Original Article

Formulation and development of botanicals-based herbal serum

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ABSTRACT

Aim and Objective: Various botanicals are used in the management of various skin diseases such as *Aloe vera*, Green Tea, Wheatgrass, etc. Hence, in the treatment of skin diseases, various topical retinoids salicylic acid is used. Therefore, it is also found that botanicals such as green tea, *A. vera*, wheatgrass, etc have an anti-bacterial activity which helps in the management of skin problems. **Materials and Methods:** In this formulation, five batches are made with the change in concentrations of botanicals, i.e., *A. vera* and green tea. The botanicals which were used in the formulation were first checked for their phenolic content by high-performance thin-layer chromatography (HPTLC). In this, the standard of Gallic acid was taken and both the extracts samples were analyzed by HPTLC for the confirmation of the presence of gallic acid. The formulation was divided into two phases, i.e., oil phase and aqueous phase. Both the phases are mixed properly Then, extract of *A. vera* and Green tea was added with continuous stirring. After that formulation was kept in a closed container for further study. **Results:** The result of the study revealed that the presence of Gallic acid in both the extracts was confirmed by HPTLC. Further, all the batches were evaluated for their pH, viscosity, and physical appearance and their results suggest that pH was 6.5, viscosity was 6580. On this basis, it was confirmed that batch F4 was a satisfactory formulation. In last it was concluded that F5 is a satisfactory herbal serum formulation. **Conclusion:** On this basis, it was confirmed that batch F4 was a satisfactory formulation. In last it was concluded that F5 is a satisfactory herbal serum formulation.

Keywords: *Aloe vera*, green tea, fingureprinting, herbal formulation, serum formulation

INTRODUCTION

Acne vulgaris is a multifarious chronic inflammatory skin disease that includes symptomatic discomfort, scarring, emotional and psychosocial distress, occupational consequences and potential psychiatric disturbances including depression and suicide.[1] They usually take place during young age and adolescence. It is a disease that affects the pilosebaceous unit (PSU) of the skin that may cause the formation of inflammatory and non-inflammatory lesions.[2] It affects areas of the skin having a high density of sebaceous follicles.

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Acne is most commonly present on the face, the upper part of the chest, and the back.[3] Numerous factors may cause acne production or proliferation in its severity. Some of these factors are genetics, sex, youth, stress, and smoking as well as comedogenic factors such as androgen, halogen, corticosteroids, and pore-clogging cosmetic products.[4] Acne is present in the form of lesions including comedones, papules, pustules, cysts, inflamed nodules and they further develop into deep, purulent lesions in severe cases. Comedone is keratin-filled plugs present on the superficial layer of skin, they can further differentiate into open and closed comedones.[5] Where open comedones are known as blackheads and close comedones are known as whiteheads. A papule is a solid lesion that does not contain fluid. The pustule is a pus-filled lesion formed due to increased follicular inflammation and accumulation of inflammatory cells. A cyst is a lesion found on the deeper layer of skin filled with keratin.[5] A nodule

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is a large, solid lesion filled with keratin and pus formed due to the destruction of follicle wall and inflammation. *Propionibacterium acne* and *Staphylococcus epidermidis* are considered as the central factors driving acne by taking part in the inflammatory response of the skin.[6] The major steps in the control of acne are the prevention of bacterial colonization and inflammation of the PSU. *A. vulgaris* is a skin disease associated with microbial infection and anti-microbial agents are required for its treatment.[7]

Various anti-microbial agents are used in cosmetic preparation for the treatment of acne based on natural and synthetic origin. Usually, synthetic materials are used in cosmetics due to their low cost but they may give adverse effects on the human skin environment.[8] The use of natural products for the treatment of acne is now used in industrialized societies. Herbs having anti-microbial, inflammation-modulating, anti-comedogenic and in certain cases, hormone-balancing activity are also useful in the treatment of acne.[9]

There are various types of treatment and management methods used for acne such as topical, systemic and hormonal treatments are used, they are mentioned here:

