



Development and Evaluation of Polyherbal Based Novel Antiaging Synergistic Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present work was aimed to develop and evaluate polyherbal-based novel antiaging formulation.

Place and Duration of Study: Faculty of pharmacy, B N University Udaipur, Department of Pharmacognosy, Divine College of pharmacy Satana between Feb 2019 to Apr 2021.

Methods: The selected plant extract *Moringa oleifera* hydroalcoholic extract (2%), *Juglans regia* aqueous extract (1%), *Vitis vinifera* ethanolic extract (1.5%), *Camellia sinensis* cold water extract (1.8%), *Punica granatum* aqueous extract (2%) were optimized in o/w type herbal cream by incorporation of different concentration of stearic acid and Tween 60. The total 9 formulations were evaluated on the basis of preliminary, phytochemical screening, and accelerated stability study and confirm the C7 polyherbal formulation (PHF) were more stable, safe, and showed pseudo plastic flow.

Results: The *In-vitro* free radical scavenging assay (DPPH Assay - IC₅₀ 56 ± 0.04 µg/ml and H₂O₂ scavenging assay - IC₅₀ = 67±0.68 µg/ml) compared with standard Ascorbic acid and *In-vitro* anti-collagenase and anti-elastase activity validate PHF as antioxidant and antiaging activity. The *in vitro* Anti-collagenase activity showed 89.5% inhibition at 100 µg/mL concentration of C7 formulation (IC₅₀ = 54.62 µg/mL) and the standard Gallic acid showed 74.6% inhibition at 100 µg/mL (IC₅₀ = 67.83 µg/mL). The *in vitro* Anti-elastase activity showed 67.5% inhibition at 250

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µg/mL of C7 formulation (IC₅₀ = 193.65 µg/mL) and copper sulphate solution used as standard showed 70.6% inhibition (IC₅₀ = 772.42 µg/mL). The percent inhibition activity was observed that the C7 formulation is potential antiaging activity as compared to positive control.

Conclusion: These studies conclude that the composition of PHF with a cream base increases the production of collagen and elastin which is responsible for the noteworthy synergistic antiaging activity.

Keywords: Anti-aging; collagenase; cream; elastase; polyherbal; skin aging.

1. INTRODUCTION

Topically applied products capable of making changes to the skin are called cosmeceutical that is considered neither drug nor cosmetic. Cosmeceuticals are nothing but the natural origin that is free from harmful synthetic. The new trend in modern pharmacognosy is the utilization of herb product development to achieve potent action with reduced adverse effects. Also, the now developed skincare and hair care products are of herbal origin. This reduces the skin problems such as hypersensitivity, hyperpigmentation, skin wrinkling, skin aging, and rough skin texture, etc. Most shreds of evidence describe herbal cosmetics are potential products than comparatively to synthetic cosmetics [1].

Aging is a complex process influenced by many biochemical pathways. The long-term damage to tissue and cellular macromolecules results in the aging of the skin layer. During the biochemical reactions, the reactive oxygen species (ROS) are continuously generated at a cellular level. ROS scavenge the cellular membrane because of the reduced elasticity and plasticity of the skin by degrading molecules like collagen, elastin, etc. So, to find out the plant-based product which is a healthy nutrient for skin rejuvenating is the goal of this study. The more than two plant extract will be more potential to treat disease or disorder. This concept is called Polyherbalism. The Polyherbalism concept was since from Ayurveda where the effective and potential Ayurvedic products for mankind are developed with the number of natural sources in a single formulation could be getting synergism [2, 3].

Moringa oleifera (family Moringaceae) is often called the "tree of life" and "Miracle Tree" because it contains high numbers of skin health-promoting nutrients and antioxidants. The aqueous extract of leaves of *M. oleifera* nourishes the dermal layer and complete protection from free tissue-damaging radicals by increasing the activity of endogenous antioxidant

enzymes. It raises the expression of stress resistance proteins all the way through the FOXO transcription factor [4]. *Juglans regia* L. (family Juglandaceae) is found to be a good source of flavonoids, sterols, phenolic acids, and related polyphenols. It is reported that *J. regia* inhibits oxidative damages in the stages of photoaging [5]. *Vitis vinifera* (family Vitaceae) was found to be effective for improving the clinical sign of aging skin. Because of the strong antioxidants present in *V. vinifera*, the antiaging and antioxidant properties could be as property of cream. The grape peel extract (GPE) was investigated as protective capacity on Ultraviolet (UV) induced skin wrinkle formation. This activity is exhibited by the total phenolic, anthocyanin, and total flavonoids content in GPE [6]. *Camellia sinensis* (L.) (Family Theaceae) Tea can be processed in different prepared forms like green tea, black tea (tea dust), or white tea. Green tea and white tea both are unfermented forms contain polyphenol, catechin especially EGCG. So, these teas show strong antioxidant activity [7]. *Punica granatum* (family Punicaceae) reported that viscoelasticity and mechanical parameters are improved. Moreover, tea leaves are lessening the malondialdehyde (biomarker for oxidative stress) level [8].

Oxidative stress is created at the cellular level caused by the generation of free radicals. The formulation which reduces oxidative stress will delay the skin aging process. The polyphenols, flavonoids, sources of vitamin C, vitamin E like phytoconstituents was confirmed with their skincare activity to support the strength, integrity, and texture of the skin. However, the majority of natural-origin fat, oil, and waxes are used as moisturizers [9]. Collagenase is a proteolysis enzyme that is accountably depleting the collagen matrix in the skin layer. These enzymes are of three types which are found in humans, MMP-1 (Matrixmetallo protease), MMP-8, and MMP-13. These enzymes are increased in response to UV radiation and accelerate the photoaging process. Elastase is another enzyme in the skin layer that degrades the elastin

molecule which is responsible for the elasticity and toughness of skin tissue. So, the phytoconstituents which claim the antiaging and antiwrinkle effect by inhibiting the elastase and collagenase enzymes are evaluated by antioxidant and anti collagenase activity [10].

