

Formulation, Standardization, And Evaluation of A Polyherbal Antiaging Remedy

Lata Gupta¹, Meenakshi Gupta², Jay Kumar¹, Nisha Sharma¹, Abhishek Kumar Singh¹, Kalpana¹,
Swarnakshi Upadhyay¹, Mamta tiwari¹

School of Pharmaceutical Sciences, Chhatrapati Sahuji Maharaj University, Kalyanpur,
Kanpur

*Corresponding author's Name: Lata Gupta, Jay Kumar

*E-mail: latagupta528@gmail.com

*E-Mail: jaysaxena274403@gmail.com

ORCID ID: 0009-0006-6388-2639

*Telephone: 9005038077

*Address: School of Pharmaceutical Sciences, Chhatrapati Shahu Ji Maharaj University,
Kalyanpur, Kanpur

Abstract

The current research project aimed to formulate and characterize an affordable herbal vanishing cream that contains liquorice extract and aloe vera gel. For thousands of years, aloe vera, a plant that resembles a cactus and is a member of the Asphodelaceae (Liliaceae) family, has been utilized in traditional medicine. Aloe, which comes in more than 300 species, appears to help with burns, wounds, and skin inflammation. It also has antiinflammatory, antiseptic, and antimicrobial properties, as well as anticancer, antiaging, antidiabetic, antibacterial, and antiviral properties. Liquorice, a member of the Leguminosae family, has seen a significant rise in its importance in pharmaceutical industries and cosmetic formulations due to the medicinal properties found in its rhizomes and roots. Glycyrrhiza extracts are commonly used in cosmetic products for their effective whitening properties, as well as their various cosmeceutical benefits such as skin antiaging, photoprotective effects, hair care, and antiacne properties. Three formulations F1, F2 and F3 were prepared using different concentrations of Aloe vera and liquorice extract. The characterization of the formulated creams was conducted using standard methods, as the tests for spreadability, irritancy, homogeneity, viscosity and PH. The affordable herbal creams made with extracts have moisturizing qualities are beneficial for skin. liquorice and aloe vera extract increase the effectiveness and concentration of the cream.

Keywords: Aloe vera, liquorice, moisturizing, polyherbal, anti-ageing, cosmetic

1. INTRODUCTION

Indian traditional medicine has a long history. Ayurveda, Siddha, Unani, and homoeopathy are only a few of the systems upon which traditional Indian medicine is based. The appraisal of the rich history of traditional medicine is crucial given the growing desire around the world to adopt and study the traditional systems and to take use of their potential based on various healthcare systems. The development of new and effective medications in modern medicine often begins with the usage of medicinal plants. The contribution of higher plants to the discovery of novel medications is only about 2%, despite the fact that many new drugs, whether synthetic or of natural origin, are occasionally discovered [1].

Their earlier research reveals the relationship between males and perfection care as follows: "The relationship between men and magnificence care can be considered as a fundamental connection to his appearance. They must feel good about themselves, be kind, and achieve psychological and physical balance. Men should be appreciative of their bodies. They do this by using objects to beautify themselves. [2].

Naturally derived cosmetic items are becoming more popular within this quickly expanding industry, and various cosmetics companies are expanding their global presence in the market for organic goods [3]. The modern usage of plant extract in skin product underlines the consumer need for environmentally friendly products, despite the use of plant for therapeutic motive is widely documented [4]. It becomes increasingly practical to produce a natural, affordable cosmetic that protects against the sun and aims to improve skin quality by lowering skin pigmentation as more people become aware of the connection between exposure to sunshine and pigmentation. In response, the South African cosmetics industry has created items using materials like liquorice [5].

Aging is an inescapable procedure for every living creature. Skin and other connective tissues go through significant changes and maturation. Epidermal thinning and the presence of wrinkles and lines on the face serve as indicators of these changes. There are two different types of skin ageing: age-related/sequential maturing and premature maturing/photographic maturing [6]. The latter is caused by external sources and includes symptoms including rough skin, light or dull pigmentation, and deep creases [7].

Various environmental factors play a significant role in contributing to degenerative changes, a decrease in physical strength, and the efficiency of bodily organs. These factors include biological aspects such as diet, exercise, leisure activities, and previous illnesses; psychological elements like depression, attitude, self-satisfaction, and adherence to a healthy lifestyle; cognitive issues such as diminished working memory; social influences including smoking and alcohol consumption; and external conditions like exposure to ultraviolet light, heat, insufficient oxygen, toxins, and poor nutrition. External and internal causes of skin ageing are described [8]. Wrinkling on the skin is a sign of natural ageing [9]. The term "photo ageing" refers to pigmented and rough skin on the hands and face, particularly in those who live in certain geographic areas because of their excessive exposure to sunshine. On the other hand, dry, pale skin with small, smooth wrinkles reveals the signs of innate ageing [10]. Microscopically, the photo-aged skin also has a thicker epidermis [11]. Clearly, the arrangement of collagen fibrils and elastin in the dermis determines the strength of skin and a lack of collagen accelerates the ageing process [12].



Fig. 1 External and Internal factors of skin aging

whereas elastin accumulation in skin that has been exposed to ageing photos is shown right below the dermal-epidermal junction [13]. Furthermore, the breakdown of the extracellular matrix has been associated with the aging of the skin and is correlated with increased levels of various enzymes in the body, such as collagenase, elastase, and hyaluronidase. [14].

Reactive oxygen species (ROS) are crucial in various cellular processes [15]. Chronic ultra violet exposure causes the skin to absorb the radiation, which produces ROS and causes oxidative stress. Elastase, collagenase, and hyaluronans levels are decreased by ROS, which can speed up the ageing process of the skin [16]. Tyrosinase, a mono oxygenase enzyme that contains copper, is a crucial component that catalyses the amalgamation of melanin in melanocytes. The significance of tyrosinase inhibitors has increased in both medical and cosmetic fields, primarily due to the buildup of excessive epidermal pigmentation,

which leads to a range of skin conditions. These include age-related melasma, freckles, age spots, and areas affected by actinic damage. These inhibitors block hyperpigmentation by hindering the process of enzymatic oxidation[17]. The synthesis and breakdown of these matrix proteins are balanced in healthy skin. Aging, UV radiation, stress, and an unhealthy lifestyle all disturb this delicate equilibrium [18]. The creation of matrix proteins in the skin can be encouraged, or matrix protein-degrading enzymes like hyaluronidase and elastase can be inhibited, to maintain the skin's youthful appearance [19]. The skin possesses an intricate barrier mechanism designed to regulate harmful natural and synthetic substances. However, prolonged or excessive exposure can surpass this system's capacity, resulting in oxidative stress and subsequent oxidative damage [20]. One of the harmful effects of regular cell metabolism is the production of oxidative free radicals. Antioxidants, which are organic compounds found in our cells, absorb and neutralise these harmful free radicals. However, those who avoid this cleansing process risk damaging DNA, proteins, and mitochondria. Over time, this damage, also known as oxidative damage, builds up [19]. Changes in the molecular structure of DNA,

$$\frac{\text{Percentage yield of extract}}{\text{Weight of powder taken for extraction}} = \frac{\text{Weight of solid extract}}{\text{Weight of powder taken for extraction}} \times 100$$

proteins, lipids, and prostaglandins are related to ageing. All are indicators of oxidative pressure, although not all progressions are caused solely by oxidation; there are other factors as well, such as spontaneous mistakes and other protein modifications [21]. Cellular ageing is caused by the build-up of these molecular alterations, particularly in proteins. But it's also understood that the generation of ROS, which participate in regular signalling procedures, is necessary to preserve homeostasis and cellular responsiveness [22].

2. MATERIAL & METHODS

In the current study, aloe vera leaves and liquorice roots were obtained from an herbal garden. 2.1.

Method of Extraction of *Aloe barbadensis*

Preparation of *Aloe barbadensis* (leaf powder) The extraction process was conducted following the methodology outlined by Arunkumar and Muthuselvam [23]. Utilizing the Soxhlet extraction technique, 25 grams of dried plant powder were placed in a glass thimble and subjected to extraction with 250 ml of various solvents, specifically methanol and ethanol, separately. The extraction continued until the solvent in the siphon tube of the Soxhlet apparatus became colorless. Subsequently, the extract was concentrated using a rotary vacuum evaporator at 35°C until all solvent was removed. The resulting dried plant crude extract was stored in a refrigerator at 2-8°C for future use [24].

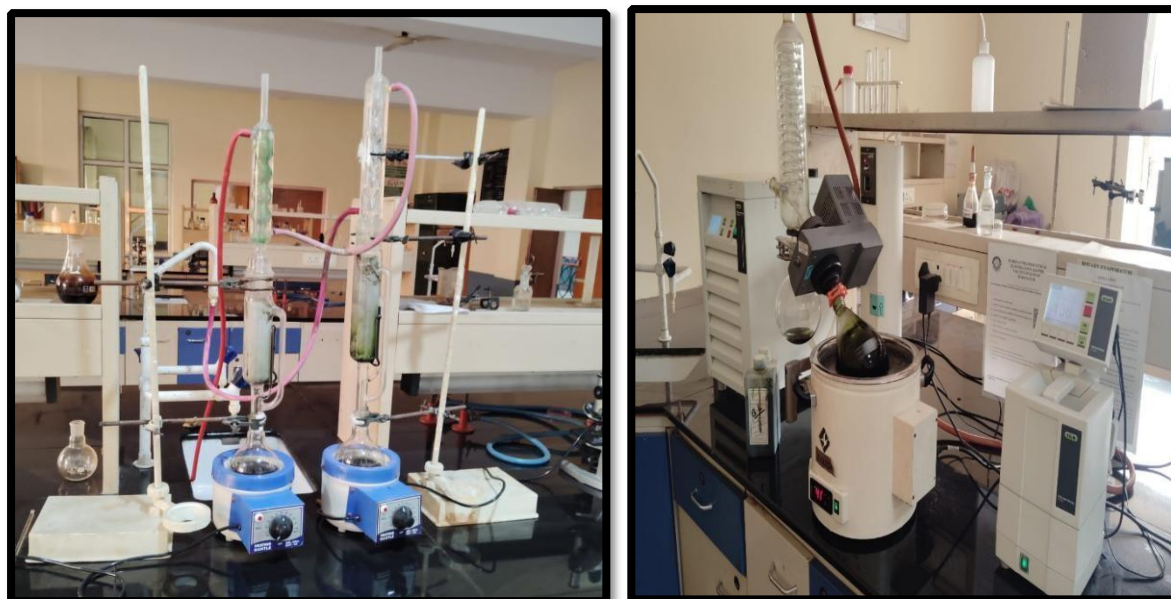


Fig. 2 Extraction from Soxhlet assembly and vacuum evaporation by rotary evaporator 2.2.
Method of extraction of *Glycyrrhiza glabra*

The extraction of glabridin from *Glycyrrhiza glabra*, the extraction was performed utilizing various solvents, including hexane, chloroform, ethanol, and methanol. A total of 25 grams of *Glycyrrhiza glabra* powder was introduced into 250 ml of each solvent through a Soxhlet apparatus. Subsequently, the solvent from the extracted material was eliminated at a low temperature using a rotary vacuum evaporator, and the resulting dry extract was prepared for further analysis.

