INTERNATIONAL JOURNAL OF
Clinical and Pharmaceutical Innovations
An International Peer Reviewed Open Access Journal

Research Article

ISSN (Online): 3142-8665
CODEN (USA): IJCPSM

2026, Volume 1, Issue 5, Page No: 117-130.

FORMULATION AND EVALUATION OF ANTI-ACNE HERBAL SERUM

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Article Info: **Received:** 17 June 2026, **Revised:** 07 July 2026, **Accepted:** 27 July 2026

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Citation:
Nithyapriya Karuppusamy*[1], Sutha Ponnusamy[2], Sangameswaran Balakrishana[3], Arunkumar K.[4], Aswini R. C.[5], Athithevan E.[6], Ayesha M.[7]. (2026). Formulation And Evaluation Of Anti-Acne Herbal Serum. International Journal of Clinical and Pharmaceutical Innovations, 1(5), 117-130.
DOI: https://doi.org/10.5281/zenodo.21705980

ABSTRACT
Acne vulgaris is a common skin disorder that affects millions of people worldwide, often causing physical illness and psychosocial problems. In recent years, the interest in natural skin care products has increased, which brought to light the investigation of potential anti-acne plant compounds, such as Marigold flower extract, as promising due to its rich phytochemical composition and traditional use in skin care. It was to formulate and find a suitable anti-acne herbal serum. Each of the five-formulation included marigold flower extract along with other plant extract known for their anti-inflammatory and antioxidants properties, like Mint and Aloe vera. To ensure quality and acceptability, the serum solution was thoroughly inspected in physiology, including appearance, texture, scent and pH, Chemical analysis revealed that F5 was the best option; it demonstrated strong efficacy and anti-acne qualities while keeping a light, non-sticky consistency that was ideal for all skin types, This study offers a successful all-natural method of treating acne by examining the advantages of plant and marigold flower extracts for skin.

KEYWORDS: Marigold flower, Mint, Aloe vera, Acne, pH, Viscosity.

சித்த மருத்துவ மூலிகைத் தோட்டம்
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Sl. No.	Botanical Name	Family	Part	Code
1.	*Tagetes erecta* L.	Asteraceae	Aerial parts	T300126256E

T300126256E

*Cultivar

30/01/2026

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Sl. No.	Botanical Name	Family	Part	Code
1.	*Mentha piperita* L.	Lamiaceae	Leaves	M300126257P

M300126257P

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Sl. No.	Botanical Name	Family	Part	Code
1.	*Aloe vera* (L.) Burm.f. Syn: *Aloe barbadensis* Mill.	Asphodelaceae	Whole plant	A300126258V

A300126258V

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INTRODUCTION

Acne is one of the most prevalent skin conditions, affecting more than 85% of teenagers. Some people have acne into their 40s and50s, but it usually starts in adolescence and goes away by the age of 20.

Even though it appears to be cosmetic, its effects can go far beyond the skin's surface, causing patients to experience severe emotional and psychological distress that may be significantly worse than the physical signs and symptoms. Suicidal thoughts and problem at work, both personal and functional. The reduction in quality of life is estimated to be like that brought on by diabetes,

arthritis, asthma or epilepsy.
Four factors are thought to be responsible for acne: excessive sebum production colonization, a host inflammatory reaction, and abnormal keratinocyte proliferation and differentiation in the hair follicle. It is thought that the skin commensal Propionibacterium acnes cause an inflammatory response that leads to subclinical and inflammatory acne lesions.

A group of symptoms linked to enlarged, irritated, or damaged sebaceous units make up the clinical aspects of acne. Lesional polymorphism, which is more commonly seen on the face, back, and chest, is the primary feature. Seborrhea is the most prevalent feature. Distended pilosebaceous units can exhibit inflammatory lesions such as pustules, papules, nodules, and cysts.

These lesions may appear as open or closed comedones. In more severe cases, a variety of inflammatory papules and nodules come together to form draining sinuses, which can result in permanent scarring and, in rare cases, malignant changes. Post-inflammatory lesions can also manifest as macular pigmentation and scars (hypertrophic, keloids, ice pick scars, depressed fibrotic and atrophic macules, and perifollicular elastolysis). Post-inflammatory hyperpigmentation is often seen in pigmented skin.