Topical retinoids have broad anti-acne activity and safety aspects which justify their use in first-line treatment of mild to moderate forms of acne especially for the treatment of comedone as well as maintenance therapy and known as comedolytic agents.[9] They eject mature comedones, reduce the formation of micro-comedones by normalizing g desquamation of follicular epithelium and exert anti-inflammatory action. The formulation technology associated with topical retinoids has concentrated on providing greater penetration of the drug into deeper layers of skin and providing stability to the retinoid molecules to afford tolerability at the lower concentrations. They are contraindicated in pregnancy and females of childbearing age.[10] The side effect with retinoids is erythema, dryness, and itching during the early phase of treatment. Oral retinoid is first-generation retinoid isotretinoin that plays important role in the treatment of *A. vulgaris*.[11] It acts by reducing sebum production in the sebaceous gland, regularizes follicular keratinization, and decreases microbial colonization of *P. acne*. Isotretinoin shows its anti-inflammatory action by normalizing the exaggerated immune response to *P. acne*.[12]

Topical antibiotics act directly on the *P. acnes* colonization and show proinflammatory action on comedones by reducing the production of interleukin-1.[2] The mild anti-inflammatory action of topical antibiotics is due to suppressing leukocyte chemotaxis.[13] Commonly used antibiotics for topical treatment are clindamycin and erythromycin. They are available in the form of solutions, lotions, gels, and saturated pads.

Hormonal treatment is also used for the management of acne. In this, Androgen plays an important role in the formation and severity of acne.[14] The disease associated with endocrinopathies like polycystic ovarian syndrome, ovarian tumor, and adrenal hyperplasia is also perceived in some patients with acne, especially when acne is formed with sudden onset.[15] In these cases, serum-free and total testosterone, dehydroepiandrosterone sulphate, luteinizing hormone, and follicle-stimulating hormone should be checked. Androgen receptor blocker: spironolactone, cyproterone acetate, chlormadinone, and flutamide Adrenal androgen production blockers:[16] Glucocorticoids Ovarian androgen blocker: gonadotropin-releasing agonists and oral contraceptives.

The use of cosmetic products is increasing around the world and the variety of chemical compounds used in the manufacture of these products grows at the same time.[17] In this way, the risk of intoxication, allergic processes, prolonged chemical exposure, side effects, and indiscriminate use is also increased. Therefore, various chemical ingredients that are harmful to the skin are mentioned.

Benzyl alcohol

Benzyl alcohol is an aromatic chemical compound used for the improvement of active substance solubility and added as an antibacteriostatic compound.[18] Therefore, there are various toxic effects are reported of benzyl alcohol, which is: Benzoic acid is a mucous membrane irritant, and also they show carcinogenic properties.

Sodium lauryl sulfate (SLS)

SLS is an alkaline, anionic surfactant. In medicinal products, SLS has several functional uses as an emulsifying agent, modified-release agent, penetration enhancer, solubilizing agent.[19] The highest risk of using products with SLS is irritation to your eyes, skin, mouth, and lungs. For people with sensitive skin, sulphates may also clog pores and cause acne. Furthermore, there are adverse reactions to SLS in cosmetics, and pharmaceutical formulations mainly are reports of irritation to the skin following prolonged topical application particularly with emollients. Paradoxically, dermatitis is made worse with hydrocortisone cream containing SLS.[20]

Potassium sorbate

Allergies to potassium sorbate are more common with cosmetics and personal products, where it can cause skin or scalp irritation.[21]

Natural ingredients have been conventionally used for centuries for skincare purposes; they are becoming more prevalent in contemporary formulations. The term "natural" is defined as something or an ingredient that is produced by nature or found in nature and is directly extracted from plants or animal products. Sources of natural ingredients can include herbs, fruits, flowers, leaves, minerals, water, and land. The effect of natural ingredients in skin care products depends on their *in-vitro* and *in-vivo* efficacy and the type of dermatological base where they are incorporated.[22] The use of bioactive extracts or phytochemicals from a variety of botanicals in cosmetics accomplishes two functions: care of the body and as ingredients to influence the biological functions of the skin, providing the nutrients for healthy skin. Generally, botanical products are a rich source of vitamins, antioxidants, essential oils and oils, hydrocolloids, proteins, terpenoids, and other bioactive compounds. According to their composition, these extracts can provide different properties. Therefore, various herbal formulations are used in skin disease including cream, gel, serum, etc.