2.3. Percentage yield

Percentage yield represents the ratio of actual yield to theoretical yield expressed as a percentage. It is determined by dividing the experimental yield by the theoretical yield and then multiplying the result by 100%. when the actual and theoretical yield are the same, the percentage yield is 100%.

2.4. Phytochemical screening of the plants extract

The extract underwent phytochemical screening to detect the presence of alkaloids, tannins, saponins, glycosides, and carbohydrates. The extracts were heated with water in a steam bath for 30 minutes and subsequently filtered. Following filtration, the resulting filtrate was analyzed for chemical constituents using specific reagents and chemicals. For instance, alkaloids were recognized through Dragendorff's, Mayer's, and Wagner's test and flavonoid were identified by the use of alkaline reagent, tannin with ferric chloride and lead acetate test [25].

2.4.1. Analysis for alkaloids

A small amount of the residue from each extract was individually dissolved in 5 ml of ethanol and subsequently filtered. The resulting filtrates were utilized for the detection of alkaloids.

a. Dragendorff's Test: Dragendorff reagent is created by combining solution A, which consists of 17 grams of bismuth subnitrate, 200 grams of tartaric acid, and 800 milliliters of distilled water, with solution B, made up of 160 grams of potassium iodide and 400 milliliters of distilled water. When the extract is mixed with a few drops of the Dragendorff reagent, the appearance of a reddish-brown color indicates the presence of alkaloids.

b. Mayer's Test: 1.36 grams of mercuric chloride were dissolved in 60 milliliters of water, while 5 grams of potassium iodide were dissolved in 10 milliliters of distilled water. The two solutions were combined and then diluted to achieve a final volume of 100 milliliters. A small amount of each extract was placed in a watch glass containing dilute hydrochloric acid, to which a few drops of Mayer's reagent were added. The appearance of a cream-colored precipitate indicates the presence of alkaloids[26].

c. Wagner's Test: To prepare Wagner's reagent, 1.27 grams of iodine and 2 grams of potassium iodide were dissolved in 5 milliliters of water, and the resulting solution was then diluted to a final volume of 100 milliliters with water. When a few drops of Wagner's reagent were added to the test filtrate, a brown precipitate formed, indicating the presence of alkaloids.

d. Hager's Test: A saturated aqueous solution of picric acid was utilized for this experiment. The addition of Hager's reagent to the test filtrate resulted in the formation of an orange-yellow precipitate, signifying the presence of alkaloids.

2.4.2. Analysis for Phenolic Compounds and Tannins

The residue from each extract was individually dissolved in ethanol and subsequently filtered. The filtrate was then subjected to tests using the specified reagent.

a. Ferric Chloride Test: A 5% solution of ferric chloride was created using 90% alcohol as the solvent. A few drops of this ferric chloride solution were introduced to a small amount of the previously prepared filtrate. The presence of tannins is indicated by the development of a dark green or deep violet color.

b. Lead acetate test: A 10% weight/volume solution of lead acetate in distilled water was incorporated into the test filtrate. If a precipitate is obtained, tannins are present.

2.4.3. Analysis for flavonoids

a. Alkaline reagent test: A mixture of 2 ml of 2% sodium hydroxide solution and plant crude extract produced a vibrant yellow color. Upon the addition of two drops of diluted acid to the solution, the color changed to colorless. This observation suggests the presence of flavonoids.

b. Shinoda test: Magnesium ribbon pieces were combined with concentrated HCl and then mixed with a crude plant extract. After a few minutes, a pink color emerged, indicating the presence of flavonoids.

2.4.4. Analysis for protein

a. Millon's test: Millon's test: When 2 ml of Millon's reagent was combined with the complete crude extract of the plant, a white precipitate formed. Upon gentle heating, the substance transformed into a red hue, signifying the presence of protein in the plant.

2.4.5. Analysis for carbohydrates

a. Molisch's test: Mix 2 ml of Molisch's solution with the crude plant extract, then carefully add 2 ml of concentrated sulfuric acid along the inner wall of the test tube. The formation of a violet ring at the interface of the two solutions indicates the presence of carbohydrates.

b. Iodine test: A mixture of 2 ml of iodine solution with crude plant extract results in purple or dark blue hues, indicating the presence of carbohydrates.

c. Benedict's test: Heat 2 ml of Benedict's reagent together with a crude extract; a reddish-brown hue will signify the presence of carbohydrates.

d. Fehling's test: A mixture of equal volumes of Fehling solutions A and B was heated together with the crude plant extract. The appearance of a red precipitate signified the presence of reducing sugars.

2.4.6. Analysis for amino acid

a. Ninhydrin test: Heat 2 ml of a 0.2% Ninhydrin solution along with the complete crude extract of the plant. The development of a violet color signifies the presence of proteins and amino acids.

2.4.7. Analysis for saponins 5 ml of distilled water were introduced to the crude plant extract in a test tube, followed by vigorous shaking. The resulting foam indicated the presence of saponins.

2.5. Assessment of Total Phenolic Content (TPC)

The Folin-Ciocalteu colorimetric method, as described by Singleton et al. [45], was used with some modifications to determine the total phenolic content (TPC) in the leaf and bark extracts. A standard solution of gallic acid was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol, resulting in a concentration of 1 mg/mL. From the standard solution, different concentrations of gallic acid solutions in methanol (25, 50, 75, and 100 µg/ml) were prepared, each of which was supplemented with 5 mL of 10% Folin-Ciocalteu reagent (FCR) and 4 ml of 7% Na₂CO₃ were added, resulting in a 10 ml final amount. As a result, the blue-colored liquid was well agitated and then incubated in a water bath at 40°C for 30 minutes. The absorbance was then measured against a blank at 760 nm. A UV-visible spectrophotometer is used to measure the dark blue color that results from the oxidation of phenols found in plant extracts, which is aided by the FCR reagent. Every experiment was carried out in triplicate, and the calibration curve was created using the mean absorbance values obtained at different gallic acid concentrations [27].

Preparation of Samples for Assessing Total Phenolic Content.

Extracts were prepared at different concentrations of 25, 50, 75, and 100 µg/ml. The method outlined for standard gallic acid was adhered to, and the absorbance for each extract concentration was measured. Samples were prepared in triplicate for each analysis, and the mean absorbance value was utilized to construct the calibration curve for assessing the phenolic content in the extracts. The total phenolic content of the extracts was quantified as milligrams of gallic acid equivalents (GAE) per gram of dry sample weight (mg/g). The total phenolic concentrations in all samples were determined using the specified formula:

$$C = c V / m,$$

C represents the total phenolic content measured in mg GAE per gram of dry extract, c denotes the concentration of gallic acid derived from the calibration curve in mg/mL, V indicates the volume of the extract in mL, and m signifies the mass of the extract in grams.

2.6. Antioxidant Activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Neutralization Activity. The DPPH free radical scavenging experiment was used to evaluate the extracts' antioxidant properties in vitro, with some modifications [28]. This is a simple and effective method for evaluating antioxidants' scavenging ability. When DPPH's oxidized form is dissolved in methanol, it takes on a deep violet color. DPPH is reduced when one electron is donated to it by an antioxidant chemical, changing its hue from deep violet to yellow. DPPH solutions have a strong absorption at 517 nm, which appears as a deep violet color. The ability to scavenge the DPPH free radical evaluates the test sample's antioxidant potential or free radical scavenging ability,

demonstrating how well it works to stop, stop, and repair damage in biological systems. The ability to scavenge DPPH free radical assesses the free radical scavenging capacity or antioxidant potential of the test sample.

Preparation of a 0.1 M DPPH Solution. In a volumetric flask, 0.39 mg of DPPH was dissolved in methanol to form a 0.1 M DPPH solution. The final volume was adjusted to 100 ml. As a result, the purple DPPH free radical solution was generated and stored at -20°C for later use.

Preparation of Extract Solutions .The right amount of each extract was dissolved in the required volume of methanol to form a stock solution of different extracts at a concentration of 1 mg/mL. From the sample stock solution, solutions of each extract were made at 25, 50, 75, and 100 µg/mL.

2.7. Preparation of an herbal cream

The following steps were undertaken in the preparation of the vanishing herbal cream.

Preparation of the oil phase: In a porcelain dish, a solution of 20% stearic acid, 3% cetyl alcohol, and 1.4% potassium hydroxide was heated to 70°C until it melted.

Preparation of the aqueous phase: A separate porcelain dish was filled with a 1% alcoholic extract of crude medicines, 4% glycerin, and 80% water. The mixture was then heated to 70°C.

Incorporation of the aqueous phase into the oil phase: At 70°C, the aqueous phase was mixed with the oil phase while being constantly stirred. After the transfer was finished, it was allowed to come to room temperature while being constantly agitated. Just before the final product was moved to an appropriate container, perfume (0.5%) was finally applied. The cream was then assessed based on a number of physical factors.



Fig. no.3: Preparation of an herbal cream

Table no. 1: Preparation of an herbal cream

S no.	Aloe extract	Liquorice extract	Methyl paraben	Stearic acid	Cetyl alcohol	KOH	glycerine	Triethanolamine	Water
F1	1%		0.02	20gm	3gm	1.4gm	4ml	Qs	Qs to 100
F2		1%	0.02	20gm	3gm	1.4gm	4ml	Qs	Qs to 100
F3	0.5%	0.5%	0.02	20gm	3gm	1.4gm	4ml	Qs	Qs to 100

2.8. Evaluation of polyherbal vanishing cream

Vanishing creams after formulation, may experience physical and chemical changes that may alter their stability. Therefore, the vanishing creams ought to be assessed for their stability before dispensing to ensure their stability all through their shelf-life. Evaluation of vanishing creams can be done by the following tests.

