. MECHANISMS OF ACTION IN DERMATOLOGY

The age, pigmentation, and barrier modulation of certain cell pathways are a measure of the efficacy of an herbal face serum. These pathways are addressed by the Ashwagandha and Orange peel combination; direct inhibition of enzymes and signal transduction. [18, 34, 35]

Collagen Synthesis and Extracellular Matrix Protection

It is considered that aging of the skin is essentially characterized by the deterioration of the extracellular matrix (ECM). The mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- B) pathways are activated by reactive oxygen species (ROS) caused by UV radiation. This stimulation causes the expression of Matrix Metalloproteinase (MMPs), that is, MMP-1, which breaks down collagen and elastin fibers enzymatically. Ashwagandha and Orange peel have a dual-layered protection. [21, 24] Citrus sinensis extract was shown to suppress the effect of collagenase and elastase and at the same time stimulate the synthesis of hyaluronic acid which increases skin hydration and elasticity in 60 days.26 Clinical trials on 8% Ashwagandha extract showed significant skin improvements in wrinkles, pores, and skin brightness after 60 days. [26, 27]

Regulation of Melanogenesis and Hyperpigmentation

Hyperpigmentation is often treated with tyrosinase inhibitors; however, the Ashwagandha-Orange serum offers a more nuanced mechanism. Ashwagandha extract (WSE) and its bioactive compound Withaferin A (WFA) impair the phosphorylation of extracellular signal-regulated kinase (ERK), which is essential for the activation of microphthalmia-associated transcription factor (MITF). By interrupting the signaling downstream of endothelin-1 (EDN-1) and stem cell factor (SCF), WSE reduces melanin content without direct tyrosinase cytotoxicity, thereby avoiding the risk of hypopigmentation often associated with synthetic agents. Vitamin C from orange peel complements this by acting as a traditional antioxidant and brightening agent. [12, 28, 29]

III. STANDARDIZED EXTRACTION METHODOLOGIES

The pharmaceutical quality of an herbal serum is contingent upon the precision of the extraction processes. For the Ashwagandha-Orange serum, distinct methods are employed to isolate steroidal lactones from the roots and essential oils from the peel. [20, 23, 37]

Processing and Standardization of Ashwagandha Roots

The concentration of active withanolides is highly sensitive to the method of drying and extraction. Harvested roots are typically sized into 2–4-inch

pieces and subjected to controlled drying to reach a moisture content of $< 6\%$.Research indicates that hot air oven drying at $50^{\circ}C$ for 12 hours is optimal, yielding the highest retention of Withanolide A compared to sun or shade drying. [30, 31, 34]
Following drying, the roots are pulverized and subjected to hydroalcoholic extraction. In industrial pharmacy practice, the "Ashwagandha Extract 2.5%" or the high-concentration "WS-35" (35% withanolide glycosides) are used.[15] These extracts are produced through a cycle of maceration, filtration, and vacuum evaporation at low temperatures to prevent thermal degradation of the steroidal lactones. [32, 35]

Table 3: Impact of Drying Protocols on Withanolide a Retention [34, 36, 38]

Method	Temperature (∘C)	Time	Moisture (%)	Withanolide A (%)
Hot Air Oven	50	12 h	6.0	0.021
Hot Air Oven	60	12 h	5.8	0.018
Sun Drying	32 – 42	6 d	5.8	0.010
Shade Drying	25 – 37	15 d	5.8	0.009

Hydrodistillation via Clevenger Apparatus
The extraction of orange peel oil is conducted using hydrodistillation in a Clevenger apparatus. This method utilizes water as a solvent and operates on the principle of azeotropic distillation. The orange peel must be prepared by separating the flavedo from the white albedo to ensure high oil purity and concentration. [40]

The standardized laboratory procedure involves:
1. Charging: 225g of crushed orange peel is placed in a 1000ml round-bottom flask.
2. Solvent Ratio: 720ml of distilled water is added (approximately 1:10 solid-to-solvent ratio).
3. Heating: The mixture is heated to $95 - 100^{\circ}C$ for 3 hours until the volume of essential oil in the graduated receiver stabilizes.
4. Recovery: The oil is separated from the hydrosol, dried using anhydrous sodium sulfate, and stored in amber vials at $< 5^{\circ}C$. [39, 41, 42]

IV. FORMULATION STRATEGY AND EXCIPIENT RATIONALE

Developing a facial serum requires a balance between rapid dermal penetration and sustained stability. The formulation is typically a biphasic emulsion (oil-in-water) that leverages both hydrophobic and hydrophilic actives.[43]

Selection and Role of Excipients [43, 44]
- Humectants (Glycerin):

Glycerin is used at concentrations of 2.5% to 10% to provide deep hydration and prevent transepidermal water loss. Its presence also enhances the smooth texture and spreadability of the serum.