A serum is a product typified by its rapid absorption and ability to penetrate the deeper layers of the skin, together with its non-greasy finish and the intensive formula with a very high concentration of active substances.[22] The serum is a skincare product containing a gel or lightweight lotion or moisturizing consistency that can penetrate deeper to deliver active ingredients into the skin.[23] A good skin serum may provide your skin with a firmer, smoother texture, make pores appear smaller, and increase moisture levels. Various serum formulations are used for topical skin diseases. The serum formulations can be made of topical antibiotics, topical retinoids, etc.

Retinoids define a class of substances comprising Vitamin A (retinol) and its natural and synthetic derivatives. As lipophilic molecules, they can diffuse through cellular and other phospholipid membranes. Inside the cells, they bind to nuclear receptors (RAR-α, -β, -γ, and RXR-α, -β, -γ) then the ligand-receptor complexes modulate the expression of genes involved in cellular differentiation and proliferation.[24] Retinol is produced in the small intestine either by hydrolysis of retinyl esters or by oxidation of various carotenoids. Retinol can be oxidized into retinaldehyde, and then into retinoic acid, the biologically active form of Vitamin A.[25] Therefore, retinol is most frequently used in cosmeceutical treatment. It is very stable in product formulations and well tolerated.[26]

This research paper aims to develop herbal serum based on botanicals with retinoids. Here, this formulation is formed because there are various synthetic products are available for the treatment of acne, synthetic agents are carcinogenic and they also irritate the skin. So, by these mentioned limitations we have developed a herbal product.

MATERIALS AND METHODS

Material

Plant material *Aloe vera* and green tea are collected from the in-house garden, retinol is obtained as a gift sample from S.A. Pharmachem Pvt. Ltd.

METHOD

Extraction of secondary metabolites

A. vera leaves were washed with water properly and then they are kept in an oven at 50°C for 7 days to completely dry. After that dried *A. vera* leaves were ground to a powdered form and kept in an airtight container for further use. The solvent extraction method was used for the extraction of Gallic acid from the *A. vera* leaf powder. The Soxhlet thimble was filled with the *A. vera* leaf powder and inserted into the Soxhlet main chamber and closed. One litre of 75% water was filled into the Soxhlet main chamber and attached to the Soxhlet apparatus, which was heated near to 70°C for about 8 h until solvent vapour filled the main chamber. After that, the solvent vapours were condensed into the chamber containing *A. vera* extract. The *A. vera* leaf extract using 70% water then evaporated on the water bath at 80°C and concentrated to semi-solid mass.[27]

Then, the extractive value of the *A. vera* was calculated by the formula:

$$\%\ \text{Extractive value} = \frac{\text{Weight of residue}}{\text{Weight of drug}} \times 100$$

The loss on drying (LOD) of *A. vera* extract powder was done. In this, a Petri dish was taken and washed properly then kept in an oven for ½-h to dry. After that they are kept in a desiccator, then the Petri dish was weighed initially. After that 2-g powder was filled in a Petri dish and weighed. They are kept for 4 h in an oven to dry and the final weight was taken, LOD of *A. vera* extract powder was calculated

$$\text{LOD} = \frac{W2 - W3}{W2 - W1} \times 100$$

where W1=Empty weight of Petri dish
W2=Weight of content in Petri dish before drying
W3=Weight of Petri dish after drying