Organoleptic Evaluation The properties of formulation used in the polyherbal vanishing cream were deliberated by quality, visual appearance, and characteristics. The cream was observed for color, odor, and consistency [29]. The results are listed in Table **pH:**The pH meter was firstly calibrated and then ready to measure the pH by immersing it in a beaker that contained 1 gram of the cream [30].

Assessment of Spreadability: 1 gram of the cream was positioned between two slides. A weight of 100 grams was applied to the upper slide. After the weight was taken off, any excess formulation was carefully scraped away. The lower slide was secured to the apparatus, while the upper slide was attached with a rigid

string to which a 20g weight was applied. The duration it took for the upper slide to detach was recorded [31].

Spreadability(S) equals the product of mass(m) and length(l) divided by time(t). Where S – Spreadability m- Weight tied to upper glass slide. l- Length moved on a glass slide t- Time taken.

The determinations were carried out in triplicate and the average of three readings was recorded.

Assessment of viscosity: Using a Brookfield viscometer (DV II+ Pro model) and spindle number S-64, viscosity measurements were carried out at 290°C and 10 rpm. Three readings of each measurement were taken, and the average of the three was recorded [32].

Irritancy test: The cream was administered to a 1 square centimeter area on the dorsal side of the left hand and monitored at regular intervals for a duration of 24 hours to assess for irritation, redness, and edema[33].

Smear type: The test was performed following the application of cream on the skin, resulting in a smear that was either oily or aqueous in composition[34].

Wash ability: The cream applied to the skin was removed by gently washing it under tap water with minimal pressure[35].

Antimicrobial study: We conducted observational study of antibacterial activity of F1, F2 and F3 batch which is optimised batch. First of all, The Minimum Inhibitory Concentration (MIC) of the extract was determined for Staphylococcus aureus and Escherichia coli. The herbal skin whitening cream was screened against bacterial strains of Escherichia coli by using agar disc-diffusion assay. The inhibition zone was quantified. the efficacy of the prepared herbal skin cream was compared to that of a commercially available formulation containing glycyrrhiza glabra root extract in terms of antimicrobial activity [36].

In vitro release of bioactive study by Franz diffusion cell method

Accurately weighed 1 gm gel was kept in egg membrane cell which was then secured between the donor and receptor compartments. These compartments were clamped together and positioned in a water bath maintained at 37°C. The receptor cell had a volume of 50 ml, with an effective surface area for permeation measuring 6.9062 cm². The receptor compartment is filled with an acetate buffer at a pH of 5.5. The hydrodynamics of the receptor fluid were preserved by stirring the fluid at a speed of 600 rpm using a star head magnet, followed by a diffusion period of 10 minutes. At specified time intervals of 5, 15, 30, and 60 minutes, and subsequently on an hourly basis 5 ml aliquots were taken and substituted with 5 ml of fresh acetate buffer at pH 5.5. To maintain skin, condition an equivalent volume of medium was replenished. The samples were analysed using a UV-Visible spectrophotometer at a wavelength of 739 nm to estimate the drug profile. The in vitro drug release assembly demonstrated the results.

3. RESULT AND DISCUSSION 3.1 Extractive value

Table no.2 %Age yield of both plant extract with deferent solvent.

Solvent	Aloe vera	% Yield	Liquorice	% Yield
Hexane	–	–	1.32 gm	5.28%
Chloroform	–	–	2.56 gm	10.24%
Ethanol	17.78 gm	71.12%	19.02 gm	76.08%
Methanol	–	–	5.63 gm	22.52%

The effects of hexane, chloroform, ethanol, and methanol on the extraction yield of aloe vera and liquorice were examined, revealing a significant variation in extraction yields depending on the solvent used. Of the solvents tested, ethanol produced the highest extraction yield for aloe vera. (71.12%) and Liquorice (76.08%) followed by methanol liquorice showed (22.52%).



Fig. 4 Extracted material of *Aloe barbadensis* and *Glycyrrhiza glabra*

3.2. Phytochemical test of extract

Qualitative phytochemical analysis of *Aloe barbadensis* and *Glycyrrhiza glabra* showed that major secondary metabolites were found in ethanolic extract of both plants. ethanolic extract of both plants. The ethanolic extract of *Aloe barbadensis* demonstrated the presence of phenolic compounds and flavonoids. However, alkaloids were present in all the solvent extracts of *Aloe barbadensis*. Conversely, the ethanolic extract of *Glycyrrhiza glabra* contained flavonoids, carbohydrates, phenols, and tannins. The ethanolic extract shown more extracting property in comparison to other solvent extracts, thus, this solvent extract was selected for polyherbal cream and polyherbal formulation and evaluation of anti-aging and anti-wrinkle activities

Table no.3: Phytochemical test of extract powder

S. No.	Tests	Ethanolic extract of <i>Aloe barbadensis</i>	Ethanolic extract of <i>Glycyrrhiza glabra</i>
Alkaloid	Dragendroff	+	+
	Hager	+	+
	Mayer	+	+
	Wagner	+	+
Phenolic & Tannin	FeCl ₃	+	+
	Lead Acetate	+	+
Flavonoids	Alkaline Reagent	+	+
Protein	Millon's Reagent	–	–
Carbohydrates	Molisch Reagent	–	+
Amino Acid	Ninhydrin Reagent	–	–



Fig. 5 Presence of phytochemical in *Aloe barbadensis* extract



Fig. 6 Presence of phytochemical in *Glycyrrhiza glabra* extract

3.3. Quantitative phytochemical analysis

Total phenol content (TPC) of *Aloe barbadensis* and *Glycyrrhiza glabra* were under acceptable limit, Gallic acid served as the reference standard for total phenolic content (TPC).

3.4. Assessment of Total Phenolic Content (TPC)

Plants with redox properties that support antioxidant activity often contain significant amounts of phenolic compounds. These phenolic constituents, particularly those with hydroxyl groups, play a crucial role in scavenging free radicals. To evaluate this property, the total phenolic content (TPC) of the plant extracts was analyzed. The variation in phenolic content in the test samples was expressed as gallic acid equivalent (GAE), based on a standard curve. As shown in Figure 12, the standard curve equation is $y = 0.0072x - 0.0005$, with a correlation coefficient of $R^2 = 0.9852$, indicating a strong linear relationship. The total phenolic content of *Glycyrrhiza glabra* was found to range from 3.67 to 7.104 mg GAE/g, while *Aloe barbadensis* exhibited a lower range, from 1.56 to 2.27 mg GAE/g.

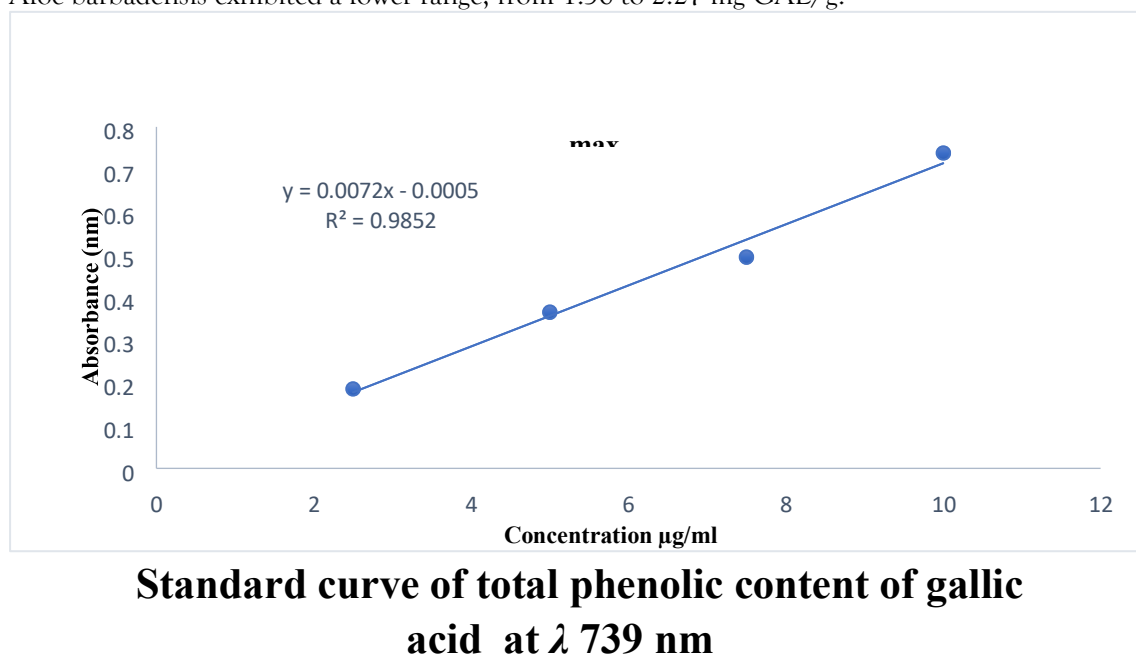


Fig. 7 Standard curve for Total Phenolic Content (measured in gallic acid)