Moderate acne can be treated with topical antibiotics like tetracycline and clindamycin, as well as retinoids like tretinoin and adapalene. Moreover, topical antibiotics like tetracycline and clindamycin can help treat moderate acne. Oral antibiotics are the standard treatment for moderate acne when topical combinations are ineffective or intolerable. First-generation tetracyclines (oxytetracycline and tetracycline hydrochloride) or second-generation tetracyclines (doxycycline, lymecycline, and minocycline) should be used in first-line oral antibiotic.

AIM AND OBJECTIVES:

Aim

- The aim of the present study is to develop and evaluate an anti-acne herbal serum with antiacne properties using selected medicinal plant extracts, and to assess its physicochemical characteristics

Objectives

- To select suitable medicinal plants with proven anti acne, antioxidant, and anti inflammatory properties based on literature review.
- To prepare herbal extracts from the selected plant materials using appropriate extraction methods.
- To formulate an anti acne herbal serum incorporating the selected herbal extracts.
- To evaluate the formulated cream for physicochemical parameters, including Appearance and homogeneity, PH, viscosity, spreadability.
- To assess the and anti-acne potential of the formulated anti acne through suitable in-vitro or in-vivo evaluation methods.

PLANT PROFILE
MARIGOLD FLOWER

❖ Synonyms
Tagetes erecte.
❖ Biological source
The biological source of marigold is the genus tagetes.
❖ Family: Asteraceae.
❖ Chemical constituents
It is rich in carotenoids like lutein and zeaxanthin.
❖ Parts used: Petals.
❖ Uses
Soothe and heal the skin, reduce acne inflammation and

improve skin tone and hydration.

❖ DESCRIPTION

Marigold is an annual flowering herb widely used in traditional medicine. The flowers possess antibacterial, anti-inflammatory, antioxidant, and wound-healing properties. Marigold extract helps reduce acne-causing bacteria, inflammation, and promotes skin repair.

❖ Active Constituents:

Flavonoids, Lutein, Carotenoids, Terpenoids, Essential oils, Phenolic compounds, Tannins, Saponins.

MINT

❖ Synonyms

Pudina.

❖ Biological source

The biological source for mint is the genus menthe.

❖ Family

Lamiaceae.

❖ Chemical constituents

Chemical constituents in mint include a variety of terpenoids and other organic compounds, with major components varying by species but often featuring menthol, menthone, methyl acetate and 1, 8-cineole.

❖ Parts used: Leaves.

❖ Uses

Soothe irritated skin, reduce acne, brighten complexion and slow signs of aging.

❖ Description

Mint is an aromatic medicinal herb known for its cooling, antimicrobial, antioxidant, and anti-inflammatory properties. It helps reduce acne-causing microorganisms, controls excess oil, and soothes irritated skin.

❖ Active Constituents

Menthol, Menthone, Menthyl acetate, Rosmarinic acid, Flavonoids, Phenolic compounds, Limonene, Cineole.

ALOE VERA

❖ Synonyms
Aloe.
❖ Biological source
Aloe is the dried juice that is extracted from the bases of the leaves of different species of aloe through incision.
❖ Family: Asphodelaceae.
❖ Chemical constituents
Salicylic acid, Aloe-emodin, Aloesin, Aloe resin A, Aloe resin E, Isobarbaloin.
❖ Part used
Leaves of Aloe.
❖ Uses
Soothes irritated skin.

❖ Description
Aloe vera is a succulent medicinal plant widely used in skincare. The gel possesses moisturizing, anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties. It hydrates the skin, reduces redness, and accelerates healing of acne lesions.

❖ Active Constituents
Acemannan, Aloin, Aloe-emodin, Glucomannan, Vitamins (A, C, E, B_{12}), Polysaccharides, Amino acids, Minerals, Saponins, Anthraquinones.

METHODOLOGY

Material Required

❖ Marigold flower extract
❖ Mint extract
❖ Aloe vera gel
❖ Tea tree oil
❖ Vitamin E
❖ Tween 20
❖ Benzyl alcohol
❖ Glycerine
❖ Purified water

Preparation of Plant Extracts

1. Marigold Extract
Wash the marigold flowers thoroughly. Dry and grind them into small pieces. Add 9g of flower to 89 ml ethanol. Keep for 3 days. Filter through filter paper. Collect the filtrate.

2. Mint Extract
Wash fresh mint leaves. Dry and grind them into small pieces**.** Add 9g of powder to 40 ml ethanol. Keep for 3 days. Filter through filter paper. Collect the filtrate.