Green tea leaves were washed with water properly and then they are kept in an oven at 45°C for 1 day to completely dry. After that dried Green Tea leaves were ground to a powdered form and kept in an airtight container for further use. The solvent extraction method was used for the extraction of Gallic acid from the *A. vera* leaf powder. The Soxhlet thimble was filled with the *A. vera* leaf powder and inserted into the Soxhlet main chamber and closed. One litre of 75% water was filled into the Soxhlet main chamber and attached to the Soxhlet apparatus, which was heated near to 75°C for about 7.5 h until solvent vapour filled the main chamber. After that, the solvent vapours were condensed into the chamber containing Green Tea extract. The Green Tea leaf extract using 70% water then evaporated on the water bath at 80°C and concentrated to semi-solid mass.[27] Then, the Extractive value of the Green Tea was calculated by the formula:

$$\%\ \text{Extractive value} = \frac{\text{Weight of residue}}{\text{Weight of drug}} \times 100$$

The LOD of Green Tea extract powder was done. In this, a Petri dish was taken and washed properly then kept in an oven for ½ h to dry. After that they are kept in a desiccator, then the Petri dish was weighed initially. After that 2-g powder was filled in a Petri dish and weighed. They are kept for 4 h in an oven to dry and the final weight was taken, LOD of Green Tea extract powder was calculated

$$\text{LOD} = \frac{W2 - W3}{W2 - W1} \times 100$$

where W1=Empty weight of Petri dish
W2=Weight of content in Petri dish before drying
W3=Weight of Petri dish after drying

Photochemical screening of secondary metabolites[28]

Test for alkaloids

a. Dragendroff's test: Take 2 ml of each extract, few drops of Dragendroff's reagent (potassium bismuth iodide solution) was added. A turbid orange/orange-red precipitate was observed in the presence of alkaloids
b. Wagner's test: A few drops of Wagner's reagent was added in 2–3 ml extract. Then, a reddish-brown precipitate was observed that confirms positive

c. Hager's test: few drops of Hager's reagent poured on the extracts and if yellow colour precipitate was observed, this confirms the test positive.

Test for Phenolic Compounds

An equal amount of 1% Ferric chloride solution and 1% Potassium ferrocyanide was mixed, 3 drops of this prepared mixture were added to the 2ml of extracts. The positive result shows the formation of a bluish colour.

Test for Tannins

a. Ferric chloride reagent test: 2–3 drops of 5% ferric chloride solution were taken and they are poured on both extracts. Then the formation of green/greenish-black colour indicates the presence of tannins
b. Potassium dichromate test: Each extract solution was taken and 1ml of 10% of aqueous potassium dichromate solution was poured. The formation of yellowish-brown precipitate confirms the presence of tannins.

Test for Flavonoids

a. Alkaline reagent test: 1ml of 10% solution hydroxide solution was taken and added to the extracts to form yellow colour, which confirms the presence of flavonoids in the sample
b. Lead acetate test: both extracts were taken and few drops of 10% lead acetate solution were added to it, this forms a yellow colour precipitate which suggests the presence pf flavonoids.

Figure Printing Profiling by High-performance Thin-layer chromatography (HPTLC)

A. vera (gallic acid)

Sample preparation

The dried aqueous extract of *A. vera* 100 mg was weighed accurately and dissolved in 10 ml of methanol to make a final concentration of 10 mg/ml. Then, 10 mg of gallic acid was dissolved in 10 ml of methanol to give 1 mg/ml standard solution.[29]

Chromatographic conditions

Sample for analysis was applied on precoated silica gel plates using Linomat V sample applicator (CAMAG). Optimization of the mobile phase was done, but a satisfactory resolution was obtained in the solvent ethyl acetate: toluene: formic acid; 6:3:1, v/v/v. The chromatograms were developed and scanned at 254 nm using Camag Scanner.[30]

Green Tea (Gallic Acid)

Sample preparation

The dried aqueous extract of Green Tea 200 mg was weighed accurately and dissolved in 10 ml of methanol to make a final concentration of 20 mg/ml. Then, 10 mg of gallic acid was dissolved in 10 ml of methanol to give 1 mg/ml standard solution.