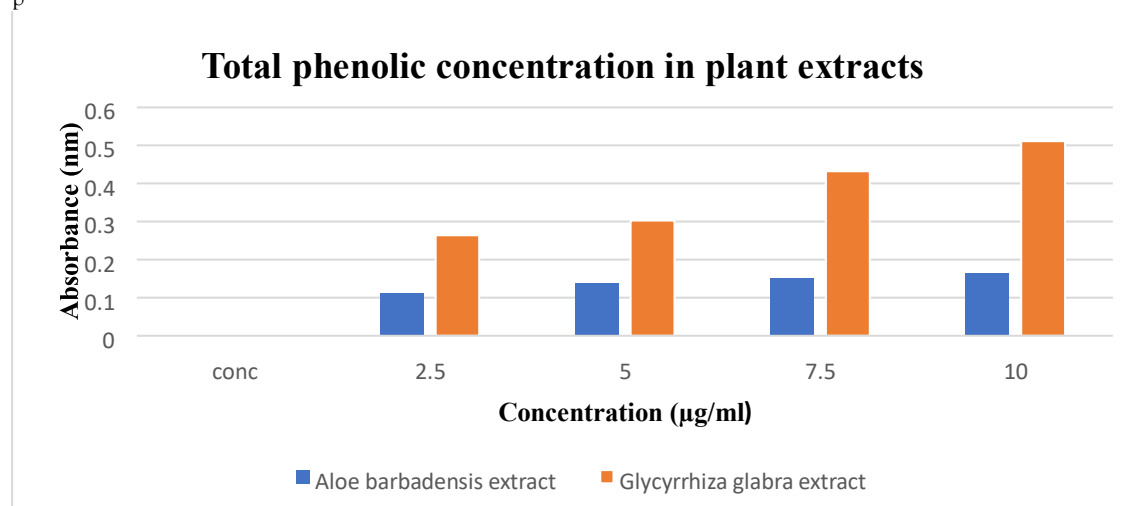


Fig. 8 Total Phenolic Concentration of both botanical extracts

Table no.4: Total phenolic concentration of Aloe barbadensis

S. No.	Concentration (µg/ml)	Absorbance	TPC value (µg)
1	2.5	0.112	1.56
2	5	0.138	1.92
3	7.5	0.152	2.11
4	10	0.163	2.27

Table no.5: Total phenol content of Glycyrrhiza glabra

S. No.	Concentration(µg/ml)	Absorbance	TPC value (µg)
1	2.5	0.264	3.67
2	5	0.303	4.21
3	7.5	0.432	6.006
4	10	0.511	7.104

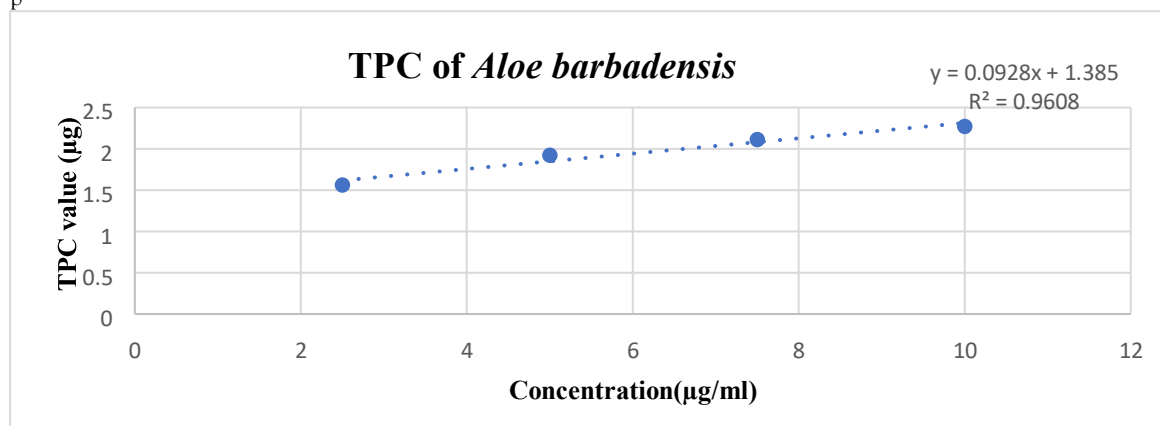


Fig. 9 TPC of *Aloe barbadensis*

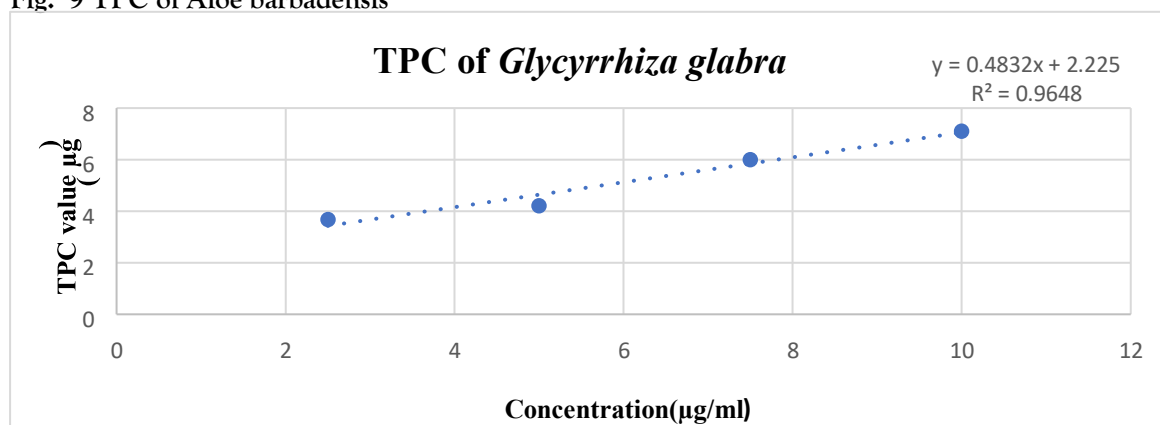


Fig.10 TPC of *Glycyrrhiza glabra*

3.5. DPPH Antioxidant activity

The antioxidant activities observed in vitro of the *Aloe barbadensis* and *Glycyrrhiza glabra*. Free radicals can harm the skin by disrupting the lipid composition of cellular membranes and interfering with the transport of nutrients and other essential components within the cell structure. This disruption also affects the primary protein content of healthy collagen and elastin fibers, leading to the development of wrinkles, sagging, and a decline in skin tone.

DPPH (di-phenyl picryl hydrazine) Serves as a reagent for examining the free radical scavenging properties of various compounds. In our study, *Aloe barbadensis* and *Glycyrrhiza glabra* both showed notable DPPH radical scavenging activity when compared to ascorbic acid as a reference standard. Results showed that The DPPH radical scavenging activity exhibited a concentration-dependent relationship, with greater concentrations demonstrating enhanced scavenging effects. Notably, this activity was significantly higher when compared to ascorbic acid. At the peak concentration (100 µg), *Aloe barbadensis* and *Glycyrrhiza glabra* have the DPPH scavenging activity was found to be superior when compared to ascorbic acid. (48.4%, 49.9% and 78.2% 1. in that order). The IC₅₀ value of ascorbic acid for DPPH was 198 µg/ml and extracts of *Aloe barbadensis* and *Glycyrrhiza glabra* showed IC₅₀ i.e., 32.52 µg/ml and 10.15 µg/ml, respectively. Results revealed that the extract of *Glycyrrhiza glabra* exhibited higher antioxidant activity than *Aloe barbadensis* when compared to standard antioxidant ascorbic acid. The inhibitory concentration (IC₅₀) value was calculated by extrapolating the graph that plots percentage inhibition against extract concentration, utilizing linear regression analysis. This value represents the quantity of antioxidant required to decrease the initial radical concentration by 50%. A lower IC₅₀ value indicates stronger antioxidant effects.

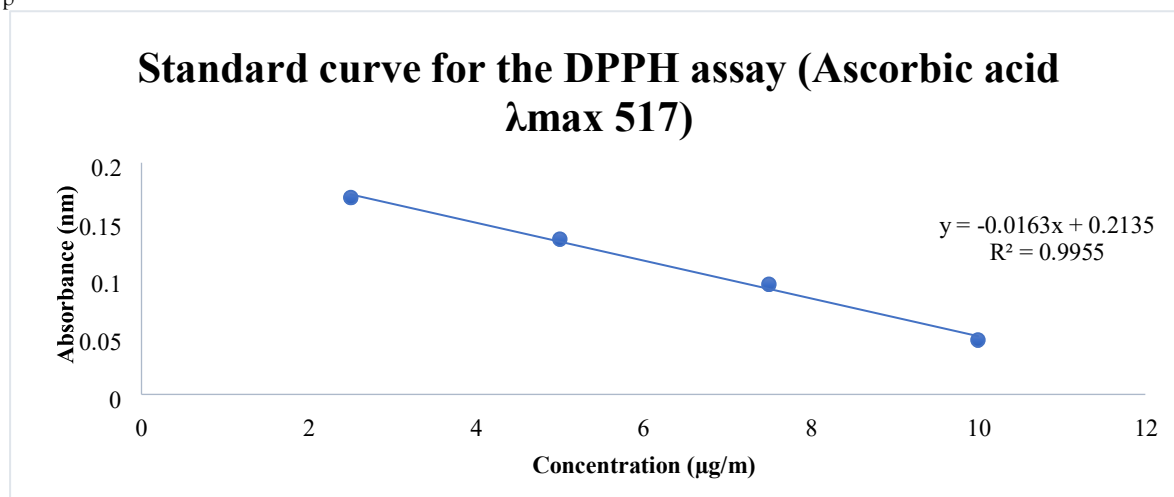


Fig. no. 11: Standard curve for the DPPH (Ascorbic acid)

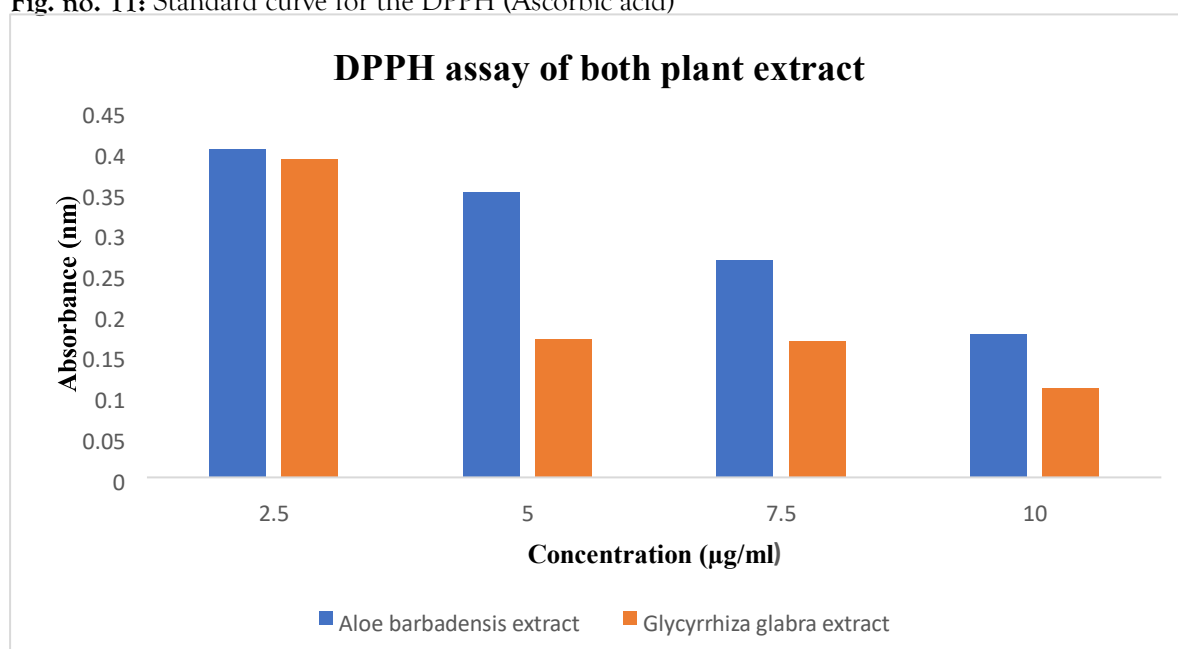


Fig. 12 DPPH assay of both plant extracts

Table no.6: Percentage Inhibition of DPPH Assay

Concentration($\mu\text{g/ml}$)	Percentage Inhibition of Ascorbic Acid	Percentage Inhibition of Aloe barbadensis	Percentage Inhibition of Glycyrrhiza glabra
2.5	78.2%	73.2%	49.9%
5	82.8%	81.7%	78.2%
7.5	87.8%	85.5%	78.6%
10	93.9%	87.4%	87.1%