- Gelling Agents (Xanthan Gum):

Functioning as a viscosity modifier, xanthan gum (0.5%–1.0%) ensures the serum has sufficient body to be dispensed while exhibiting shear-thinning (pseudoplastic) properties for easy application.

- Emulsifiers (Polysorbate 40):

With an HLB value of 15.6, Polysorbate 40 (Tween 40) is ideal for stabilizing the essential oil within the aqueous base. It works through interfacial competition, preventing the coalescence of oil globules.

- Antioxidants (Vitamin E):

Added to the oil phase to prevent the oxidation of D-limonene and other unsaturated terpenes, Vitamin E also provides direct antioxidant benefits to the skin.

- Preservatives (Sodium Benzoate):

Effective against bacteria and fungi at a pH of 4.5 to 5.5, sodium benzoate ensures microbial stability over the product's shelf life.

Table 4: Formulation Master Table for Ashwagandha-Orange Face Serum [23, 28, 36]

Ingredient	Role	Batch F1 (%)	Batch F2 (%)	Batch F3 (%)
Ashwagandha Extract	Active Agent	5.0	10.0	15.0
Orange Peel Oil	Active Agent	2.0	2.0	1.0
Glycerin	Humectant	5.0	8.33	10.0
Xanthan Gum	Gelling Agent	0.6	0.8	1.0
Polysorbate 40	Emulsifier	1.0	1.5	2.0
Vitamin E	Antioxidant	0.1	0.1	0.1
Sodium Benzoate	Preservative	0.2	0.2	0.2
Distilled Water	Solvent	q.s. 100	q.s. 100	q.s. 100

V. EVALUATION AND QUALITY ASSURANCE PARAMETERS

The pharmaceutical evaluation of the formulated serum is conducted to verify its safety, stability, and efficacy. These parameters must be assessed following ICH (International Council for Harmonization) guidelines. [6, 34]

Physicochemical Characterization

Physical analysis includes evaluation of the organoleptic characteristics. An ordinary herbal serum must be milky-white in colour and non-greasy. Homogeneity is determined by smearing the serum on a glass plate; grit or phase separation is not observed. A pH of the serum is an important safety parameter. Given that human skin is kept at an acidic range (4.167), the serum should have a range of 4.5-6.0 to reduce skin irritation and preserve serum preservative power of sodium benzoate. The measure of viscosity is done on a Brookfield viscometer at 20 rpm; the best values are 800-14,000 cps depending on the desirable consistency. [39, 42]

Spreadability and Dermal Adhesion

The spreadability of the serum determines the ease of application and uniform distribution of the medicament. It is measured using the slip-and-drag metho

d, where 0.5 ml of the serum is placed between two glass slides under a 150g weight for one minute. The spreadability index is calculated as follows:

$$S = \frac{m \cdot L}{t}$$

Where S is spreadability, m is the weight, L is the length of spread, and t is the time. A spreadability of 5-7 cm is considered ideal for a comfortable topical application. [44, 46]

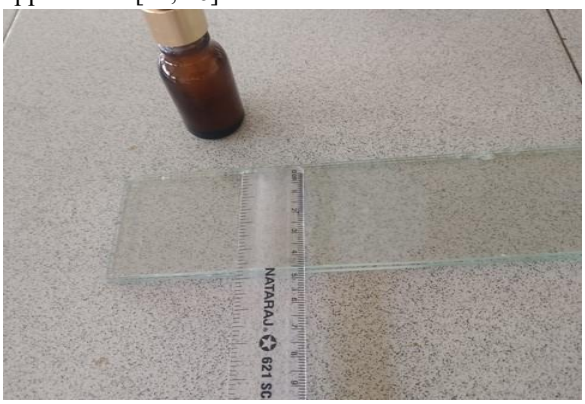

Fig: 03: Spreadabilty analysis of the formulated serum

Table 5: Physicochemical Properties of the Optimized Formulation (Batch F2) [36, 38, 40]