Chromatographic conditions

Sample for analysis was applied on precoated silica gel plates using Linomat V sample applicator (CAMAG). Optimization of the mobile phase was done, but a satisfactory resolution was obtained in the solvent ethyl acetate: toluene: formic acid; 5:3:2, v/v/v. The chromatograms were developed and scanned at 280 nm using Camag Scanner.[31]

Formulation of herbal serum

The formulation of different herbal serum was prepared as listed in Table 1. The formulation was divided into two phases i.e. aqueous phase and oil phase. First, the oil phase was taken in a beaker and homogenized perfectly for 15 min at 1000 rpm and then emulsifying agent (Tween 80) was added to that. After that aqueous phase was added slowly with continuous stirring of the oil phase. Then both the phases are homogenized properly for more than 20 min at the same rpm, then *A. vera* leaf extract and Green tea extract was added into the formulation and homogenized for 15 min at the same rpm to get a serum formulation.[32]

Evaluation parameters

Appearance and homogeneity

The prepared gels were tested for physical appearance and homogeneity by visual inspection.

pH

The pH of all the formulations was determined by using the digital pH meter (U- Tech, SSI-302). 2 g of serum was accurately weighed and dispersed into 20 ml of distilled water and stored for 2 h. Then, the measurement of pH of each formulation was done and recorded in triplicate.

Viscosity and rheological studies

All five serum formulations were tested for their rheological parameters at 25°C using Brookfield Viscometer. The measurement was made over a whole range of speed settings from 10rpm with the 30s between two successive speeds and then in descending order.

RESULTS AND DISCUSSION

Extraction of secondary metabolites

a. Extractive value: The Extractive value of both the herbs are done and mentioned in Table 2. The results show that they are under the limit

Table 1: Formulation of herbal serum

Formulation/ingredient (%)	F1	F2	F3	F4	F5
Cetostearyl alcohol	0	2	2	2	2
PEG 6000	2	2	2	2	2
Coconut oil	3	3	3	3	3
Tween 80	2	2	2	2	2
1% Sodium citrate	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Vitamin A	1.5	1	1.5	1.5	1.5
Aloe vera	1	2	1	2	2
Green Tea	0.5	0.5	1	2	1
Salicylic acid	2	2	2	2	2
Potassium sorbate	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.8	0.8	0.8	0.8	0.8
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

b. LOD: The LOD of both the drugs are done and mentioned in Table 2. The results confirm that LOD is under the standard limit.

Phytochemical screening of secondary metabolites

The results of phytochemical screening of secondary metabolites of dried leaves powder of *A. vera* and Green tea revealed the presence of alkaloids, flavonoids, tannins and phenolic compounds. The presence of alkaloids was confirmed through Dragendroff's, Wagner's and Hager's reagent test. The presence of phenols and flavonoids are confirmed by Alkaline reagents, and these results are mentioned in Table 3.

Fingerprinting profiling by HPTLC

The HPTLC method was used to confirm the presence of gallic acid in *A. vera* and Green tea. The standard chromatogram of gallic acid is shown in Figure 1, from that confirmation of gallic acid in the samples, are determined. The chromatograms of test samples i.e. *A. vera* and green tea are shown in Figures 2 and 3 respectively, which confirms the presence of gallic acid in both the extracts. The Rf value of standard and samples are mentioned in Table 4. The confirmation of gallic acid in both the extracts means that they both have an anti-bacterial activity which will help in the management of acne.

Table 2: Extractive value and loss on drying of *Aloe vera* and green tea

S. No.	Herb	%Extractive value (NLT 12%)	Loss on drying (NMT 5%)
1.	*Aloe vera*	12.93	4.76
2.	Green Tea	12.98	3.32

Table 3: Phytochemical screening of secondary metabolites

S. No.	Test	Reagent	*Aloe vera* extract	Green tea extract
1.	Alkaloid	Dragendroff's	+	+
		Wagner's	+	+
		Hager's	-	+
2.	Phenolic compound		+	+
3.	Tannins	Ferric chloride	+	+
		Potassium dichromate	+	-
4.	Flavonoids	Alkaline	+	-
		Lead acetate	+	+

+= Present; −=Absent

Table 4: Chromatographic profile of *Aloe vera* and green tea extract by high-performance thin-layer chromatography method