3.6. In Vitro Anti- Bacterial Activity of extracts Using Agar Disc Diffusion Method

The antibacterial properties of Aloe barbadensis extract and Glycyrrhiza glabra were evaluated against *S. aureus* and *E. coli*. The highest antibacterial effects were noted in the ethanol extract at a concentration of 1000 $\mu\text{g/ml}$. Notably, the most significant inhibition of growth was recorded for both *S. aureus* and *E. coli*. Results are presented in Aloe barbadensis extract and Glycyrrhiza glabra can inhibit the proliferation of Streptococcus aureus and E coli.

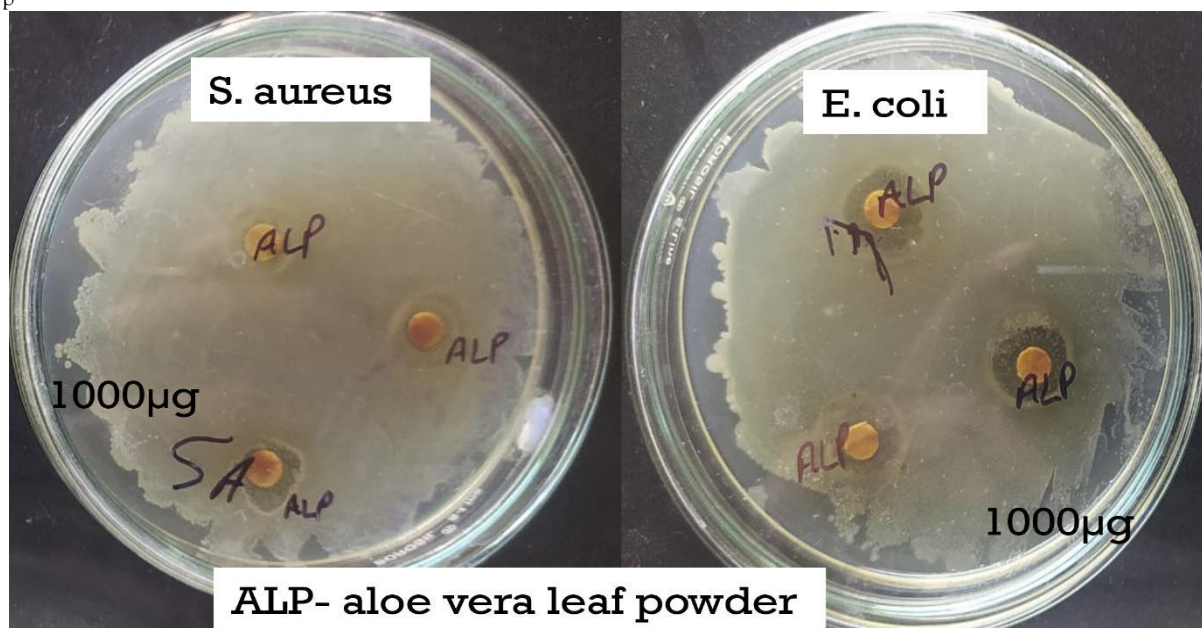


Fig. 13: Inhibition zone of *Aloe barbadensis* plant extract against *Staphylococcus aureus* and *E. coli*

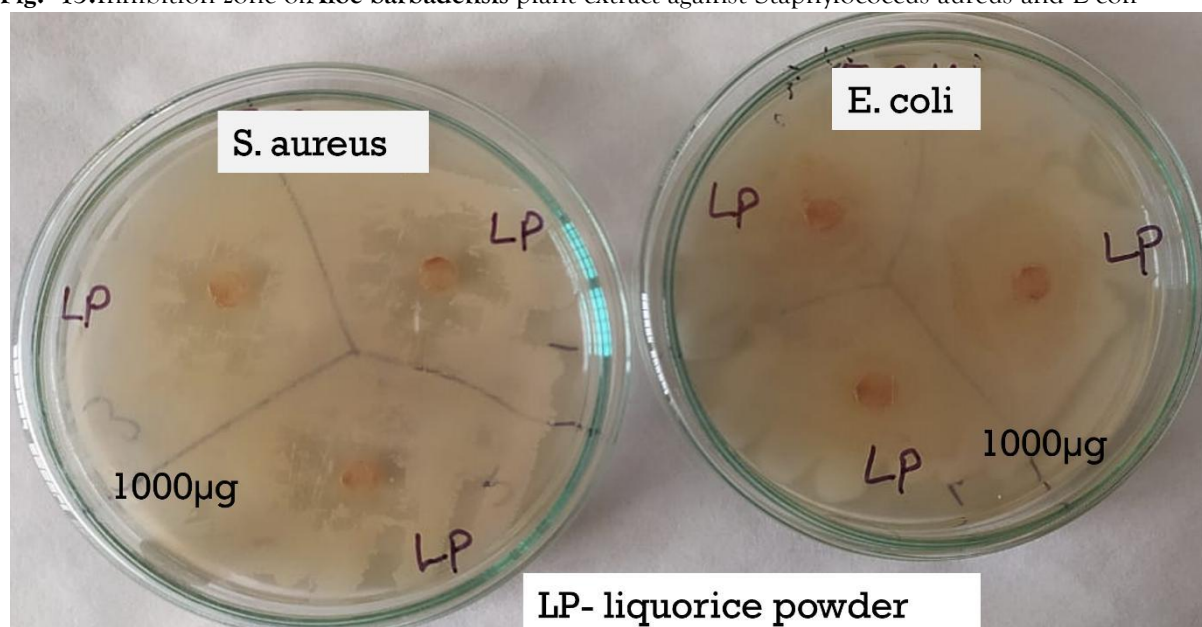


Fig.14 Inhibition zone of *Glycyrrhiza glabra* extract against *Staphylococcus aureus* and *Escherichia coli*.

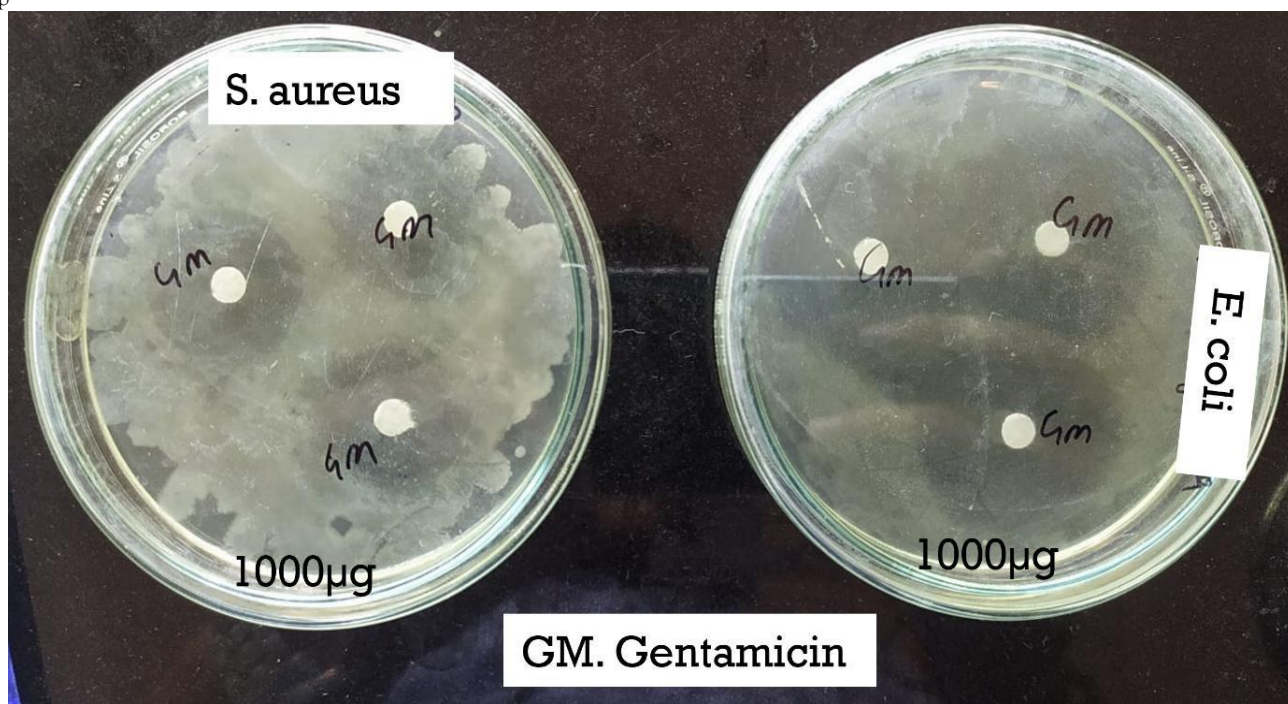


Fig.15 Inhibition zone of standard gentamicin against *S. aureus* and *E. coli*

Table no.7 In-Vitro anti- bacterial activity and zone of inhibition of plant extract and standard drug.