Preparation of Aloe vera gel
Separate aloe vera pulp from the fresh aloe vera plant. Wash it about 5 to 6 times in water to avoid skin irritation. Boil it with dm water with a small beaker kept in its centre. The extract is evaporated and collected in the small beaker.

PREFORMULTION STUDIES

Pre-formulation is the study of physical, chemical, and mechanical properties of a drug substance before formulation development. It helps in selecting suitable excipients., dosage forms, and manufacturing processes to ensure the safety, stability, efficacy, and quality of the final pharmaceutical product.

Pre-formulation studies include the following:

- Phytochemical studies
- Description
- Hygroscopic nature
- Solubility

PHYTOCHEMICAL SCREENING OF EXTRACTS

1. Preliminary phytochemical screening of Marigold extract

The phytochemical analysis of the ethanolic extract of Marigold were performed individually for the presence of Flavonoids, Phenolics & Tannins, Alkaloids, Saponins, Glycosides, Terpenoids.

Test for alkaloids

- Dragendroff's test:

1ml of the extract was added to test tube to that add 1ml of Dragendroffs reagent (potassium bismuth iodine solution). An orange red precipitate confirms the presence of alkaloids.

Test for flavonoids

1ml of extract was added to the test tube and few drops of dilute solution of ferric chloride was added. The appearance of reddish-brown colour indicates the presence of flavonoids.

Test for phenols

- Ferric chloride test:

The extract was added to the test tube to that 5% of ferric chloride was added and observed for the formation of deep blue colour.

Test for tannins

To 5ml of extract add 1ml of lead acetate solution. Th brown colour precipitate indicates the presence of tannins.

Test for saponins

- Foam test:

1ml of extract was added to the test tube and 5ml of distilled water and few drops of olive oil is added and shaken well for minutes. The formation of layer of foam indicates the presence of saponins.

Test for glycosides

To the extract add 1ml of glacial acetic acid and ferric chloride solution and 1ml of conc. Sulphuric acid the appearance of reddish-brown colour indicates the presence of glycosides.

Test for terpenoids

- Salkowski's test

To the 2ml of extract was shaken with chloroform, to the chloroform layer sulfuric acid was added slowly to the side of the test tube. The formation of red colour indicates the presence of terpenoids.

2. Preliminary phytochemical screening of mint extract

The phytochemical analysis of the ethanolic extract of mint was performed individually of the presence of flavonoids, phenolics, terpenoids, tannins and saponins was carried out.

Test for flavonoids

1ml of the extract was added to the test tube and few drops of dilute solution of ferric chloride were added. The appearance of reddish-brown colour indicates the presence of flavonoids.

Test for phenols

- Ferric chloride test

The extract was added to the test tube to the 5% ferric chloride was added and observed for the formation of deep blue colour.

Test for terpenoids

- Salkowski's test

To the 2ml of extract was shaken with chloroform, to the chloroform layer sulfuric acid was added slowly to the side of the test tube. The formation of red colour indicates the presence of terpenoids.

Test for tannins

To 5ml of extract add 1ml of lead acetate solution. Th brown colour precipitate indicates the presence of tannins.

Test for saponins

- Foam test

1ml of extract was added to the test tube and 5ml of distilled water and few drops of olive oil is added and shaken well for minutes. The formation of layer of foam indicates the presence of saponins.

3. Preliminary phytochemical screening of aloe vera

The phytochemical analysis of aloe extract was performed individually for the presence of carbohydrates, saponins, phenolics and proteins were carried out.

Test for carbohydrates

2ml of extract was added to the test tube and add 2-3 drops of Molisch reagent. Carefully add 1-2 drops of conc. Sulphuric acid along the side of the test tube with mixing. Formation of a violet ring at the interface indicates the presence of carbohydrates.

Test for glycosides

To the extract add 1ml of glacial acetic acid and ferric chloride solution and 1ml of conc. Sulphuric acid the

appearance of reddish-brown colour indicates the presence of glycosides.

Test for saponins

- Foam test

1ml of extract was added to the test tube and 5ml of distilled water and few drops of olive oil is added and shaken well for 10 mins. The formation of layer of foam indicates the presence.

Test for phenols

- Ferric chloride test

The extract was added to the test tube to the 5% ferric chloride was added and observed for the formation of deep blue colour.