Parameter	Observed Value	Acceptance Limit
Physical Appearance	Milky white, Glossy	Translucent to White
Texture	Smooth, Non-greasy	Homogenous
pH	5.4	4.5 – 6.5
Viscosity	13,759 cPs	800 – 15,000 cPs
Spreadability	6.4 cm	5.0 – 7.0 cm
Phase Separation	None	No separation (3000 rpm)
Microbial Content	Negative	< 100 CFU/g

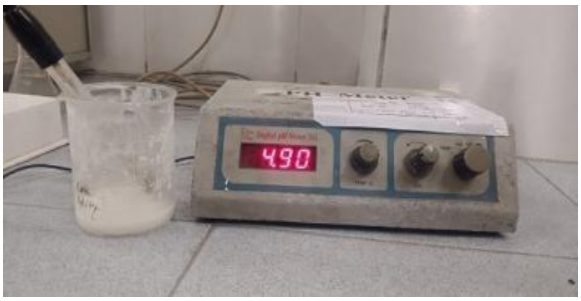

Fig 04: pH determination of formulated herbal face serum

Fig 05: Viscosity determination of formulated herbal face serum using a viscometer

TABLE 6: COMPARATIVE STUDIES AND INDUSTRIAL CONSIDERATIONS [15, 30]

Constituent	Egyptian Extract (%)	Indian Extract (%)	Therapeutic Application
Campesterol	28.70	18.25	Barrier Repair
Stigmasterol	16.11	10.40	Anti-inflammatory
β-Sitosterol	17.66	20.34	Hydration
Oleic Acid	4.66	9.14	Skin Softening
Atropine	1.49	Trace	Neuro-signaling

The industrial viability of an herbal serum is often determined by the source and standardization of its ingredients. Bioavailability studies in humans have shown that standardized Ashwagandha extracts like WS-35 (high glycoside content) are up to 280 times more bioavailable than low-standardization variants. Furthermore, the geographical origin of the botanical material influences the phytochemical density.

Comparative GC-MS Profile of Ashwagandha Roots (Indian vs. Egyptian) [14, 18, 19]

Egyptian Ashwagandha typically contains higher area percentages of phytosterols like campesterol and stigmasterol, making it particularly suitable for anti-aging and barrier-repair formulations. Conversely, Indian varieties are rich in sitosterol and oleic acid, which are preferred for deep-hydration serums.

VI. STABILITY TESTING AND REGULATORY COMPLIANCE

Accelerated stability studies are conducted over a three-month period to observe any changes in physical or chemical properties under stressed conditions ($40^{\circ}C$, 75% RH). [34]

Long-term Integrity and Phase Stability

Ashwagandha-Orange serum proves to be quite stable with the right non-ionic surfactants. The optimized formula (F2) did not exhibit any significant drift over a period of 90 days in pH or phase separation. Nevertheless, it is necessary that researchers should be keen on the auto-oxidation of Polysorbates and essential oils that may cause loss of aroma and reduction in pH. UV covered packaging and addition of antioxidant such as Vitamin E is crucial to preserving the integrity of the active constituents. Laws such as the Modernization of Cosmetics Regulation Act of 2022 (MoCRA) in the USA and guidelines in Europe require stringent safety substantiation and adverse event reporting (ARE).48 Manufacturers are required to have a complete quality dossier, including the source of the herbal substance and standardized manufacturing procedures (GMP). [6, 33]

Fig 06: Stability testing of formulated herbal face serum at room temperature

CONFLICT OF INTEREST

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

VII. CONCLUSION

The development of an herbal face serum that incorporates *Withania somnifera* and *Citrus sinensis* is scientifically valid to handle multi-factorial skin issues like photoaging, hyperpigmentation and loss of hydration. The multidimensional action of withanolides and limonene promotes a complex defense system, addressing the MAPK and NF- B cascades and at the same time stabilizing the structural proteins on the skin. The highest preservation of bioactive constituents is guaranteed by technical accuracy in the Clevenger-assisted hydrodistillation of orange peel, as well as the standardized drying procedure of Ashwagandha roosts. The pharmaceutical analysis of the optimized version (Batch F2) reveals a product of the desired pH (5.4), excellent spreadability (6.4 cm), and strong stability in different environmental conditions. For the B.Pharm student, the following formula is an example of the fusion of traditional pharmacognosy to the strict pharmaceutical evaluation criteria, and meets the rising demand of effective, safe, and natural cosmeceuticals in the contemporary market. The next wave of research should be on the long-term clinical bioavailability of these botanical combinations in human beings in order to further prove their therapeutic superiority over synthetic ones.

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