S no.	Concentration of Extract	Standard drug (Gentamicin)		Aloe barbadensis		Glycyrrhiza glabra	
		<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>e. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
1	100 µg/ml	17mm	19.3mm	8mm	6.3mm	10mm	7mm
2	200 µg/ml	21mm	20mm	9.8mm	7.9mm	13mm	9mm
3	400 µg/ml	28.1mm	23.6mm	12mm	10.3mm	15mm	12mm
4	800 µg/ml	30mm	25mm	14.7mm	12mm	19.3mm	14.5mm
5	1000 µg/ml	34mm	31.4mm	18mm	16.2mm	23mm	20mm

3.7. Physical evaluation of polyherbal cream

The characteristics of cream in term of appearance, pH, viscosity, spreadability, consistency and homogeneity of polyherbal cream. The prepared cream formulation exhibited a pale yellow hue, and all variations possessed a distinct aroma. It was found that all the cream formulations were homogenous, smooth, non-greasy film on the skin surface and consistent in nature. The cream formulations were easily spreadable and moisturize the skin surface and given emollient feel.



Fig. no. 16: Diagram of formulation

Table no.8: Physical evaluation to of extract loaded polyherbal cream formulation

Formulation	Colour	Odour	Consistency	Homogeneity
FC1	Off white	Fine	Smooth	Homogeneous
FC2	Off white	Characteristic	Smooth	Homogeneous
FC3	Pale yellow	Pleasant	Smooth	Homogeneous

3.7.1. pH: The pH of the polyherbal vanishing cream was assessed using a pH meter. One gram of the cream was dissolved in 10 milliliters of distilled water and allowed to stand for two hours. The pH of each formulation was measured three times to ensure accuracy. It is shown below in table. **Table no.9:** Determination of pH to all formulation

S no.	Formulation	pH
1.	FC1	5.2
2.	FC2	5.1
3.	FC3	5.4

3.7.2. Spread-ability: The spreadability values suggest that the polyherbal cream can be easily applied with minimal shear force. The spread-ability of F1 g.cm/sec, F2.cm/sec. and F3g.cm/sec. while (F3 mix) both plant extract loaded herbal cream was g.cm/sec., indicating spread-ability of polyherbal cream containing F3 was high as compared to the extract loaded cream as shown below in table.



Fig. no.17: spread-ability

Table no.10: Result of Spread-ability study

S no.	Formulation	Spread-ability (gm.cm/sec)
1.	FC1	22
2.	FC2	27.5
3.	FC3	30.2

3.7.3. Viscosity: Viscosity serves as a crucial factor in defining the characteristics of the polyherbal cream, as it influences both its mechanical and physical properties such as spread-ability, consistency of the formulation which provide ease behaviour of application on the mucosal surface and patient compliance

drug at target site as well as release of drug the comparative viscosity estimated through Brookfield viscometer of all formulations and it reported in given below table. **Table no.11:** Viscosity of the formulation

S no.	Formulation	Viscosity
1.	FC1	1337cp
2.	FC2	1366cp
3.	FC3	1543cp

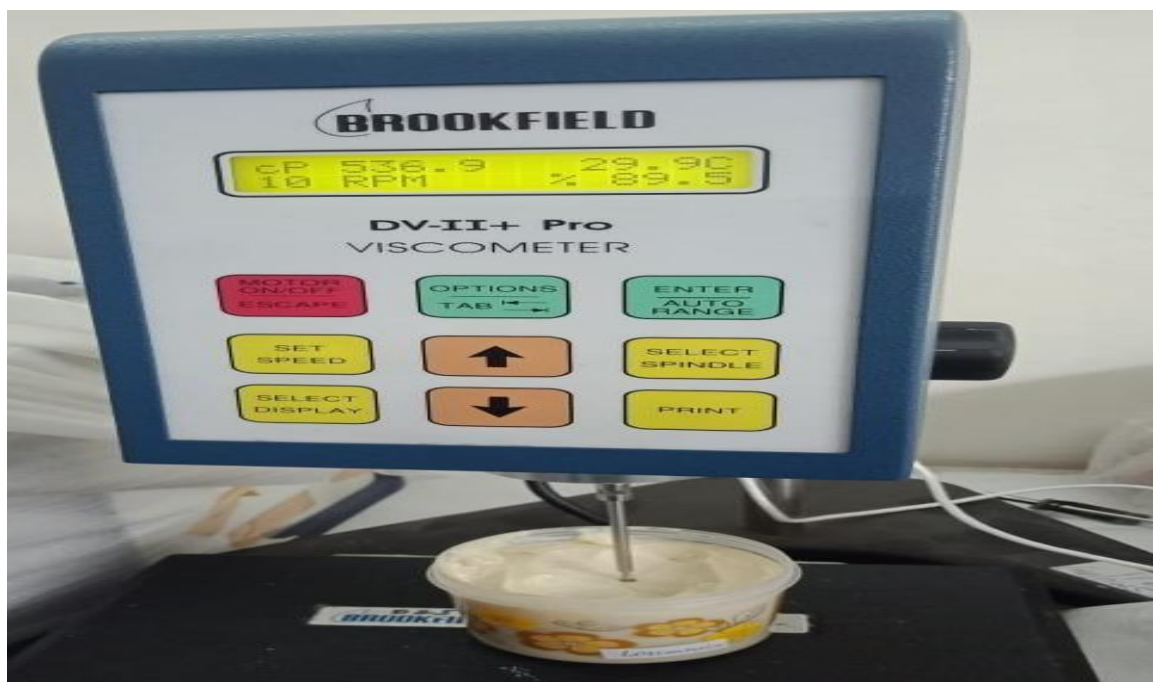


Fig. no.18: Measurement of Viscosity by Brookfield viscometer

3.7.4. Extrudability: An ideal polyherbal cream should possess optimum extrudability on application to provide patient compliance. F1, F2 and F3 exhibited may be attributed to it less viscosity which facilitated more extrusion from tube with in specified time.

Table no.12: Extrudability study of all formulation

S no.	Formulation	Extrudability (%)
1.	FC1	23
2.	FC2	22.5
3.	FC3	26.2



Fig. no.19: Extrudability

3.7.5. Anti- Bacterial Activity of formulations

In vitro anti-bacterial study revealed clear zone of inhibition and confirmed susceptibility of formulation polyherbal cream. Although formulated polyherbal creams F1, F2 and F3 showed enhance activity as compare to both marketed product (joy and Himalaya cream) may be attributed to more diffusion of drug from creams to the Petri plates. Results of in vitro anti-bacterial activity and zone of inhibition of formulated polyherbal cream was shown in figure.

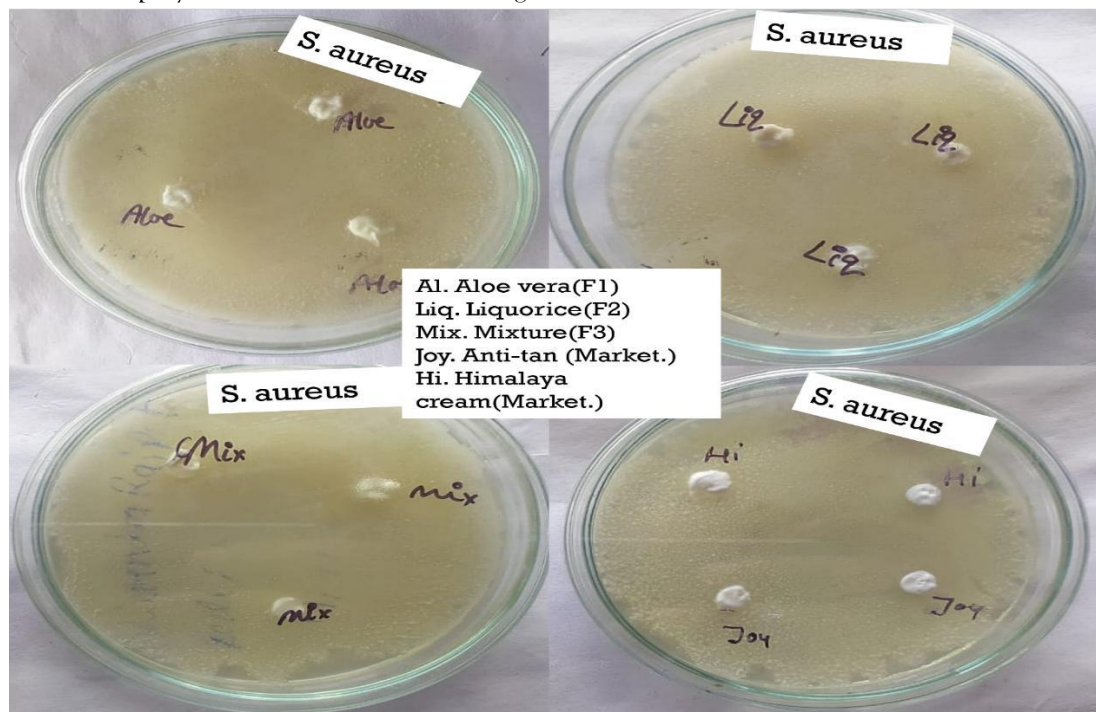


Fig. no. 20: Anti-bacterial activity using formulation and marketed product in *S. aureus*