Test for proteins

To the extract add few drops of biurets reagent the formation of violet colour indicates the presence of proteins.

FORMULATION OF ANTI-ACNE HERBAL SERUM

Ingredients	F1	F2	F3	F4	F5	F6
Marigold Extract (ml)	0.05	0.75	1.00	1.25	1.50	1.75
Aloe vera gel Extract (ml)	2.00	2.50	3.00	3050	4.00	4.50
Mint Extract (ml)	0.25	0.40	0.50	0.75	1.00	1.25
Tea tree oil (ml)	1	1	2	2	3	4
Vitamin E (ml)	0.10	0.15	0.20	0.25	0.30	0.40
Tween 20 (ml)	0.20	0.25	0.30	0.40	0.50	0.60
Benzyl Alcohol (ml)	0.10	0.15	0.20	0.25	0.25	0.30
Glycerine (ml)	0.50	0.60	0.75	0.75	1.00	1.25
Purified Water	q.s. to 15 ml	q.s. to 15 ml	q.s. to 15 ml	q.s. to 15 ml	q.s. to 15 ml	q.s. to 15 ml

PREPARATION

Accurately measure the required quantity of marigold extract and mint extract according to the formulation table. Take the measured aloe vera gel in a clean beaker and stir until a smooth gel base is obtained. Add glycerine slowly to the aloe vera gel with continuous stirring. In a separated beaker, mix Tween 20 and tea tree oil until a uniform mixture is formed. Add vitamin E to the above mixture and stir well. Slowly add the marigold and mint extract to the aloe vera base while stirring continuously. Add the Tween 20, Tea tree oil, Vitamin E mixture to the herbal gel base and mix thoroughly to obtain a homogeneous serum. Add benzyl alcohol as preservative and mix well. Make up the final volume to 15 ml with purified water (q.s. to 5 ml). Stir continuously for 10 – 15 minutes until a clear and uniform serum is obtained.

Transfer the prepared serum into clean, sterilized, amber-coloured bottles and label appropriately. Store away from direct sunlight.

IN-VITRO STUDIES

PHYSICOCHEMICAL EVALUATION

Physicochemical evaluation is the systematic testing of a substance to determine its intrinsic physical and chemical properties.

Organoleptic evaluation

Organoleptic observations were carried out visually using the five senses to describe the face cream preparations that had been made. The organoleptic test is presented.

pH test

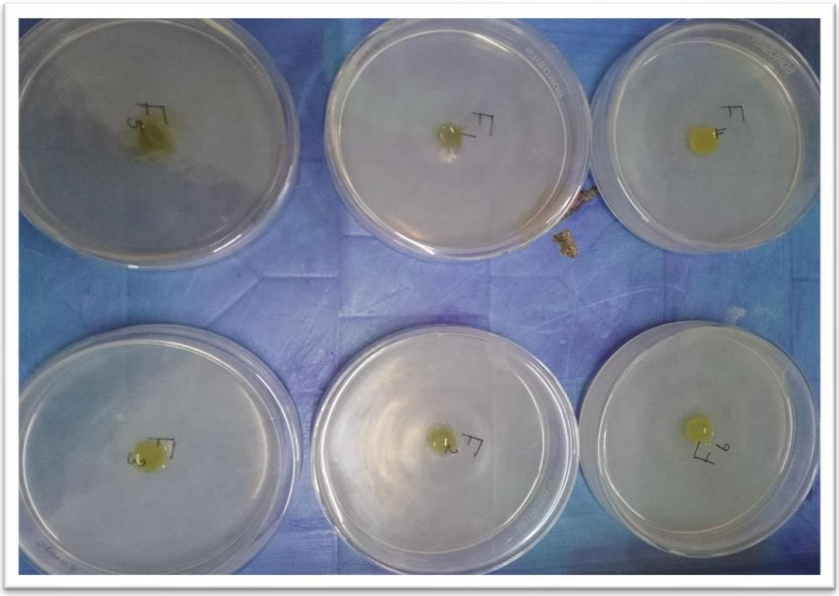
The pH meter was calibrated using standard buffer

solution. pH is calculated using calibrated pH meter or wide range pH paper.

Viscosity
Viscosity of the formulated serum of F1 to F6 are determined with a Brookfield Viscometer. Operate the viscometer at selected speed and allow the reading to stabilize. Record the viscosity value in cP and calculate the average.