S. No.	Extract/standard	Solvent system	Detection	Rf
1.	Standard Gallic acid	ethyl acetate: toluene: formic acid; 6:3:1, v/v/v	254 nm	0.81
2.	*Aloe vera* extract	ethyl acetate: toluene: formic acid; 6:3:1, v/v/v	254 nm	0.02, 0.37, 0.42, 0.46, 0.60, 0.85, 1.05, 1.21
3.	Green tea extract	ethyl acetate: toluene: formic acid; 5:3:2, v/v/v	254 nm	0.08, 0.22, 0.61, 0.81, 0.88, 1.01, 1.21

Evaluation parameters

A. vera faces serum formulation was translucent white viscous liquid preparation with a smooth homogeneous texture and glossy appearance. The formulation was re-dispersed within a second. After use, it felt emollient, slipperiness and no residues were formed and easy to wash out.

Physical appearance

Serum formulation was a translucent white color, viscous liquid preparation with a smooth homogeneous texture and glossy appearance.

pH

It was found to be in the range of 6.5–7.1 of all five batches. The data of all the formulations are mentioned in Table 5. The optimized batch selected is F4 and the mean pH was 6.5.

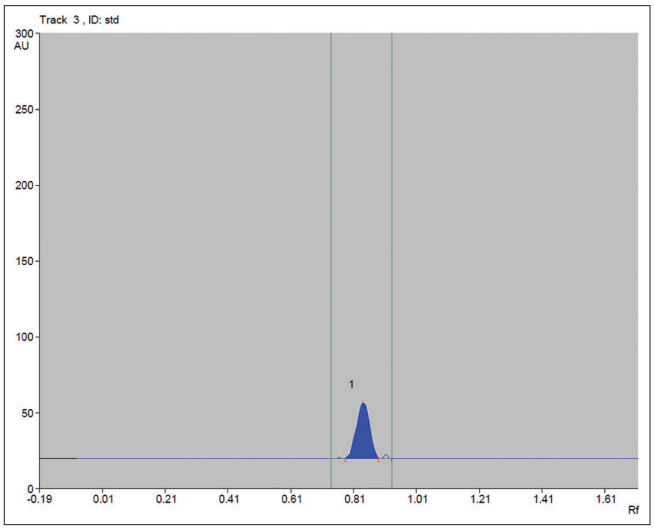

Figure 1: Thin-layer chromatography chromatogram of standard of Gallic acid at 254 nm, developed using a mobile phase (ethyl acetate: toluene: formic acid; 6:3:1, v/v/v)

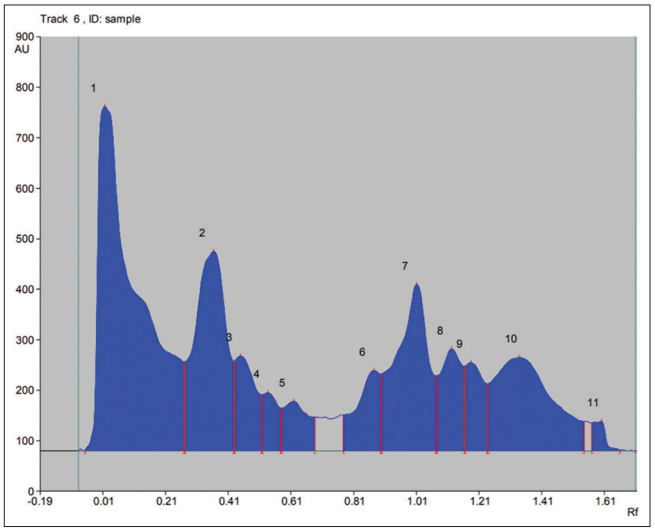

Figure 2: Thin-layer chromatography chromatogram of test sample of *Aloe vera* leaf extract at 254 nm, developed using a mobile phase (ethyl acetate: toluene: formic acid; 6:3:1, v/v/v)