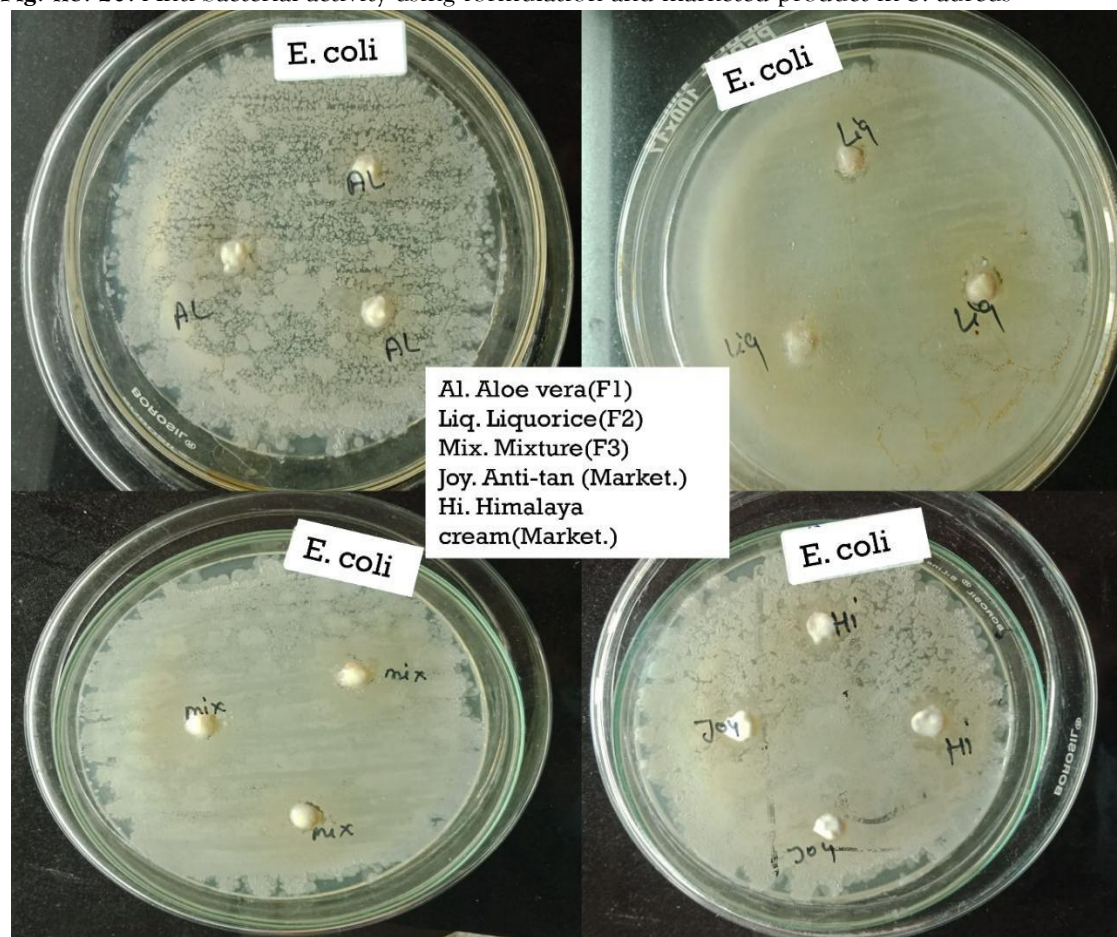


Fig. no. 21: Anti-bacterial activity using formulation and marketed product in *E. coli* **Table no.13:** Zone of inhibition

S.No.	Formulation	Inhibition zone of <i>Staphylococcus aureus</i>	Inhibition zone of <i>Escherichia coli</i>
1.	FC1	1.7	1.8
2.	FC2	1.9	2.4
3.	FC3	2.2	2.9
4.	Marketed1	1.9	1.7
5.	Marketed 2	2.1	1.8

3.7.6. Drug release

Table no.14: % Drug release

Time	FC1	FC2	FC3
0	0.24%	0.10%	0.04%
5	0.75%	0.94%	0.66%
15	1.46%	2.12%	1.74%
30	2.64%	3.67%	3.44%
60	4.49%	5.67%	5.76%
1hr30	7.06%	7.91%	10.44%
2hr30	10.46%	10.62%	17.42%
3hr30	16.25%	16.41%	27.55%
4hr30	24.26%	27.45%	40.63%
5hr30	35.13%	39.54%	54.84%
6hr30	50.44%	54.89%	70.41%
7hr30	65.07%	71.57%	86.90%
8hr30	79.53%	85.89%	97.44%

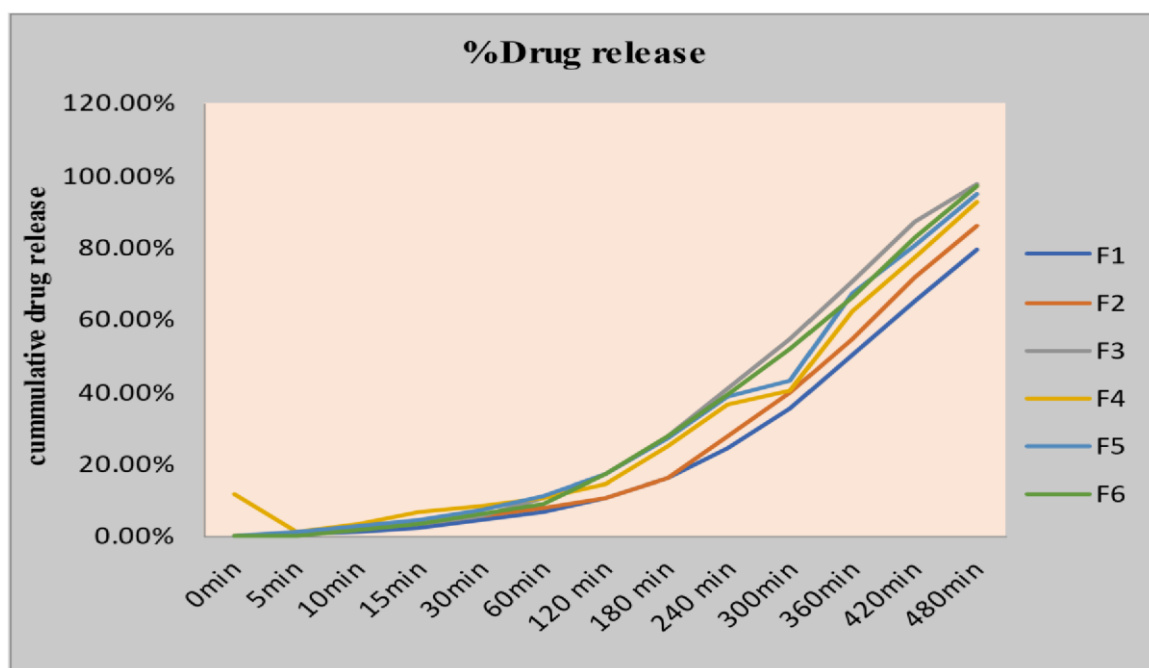


Fig. no. 22 % drug release

4. DISCUSSION

The objective of this research was to create and develop a polyherbal formulation aimed at anti-aging applications. We utilized a Soxhlet apparatus to prepare extracts from the leaves of *Aloe barbadensis* and the root of *Glycyrrhiza glabra*. The synthesized plant extracts were subjected to phytochemical screening. The findings indicated that ethanol was the most effective solvent for extracting both *Aloe barbadensis* leaves and *Glycyrrhiza glabra* root, yielding superior results compared to other solvents such as hexane and chloroform. The extractive values for the ethanolic extracts were notably higher, measuring 17.78 g for *Aloe barbadensis* and 19.02 g for *Glycyrrhiza glabra*. Furthermore, the crude extracts derived from *Aloe barbadensis* leaves and *Glycyrrhiza glabra* root revealed the presence of various phytoconstituents, including flavonoids, phenols, and tannins, particularly in the ethanolic extract.

The total phenolic content was assessed using the Folin-Ciocalteu reagent method. Among the extracts tested, *Glycyrrhiza glabra* root extract demonstrated the highest total phenolic content, with *Aloe barbadensis* leaf extract following closely behind.

The total antioxidant activity of the two plant extracts was assessed using the DPPH assay method, with ascorbic acid serving as the standard. The extract from *Aloe barbadensis* leaves demonstrated superior antioxidant activity in comparison to the root extract of *Glycyrrhiza glabra*.

The antimicrobial properties of the extract were assessed using the disc diffusion method. The bacterial strains employed for evaluating antibacterial activity included *S. aureus* and *E. coli*. The effectiveness of *Aloe barbadensis* and *Glycyrrhiza glabra* extracts against these bacteria was measured at various concentrations. *Glycyrrhiza glabra* exhibited superior antibacterial activity compared to *Aloe barbadensis*. Additionally, the results indicated that the extracts demonstrated a more pronounced antibacterial effect against *S. aureus*.

For the present work total six formulations were prepared. Three formulations of polyherbal cream (FC1 to FC3) were prepared using different concentrations of ethanolic extract of *Glycyrrhiza glabra* root and *Aloe barbadensis* leaves extracts, with 20% of stearic acid. Other three different polyherbal gel formulation (FG4 to FG6) were prepared using different concentrations of ethanolic extract of *Glycyrrhiza glabra* root and *Aloe barbadensis* leaves extracts, with 2.5% of Sodium carboxy methyl cellulose.

The prepared formulations were physical evaluation for colour, odour, consistency and homogeneous and pH. pH of the formulation ranging from (5.1-5.5). Viscosity varied in Cp according to the amount of extract that was added. The values of the spreadability, which range from 21 ± 1.2 gm/sec to 30.2 ± 2 gm/sec, showed that the formulation is readily spreadable. The result of extrudability in the range from 20 ± 2.5 to 26.2 ± 1.9 gm/cm². The In vitro anti-microbial activity had shown satisfactory results for the polyherbal formulation (*S. aureus* = 3.8 mm, *E coli* = 3.6 mm) is higher than other formulation. In vitro Bioactive release was done by Franz diffusion method using egg membrane. Release of bio-actives from F1 to F6 formulations were determine. 5.8 phosphate buffer was used for the in vitro bioactive release studies of the formulations. Among all formulations, F3 showed better release (97.44 %) in comparison with the other formulation FC1(79.53%), FC2(85.89%), FG4(92.61%), FG5(95.02) and FG6(96.80%) respectively.

5. CONCLUSION

5.1. Conclusion:

This study aimed for the preparation and evaluation of polyherbal formulation for antiaging purpose.

The ethanolic extract shown more extracting property in comparison to other solvents hexane and chloroform. Phytochemical study shown that the flavonoid, phenol and tannins are present in the plant extract. Ethanolic extract of *Aloe barbadensis* showed presence of phenols, and flavonoids.

The total phenolic content (TPC) was determined in terms of mg Gallic acid equivalent /gm (the standard curve $y = 0.0072x - 0.0005$ $R^2 = 0.9852$). The total phenol content of *Aloe barbadensis* value ranging from 1.56 – 2.27 mg (gallic acid equivalent/gm) and *Glycyrrhiza glabra* value ranging from 3.67 – 7.104 mg (gallic acid equivalent/gm).

To investigate the anti-aging potential, in-vitro DPPH radical scavenging activity was performed. At the highest concentration (10 µg/ml), *Aloe barbadensis* and *Glycyrrhiza glabra* have the highest DPPH scavenging activity (87.4% and 87.1 %) respectively as compared to Ascorbic acid 93.9%.