Spreadability
Place the known quantity of serum between two glasses. Apply the standard weight on the upper slide for the fixed time. Allow the upper slide to move under a specified load. Record the time taken and calculate spreadability.

Zone of inhibition
Prepare sterile nutrient agar plates. Spread the bacterial culture uniformly. Make wells in the agar. Add the serum formulations(F1-F6) into the wells. Incubate at 37 degree C for 24-48 hrs. Measure the zone of inhibition (mm) around each well.



Cytotoxicity
It evaluates the toxic effect of a formulation on cultured cells. (e.g., keratinocytes, fibroblasts, or other cell lines). Common assays include MTT assay, XTT assay, neutral red uptake assay, and trypan exclusion test.

Calorimetry
Switch on the colorimeter and allow it to warm up. Set the required wavelength (510–590 nm, as applicable). Calibrate the instrument using a blank solution. Fill a clean cuvette with the serum sample. Place the cuvette in the colorimeter and record the absorbance. Repeat for all formulations (F1–F6). Record the absorbance values and compare the formulations.

IN-VIVO STUDIES
Removal test
Apply the known amount of serum uniformly on a marked area of skin or a glass plate and allow it to dry. Wash the area with a measured quantity of water or a mild cleanser. Gently wipe the surface and observe the ease of removal. Record the percentage of serum removed.

Skin irritation test
Apply the serum to a shaved area of skin. Observe for redness, swelling, or irritation for 24-72 hrs.

Dermal toxicity studies
Apply a fixed amount of the anti-acne serum to a shaved area of animal skin (commonly rats or rabbit) and cover with a gauze patch. Observe the animal for 24-72 hours for sign of erythema (redness) oedema (swelling), itching, or other skin reaction. Record body weight, behavioural changes, and any sign of systemic toxicity during the study period. Calculate the irritation score and compare it with the control group to assess dermal safety.

Histopathological studies
After treatment collect skin tissue sample from control and treated groups. Fix tissues in 10% formalin, dehydrated, and embed in paraffin wax. Cut thin section (4-5 mm) and stain with haematoxylin and eosin (H&E).

Examine under a microscope for epidermal thickness, inflammatory cell infiltration, tissue damage, and healing.

Microbial reduction test

Inoculate microbial cultures on to agar plates. Apply the anti-acne serum to wells or discs placed on the inoculate agar. Incubate at appropriate temperature (usually 37 degree C) for 24-48 hours. Measure the reduction in colony count or the zone of inhibition and compare with control.

RESULT AND DISCUSSION

1. Preliminary phytochemical analysis of marigold extract

The phytochemical analysis of ethanolic extract of marigold was analysed for the presence of Alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids and the results were shown in the following table.

Phytochemicals of marigold extract

S. No	Phytochemicals	Result
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Tannins	+
5	Saponins	+
6	Glycosides	+
7	Terpenoids	+

(+) positive, (-) negative

The ethanoic extract of marigold extract contains alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids.

2. Preliminary Phytochemical Analysis of Mint

The Phytochemical analysis of ethanolic extract of Mint was analyzed for the flavonoids, phenolics, terpenoids, tannins, saponins, and the result were shown in the following table:

S.NO	Phytochemicals	Result
1	Flavonoids	+
2	Phenolics	+
3	Terpenoids	+
4	Tannins	+
5	Saponins	+

(+) positive, (-) negative

The ethanolic extract of mint contains flavonoids, phenolics, Terpenoids, Tannins, Saponins.

3. Preliminary Phytochemical analysis of Aloe vera

The phytochemical analysis of ethanolic extract of aloe vera was analyzed for the carbohydrates, glycosides, saponins, phenolics, proteins, and the results were shown in the following table.

S. No	Phytochemicals	Result
1	Carbohydrates	+
2	Glycosides	+
3	Saponins	+
4	Phenolics	+
5	Protein	+

(+) positive, (-) negative

The ethanolic extract of aloe vera contains carbohydrates, glycosides, saponins, phenolics, and proteins.