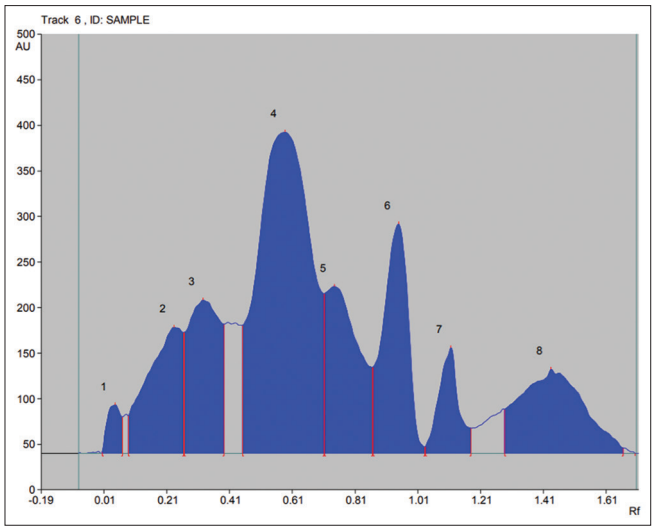

Figure 3: Thin-layer chromatography chromatogram of test sample of Green tea leaf extract at 254 nm, developed using a mobile phase (ethyl acetate: toluene: formic acid; 5:3:2, v/v/v)

Table 5: pH and Viscosity of batches (F1-F5)

S. No.	Batch	pH	Viscosity (cps)
1.	F1	6.1	6564
2.	F2	6.7	6724
3.	F3	6.2	6563
4.	F4	6.5	6479
5.	F5	6.9	6568
Average		6.5	6580

Viscosity

The viscosity of the formulation kept at room temperature was measured of all the batches, the data of the formulation are mentioned in Table 5. The optimized batch selected is F4 and the mean viscosity was 6580 cps.

CONCLUSION

The herbal serum for the management of acne with botanicals was successfully formulated and evaluated for different parameters. The result of this formulation indicates that the presence of gallic acid in both the herbs will show anti-bacterial activity, which will help in the management of acne with the retinaldehyde. The five batches were formulated and it was found that batch F4 was the optimized and satisfactory batch. It shows that it will give a better effect on the skin when used topically, it will also not irritate due to its pH is in range as required. Hence, the present investigation revealed the possibility of developing the commercial product with *A. vera* and Green in combination with retinaldehyde for the management of *A. vulgaris*, a remedy in Indian traditional medicine.