The IC₅₀ value for DPPH assay of ascorbic acid, *Aloe barbadensis* and *Glycyrrhiza glabra* was 198 µg/ml, 32.52 µg/ml and 10.15 µg/ml respectively. Thus, the extracts of *Aloe barbadensis* and *Glycyrrhiza glabra* exhibited good antioxidant activity. These findings suggested that the free radical neutralizing capacity of *Aloe barbadensis* was more than *Glycyrrhiza glabra* extract.

Both the extract *Glycyrrhiza glabra* and *Aloe barbadensis* they are showing good antimicrobial activity against *Staphylococcus aureus*, and *Escherichia coli*. The measured zone of inhibition of positive control (gentamycin), *Glycyrrhiza glabra* plant extract and *Aloe barbadensis* plant extract against *Staphylococcus aureus* at 1000 µg/ml was (34 ± 2), (23±1mm) and (18±3 mm) respectively.

The prepared formulations were physically evaluation for colour (pale yellow and dark brown in colour), odour (good, pleasant and characteristics), consistency (smooth, good, excellent) and homogeneous in nature. pH of the formulation ranging from (5.1 to 5.5). Viscosity of the polyherbal formulation was measured by Brookfield viscometer at 32.10C and 64 spindle number, found that the F1= 1337cp, F2= 1366cp, F3= 1543cp, F4= 3236cp, F5= 3259cp, F6= 3438cp.

The spread ability, which range from 21 ± 1.2 gm/sec to 30.2 ± 2 gm/sec, showed that the formulation is readily spreadable.

The result of extrudability in the range from 20 ± 2.5 to 26.2 ± 1.9 gm/cm². In vitro Antimicrobial activity gives the good zone of inhibition of the polyherbal formulation. Although formulated polyherbal creams F1, F2, F3 and polyherbal gels F4, F5and F6 showed enhance activity as compare to both marketed products. The antimicrobial activity of polyherbal formulation measured by the zone of inhibition of FC3 (*S. aureus* = 3.8 mm, *E coli* = 3.6 mm) is higher than other formulation. F3 formulation of methanolic extract of *Glycyrrhiza glabra* root and *Aloe barbadensis* leaves give the best release of bioactive (97.44 %) in comparison with the other formulation FC1(79.53%), FC2(85.89%), FG4(92.61%), FG5(95.02) and FG6(96.80%) respectively.

REFERENCE .

1. Souiden, N. & Diagne M.; Canadian and French men's consumption of cosmetics: a comparison of their attitudes and motivations; *Journal of Consumer Marketing*; 2009 Mar 20; 26(2):97-109.
2. Blanchin, S., Coffin, C., Viader, F., Ruf, J., Carayon, P., Potier, F., Portier, E., Comby, E., Allouche, S., Ollivier, Y. & Reznik Y.; Anti-thyroperoxidase antibodies from patients with Hashimoto's encephalopathy bind to cerebellar astrocytes; *Journal of neuroimmunology*; 2007 Dec 1; 192(1-2):13-20.
3. Oana, M.S., & Dinu, V.S.; "The Impact Of Globalization In The Industry Of Cosmetics," *Annals of Faculty of Economics, University of Oradea; Faculty of Economics*; 2013 December; vol. 1(2), pages 681-691.
4. Ana, S.R., Marilene, E.M., Beatriz, O. and José, S.L.; Main benefits and applicability of plant extracts in skin care products; *Cosmetics*; 2015 10 April; 48-65.
5. Mungai, C.; Cosmetics and beauty in Africa: boom in traditional products, and how skin bleaching is unexpectedly driving innovation; *Mail & Guardian Africa*; 16, 2014.
6. Sahasrabudhe, A. & Deodhar, M., Anti-hyaluronidase, Anti-elastase Activity of *Garcinia indica*; *International Journal of Botany*; 2010; 6: 299-303
7. Stipcevic, T., Piljac, J. & Berghe, D.V.; Effect of different flavonoids on collagen synthesis in human fibroblasts; *Plant foods for human nutrition*; 2006 Mar 1; 61(1):27-32.
8. Balcombe, N.R. & Sinclair, A.; Ageing: definitions, mechanisms and the magnitude of the problem; *Best Practice & Research Clinical Gastroenterology*; 2001 Dec 1; 15(6):835-49.
9. Krutmann, J., Boulou, A., Sore, G., Bernard, B.A. & Passeron, T.; The skin aging exposome; *Journal of dermatological science*; 2017 Mar 1; 85(3):152-61.
10. Gilchrist, B.A.; Skin aging and photoaging: an overview; *Journal of the American Academy of Dermatology*; 1989 Sep 1; 21(3):610-3.
11. Warren, R., Gartstein, V., Kligman, A.M., Montagna, W., Allendorf, R.A. & Ridder, G.M.; Age, sunlight, and facial skin: a histologic and quantitative study; *Journal of the American Academy of Dermatology*; 1991 Nov 1; 25(5):751-60.
12. Seo, J.Y. & Chung, J.H.; Thermal aging: a new concept of skin aging; *Journal of Dermatological Science Supplement*; 2006 Dec 1; 2(1): S13-22.
13. Mera, S.L., Lovell, C.R., Jones, R.R. & Davies, J.D.; Elastic fibres in normal and sun-damaged skin: an immune histochemical study; *British Journal of Dermatology*; 1987 Jul; 117(1):21-7.
14. Tatemoto, H., Tokeshi, I., Nakamura, S., Muto, N. & Nakada, T.; Inhibition of boar sperm hyaluronidase activity by tannic acid reduces polyspermy during in vitro fertilization of porcine oocytes; *Zygote*; 2006 Nov; 14(4):275-85.

15. Mukherjee, P.K., Maity, N., Nema, N.K. & Sarkar, B.K.; Bioactive compounds from natural resources against skin aging; *Phytomedicine*; 2011 Dec 15; 19(1):64-73.
16. Rittié, L. & Fisher, G.J.; UV-light-induced signal cascades and skin aging; *Ageing research reviews*; 2002 Sep 1; 1(4):705-20.
17. Parvez, S., Kang, M., Chung, H.S. & Bae, H.; Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetics and agriculture industries, *Phytotherapy Research; An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*; 2007 Sep; 21(9):805-16.
18. Jimenez, F., Mitts, T.F., Liu, K., Wang, Y. & Hinek, A.; Ellagic and tannic acids protect newly synthesized elastic fibers from premature enzymatic degradation in dermal fibroblast cultures; *Journal of investigative dermatology*; 2006 Jun 1; 126(6):1272-80.
19. Varani, J., Dame, M.K., Rittie, L., Fligel, S.E., Kang, S., Fisher, G.J. & Voorhees, J.J.; Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation; *The American journal of pathology*; 2006 Jun 1; 168(6):1861-8.
20. Ott, M., Gogvadze, V., Orrenius, S. & Zhivotovsky, B.; Mitochondria, oxidative stress and cell death; *Apoptosis*; 2007 May 1; 12(5):913-22.
21. Rattan, S.I.; Theories of biological aging: genes, proteins, and free radicals; *Free radical research*; 2006 Jan 1; 40(12):1230-8.
22. Ma, Y.S., Wu, S.B., Lee, W.Y., Cheng, J.S. & Wei, Y.H.; Response to the increase of oxidative stress and mutation of mitochondrial DNA in aging; *Biochimica et Biophysica Acta (BBA)-General Subjects*; 2009 Oct 1; 1790(10):1021-9.
23. Arunkumar, S., Muthuselvan, M.; Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens; *World Journal of Agricultural Science*; 2009; 5: 572-576.
24. Jaradat, N., Hussien, F., Ali, A.A.; Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra Alata Decne; *Journal of Material Environmental Science*; 2015;6(6):1771-1778.
25. Muthukrishnan, S., Sivakumar, T.; Physicochemical Evaluation, Preliminary Phytochemical Investigation, Fluorescence and TLC Analysis of Leaves of Schleicheria Oleosa (Lour.) Oken; *Indian Journal of Pharmaceutical Sciences*; Jun 30 2018;80(3):52532.
26. Hussain, I., Khattak, M.U., Muhammad, Z., Khan, N., Khan, F.A., Ullah, Z., Haider, S.; Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpaktunkhwa Pakistan; *African Journal of Pharmacy and Pharmacology*; 30 Jun, 2011; 5(6):746-50
27. V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventos, "Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent," *Oxidants and Antioxidants Part A*, vol. 299, pp. 152-178, 1999.
28. K. Nithianantham, M. Shyamala, Y. Chen, L. Y. Latha, S. L. Jothy, and S. Sasidharan, "Hepatoprotective potential of Clitoria ternatea leaf extract against paracetamol induced damage in mice," *Molecules*, vol. 16, no. 12, pp. 10134-10145, 2011.
29. Sahu AN, Jha SB, Dubey SD. Formulation & Evaluation of Curcuminoid Based Herbal Face Cream. *Indo-Global Journal of Pharmaceutical Sciences*, 2011;1(1):77-84
30. Sujith SN, Molly M, Sreena K. Formulation and Evaluation of Herbal Cream containing Curcuma longa. *International Journal of Pharmaceutical and Chemical Sciences*, 2012; 1(4).
31. A.Vijayalakshmi, A. Tripura, V. Ravichandiran. Development and Evaluation of Anti- Acne Products from Terminalia arjuna Bark, *IJCRRG* 3(1):320-327.
32. Ravindra RP, Muslim PK. Comparison of physical characteristics of vanishing Cream base, cow ghee and shata-dhautaghrita as per pharmacopoeial standards. *International Journal of Pharma and BioSciences*, 2013; 4(4):14 - 21.
33. Ashish A, Mohini K, Abhiram R. Preparation and evaluation of polyherbal cosmetic cream. *Der Pharmacia Lettre*, 2013; 5(1):83-88.
34. A. Premkumar, T. Muthukumaran, V. Ganesan, Shanmugam R, Priyanka DL. Formulation And Evaluation Of Cream Containing Antifungal Agents, Antibacterial Agents And Corticosteroids. *Hygeia.J.D.Med*, 2014; 6(2):5-16.
35. Kotta KK, Sasikanth .K, M.Sabareesh, . Formulation And Evaluation Of Diacerein Cream .*Asian J Pharm Clin Res*, 2011; 4(2):9398.
36. N. Radhakrishnan, Effect of liquorice: A skin whitening agent on black molly, *J. Appl. cosmetol.* 23, 149-158. (oct / dec 2005