OVERALL PHYTOCHEMICAL ANALYSIS TEST RESULTS

PHYTOCONSTITUENTS	MARIGOLD EXTRACT	MINT EXTRACT	ALOEVERA EXTRACT
Alkaloids	+	_	+
Flavonoids	+	+	_
Phenols	+	+	+
Tannins	+	+	_
Saponins	+	+	+
Glycosides	+	_	+
Terpenoids	+	+	_
Carbohydrates	_	_	_
Proteins	_	_	+

Formulation of face serum

The face serum which contains various polyherbal were formulated using different concentration of ethanolic extract and the oil phase remains same concentration for all the formulation (**F1, F22, F3, F4, F5, F6).**

All the prepared formulations F1, F2, F3, F4, F5 and F6 stored in the room temperature and used for the further evaluation procedure.

Evaluation of face cream

The color, odour, consistence, texture of the formulations was reported in the table.

Table: Organoleptic characters of the face serum.

S. NO	Properties	F1	F2	F3	F4	F5	F6
1	Color	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light yellow	Light yellow
2	Odour	Pleasant	Pleasant	Pleasant	Pleasant	pleasant	pleasant
3	Texture	Soft	Soft	Soft	Soft	soft	soft

All the four formulations were good in their properties such as color, odor, consistence, texture.

pH test

The pH test for the whitening face cream was performed using pH meter and the results were shown in the table.

Table: pH values of the face cream.

S.NO	Formulation	pH
1	F1	5.9
2	F2	5.8
3	F3	5.7
4	F4	5.6
5	F5	5.5
6	F6	5.4

The pH results obtained by the 6 different formulations have a pH range of 5.4-5.9. This shows that the six formulations have a pH that is safe and according to the standards based on the requirements, which ranges from 3.5-8. So, it is hoped that the formulated serum will not irritate the skin.

Viscosity

The viscosity of the whitening face cream of different formulation shows the good spreadability of the cream, and the values are interpreted in the table:

Table: Viscosity values of the face cream.

S.NO	Formulation	Viscosity (Cp)
1	F1	420
2	F2	510
3	F3	590
4	F4	670
5	F5	760
6	F6	850

Determination of spreadability

The spreadability of the formulated serums are calculated using the formula and the results are interpreted in the following table.

Removal test

The formulated serums applied on the skin surface are easily removed by the running tap water and the results are interpreted in the following table**:**

Table: Organoleptic characters of the face serum.

S.NO	Formulation	Removal test	Spreadability (sec)
1	F1	ER	7.8
2	F2	ER	7.4
3	F3	ER	7.0
4	F4	ER	6.6
5	F5	ER	6.2
6	F6	HG	5.8

NG – Non greasy, HG – Homogenous, ER – Easy removal.

ZONE OF INHIBITION

Formulation	Zone of inhibition(mm)	Activity
F1	18	Good
F2	32	Very strong
F3	35	Very strong
F4	40	Excellent /highest activity
F5	25	Strong
F6	34	Very strong

Among all these formulations, F4 exhibited the highest zone of inhibition (40 mm), indicating the maximum antibacterial activity against the test organism. Therefore, F4 was selected as the optimized formulation because it showed superior antimicrobial efficacy compared with F1, F2, F3, F5, and F6.

CALORIMETRY

Formulation	Wavelength (nm)	Absorbance
F1	550	0.50
F2	550	1.03
F3	550	1.50
F4	550	1
F5	550	1.95
F6	550	1

CONCLUSION

The present study successfully formulated and evaluated an herbal anti acne serum containing marigold (*Tagetes patula/Tagetes erecta)* extract, mint (Mentha) extract, and aloe vera gel. The selected herbal ingredients were chosen based on their well-known antibacterial, anti-inflammatory, antioxidant, soothing, and wound healing properties, which are beneficial in the management of acne. Preliminary phytochemical screening of the extract confirmed the presence of bio active constituents such as flavonoids, phenolics, terpenoids, glycosides, and saponins, which contributed to the therapeutic activity of the formulation. The prepared serum exhibited satisfactory physiochemical characteristics including acceptable appearance, homogeneity, pH, spreadability, and stability. The incorporation of marigold extract provided anti-inflammatory and antioxidant effects, while mint extract contributed to anti-microbial and cooling properties. Aloe vera gel enhanced skin hydration and promoted healing if acne lesion. The combined action of these herbal ingredients produced a formulation with potential anti acne activity and improved skin compatibility. Based on the evaluation studies, the formulated herbal serum was found to be stable, safe and suitable for topical application. Therefore, the developed anti acne serum can be considered a promising natural alternative for the management of acne and related skin condition. Further clinical studies on a larger population are recommended to establish its long-term efficacy and safety.

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