REFERENCES

1. Cassano P, Petrie SR, Hamblin MR, Henderson TA, Iosifescu DV. Review of transcranial photobiomodulation for major depressive disorder: Targeting brain metabolism, inflammation, oxidative stress, and neurogenesis. Neurophotonics 2016;3:031404.
2. Gollnick HJ. Current concepts of the pathogenesis of acne. Drugs 2003;63:1579-96.
3. Adityan B, Kumari R, Thappa DM. Scoring systems in *Acne vulgaris*. Indian J Dermatol Venereol Leprol 2009;75:323-6.
4. Fox L, Csongradi C, Aucamp M, Plessis JD, Gerber M. Treatment modalities for acne. Molecules 2016;21:1063.
5. Latter G, Grice JE, Mohammed Y, Roberts MS, Benson HA. Targeted topical delivery of retinoids in the management of *Acne vulgaris*: Current formulations and novel delivery systems. Pharmaceutics 2019;11:490.
6. Fournière M, Latire T, Souak D, Feuilloley MG, Bedoux G. *Staphylococcus epidermidis* and *Cutibacterium acnes*: Two major sentinels of skin microbiota and the influence of cosmetics. Microorganisms 2020;8:1752.
7. Maisch TJ. Anti-microbial photodynamic therapy: Useful in the future? Lasers Med Sci 2007;22:83-91.
8. Decker A, Graber EM. Over-the-counter acne treatments: A review. J Clin Aesthet Dermatol 2012;5:32.
9. Raghuwanshi K, Mishra A. Anti-acne phytoconstituents-an intensive review. Int J Rec Adv Med Pharma Res 2020;3:1.
10. Simonart T. Newer approaches to the treatment of *Acne vulgaris*. Am J Clin Dermatol 2012;13:357-64.
11. Thielitz A, Gollnick H. Topical retinoids in *Acne vulgaris*. Am J Clin Dermatol 2008;9:369-81.
12. Dispenza MC, Wolpert EB, Gilliland KL, Dai JP, Cong Z, Nelson AM, *et al*. Systemic isotretinoin therapy normalizes exaggerated TLR-2-mediated innate immune responses in acne patients. J Invest Dermatol 2012;132:2198-205.
13. Toyoda M, Morohashi MJ. An overview of topical antibiotics for acne treatment. Dermatology 1998;196:130-4.
14. Elsaie ML. Hormonal treatment of *Acne vulgaris*: An update. Clin Cosmet Investig Dermatol 2016;9:241.
15. Kim GK, Del Rosso JQ. Oral spironolactone in post-teenage female patients with *Acne vulgaris*: Practical considerations for the clinician based on current data and clinical experience. J Clin Aesthet Dermatol 2012;5:37.
16. Barros B, Thiboutot D. Hormonal therapies for acne. Clin Dermatol 2017;35:168-72.
17. Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr., Rejeski D. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol 2015;6:1769-80.
18. Tuominen A. Tannins and Other Polyphenols in *Geranium Sylvaticum*. UtuPub; 2018.
19. Bezuidenhout JJ. Development of Vitamin C and E Fixed-dose Combination, Multiple-unit, Solid Oral Delivery Systems. Potchefstroom: North-West University, South-Africa; 2019.
20. Pecquet C, Bayrou O, Vigan M, Raison N, Lauriere M. P14 hydrolysed wheat protein: A new allergen in cosmetics and food. Contact Dermat 2004;50:182-3.
21. Adams RM, Maibach HI. A five-year study of cosmetic reactions. J Am Acad Dermatol 1985;13:1062-9.
22. Burlando B, Verotta L, Cornara L, Bottini-Massa E. Herbal Principles in Cosmetics: Properties and Mechanisms of Action. United States: CRC Press; 2010.
23. Salvioni L, Morelli L, Ochoa E, Labra M, Fiandra L, Palugan L, *et al*. The emerging role of nanotechnology in skincare. Adv Coll Interface Sci 2021;293:102437.
24. Sorg O, Antille C, Kaya G, Saurat JH. Retinoids in cosmeceuticals. Dermatol Ther 2006;19:289-96.
25. Kedishvili NY. Retinoic acid synthesis and degradation. Subcell Biochem 2016;81:127-61.
26. Zasada M, Budzisz E. Retinoids: Active molecules influencing skin structure formation in cosmetic and dermatological treatments. Postepy Dermatol Alergol 2019;36:392.

27. Saniasiaya DJ. Antifungal effect of Malaysian *Aloe vera* leaf extract on selected fungal species of pathogenic otomycosis in *in vitro* culture medium. Oman Med J 2017;32:41.
28. Howlader MM, Ahmed SR, Kubra K, Bhuiyan KH. Biochemical and phytochemical evaluation of *Stevia rebaudiana*. Asian J Med Biol Res 2016;2: 121-30.
29. Karthik R. Pharmacognostical, Phytochemical Pharmacological Evaluation of Justicia Glauca. Madurai: College of Pharmacy, Madurai Medical College; 2012.
30. Pandey DK, Shahnawaz, Dey A. Comparative HPTLC analysis of antioxidant compound gallic acid from *in vitro* and naturally grown *Stevia rebaudiana*. J Biol Active Prod Nat 2015;5:397-405.
31. Pandey DK, Parida S, Dey A. Comparative HPTLC analysis of bioactive marker barbaloin from *in vitro* and naturally grown *Aloe vera*. Rev Bras Farmacogn 2016;26:161-7.
32. Nawarathne NW, Wijesekera K, Wijayaratne WM, Napagoda M. Development of novel topical cosmeceutical formulations from *Nigella sativa* L. with antimicrobial activity against acne-causing microorganisms. ScientificWorldJournal 2019;2019:5985207.