The polyherbal formulation will confer the synergism effect based on the two mechanisms, i.e. pharmacokinetics and pharmacodynamics. The pharmacokinetics parameters like absorption, distribution, metabolism and elimination will ease the drug to be effective and active metabolites with similar pharmacodynamics targeted by diverse mechanism of action will present synergism effect [11].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant

The leaves of *M. oleifera* (Moringaceae), Seed of *J. regia* (Juglandaceae), Fresh fruits of *V. vinifera* (Vitaceae), Dried leaves of *C. sinensis* (Theaceae) and Fresh fruits of *P. granatum* (Punicaceae), and flowers *R. grandiflora* (Rosaceae) were collected and authenticated by Prof. Manohar G. Gavit, Assistant professor, MVPS KANMS Art, Commerce and Science College, Satana, Dist- Nashik, Maharashtra, India. (KANMS/2020-21/62/Herbarium 1, 2, 3, 4, 5, and 6).

2.2 Preparation of Plant Extract

The fresh leaves of *Moringa oleifera* collected and drying under the sun. And then extracted with soxhlet extractor with solvent Alcohol: Water (1:1) to make the hydroalcoholic extract. The collected dried seed of walnuts (kernels covered with pellicles) were extracted with water at 25°C for 12hr. The resulting extract was filtrated using Whatman membrane and dried in a hot air oven [12]. The skin of fresh fruit of *Vitis vinifera* was extracted by shaking on a mechanical shaker for 12hr in the dark at RT with 90% Ethanol solvent. The resulting extract was centrifuged at 12,000 rpm for 10 min [13]. The dried leaves of *Camellia sinensis* (White tea) were obtained from Dagadu Teli Chandwadkar, Nashik (Maharashtra). 500 gm of dried white tea were ground in a mortar and pestle and then to sonication for 15 minutes to extract maximum components from within the cells. Then the filtrate was filtered, dried, and weighed [14]. The fresh fruit of *Punica granatum*

(Pomegranate) were dried completely and was extracted using deionized water at room temperature. The extracts were concentrated on a water bath at 50°C [15]. The petals of fresh flowers of *rosa grandiflora* were macerated using 90% ethanol as a solvent for 24 hr [14]. The extraction yield was expressed as % of dried mass compared to the total mass of crude drug used for extraction.

2.3 Preparation of Antiaging Cream

The prototype formula of polyherbal anti-aging cream was formulated. This formula contains an aqueous phase and oil phase. Oil phase like (stearic acid an emulsifier, liquid paraffin, lanoline, and others) was mixed (Part A) and heated up to 75°C. Then the aqueous phase like propylene glycol, glycerin, Rosewater (Part B) continuously added dropwise with stirring using a magnetic stirrer. The precaution is to be taken to make the homogeneous o/w cream. Finally the mixture of different plant extract like *Moringa oleifera* hydroalcoholic extract (MOHA), *Juglans regia* Aqueous extract (JRAE) *Vitis vinifera* Ethanolic extract (VVEE), *Camellia sinensis* Cold water extract (CSWE), *Punica granatum* Aqueous extract (PGAE) in the quantity (%w/w) of 2%, 1.5%, 2%, 1%, 3% respectively.

2.4 Formulation Optimization

The oil phase contains Stearic acid (10%), Tween 60 (2%), Liquid paraffin (6%), Beeswax (1.5%) while aqueous phase contains Glycerin (5%), Methyl paraben (0.05%), Triethanolamine (1.8%), Rose water up to 100% and perfume in quantity sufficient. The formulation was optimized using with Minitab Software. The formulation was optimized for the two factors (emulsifiers) with different levels (Stearic acid – 9, 10, 11 and Tween 60 - 1, 1.5, 2). By applying full factorial design 32, the total of 9 formulas was prepared and simultaneously going for evaluation.

2.5 Evaluation of Prepared Poly-Herbal Anti-aging Cream

2.5.1 Preliminary parameters

Organoleptic evaluation: The organoleptic test of formulated cream was performed usually by observing the state, color, odor, texture, clarity, etc.

Table 1. Preparation and formulation of Polyherbal Antiaging cream

Ingredients (%)	Formulation Code								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
Active Ingredients (%w/w)									
<i>Moringa oleifera</i> hydroalcoholic extract (MOHA)	2	2	2	2	2	2	2	2	2
<i>Juglans regia</i> Aqueous extract (JRAE)	1	1	1	1	1	1	1	1	1
<i>Vitis vinifera</i> Ethanolic extract (VVEE)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
<i>Camellia sinensis</i> Cold water extract (CSWE)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
<i>Punica granatum</i> Aqueous extract (PGAE)	2	2	2	2	2	2	2	2	2
Cream Base Ingredients									
Stearic acid	9	10	11	9	10	11	9	10	11
Tween 60	1	1.5	2	1.5	2	1	2	1	1.5
Liquid paraffin	30	30	30	30	30	30	30	30	30
Beeswax	1	1	1	1	1	1	1	1	1
Glycerin	10	10	10	10	10	10	10	10	10
Triethanolamine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Rosewater	100	100	100	100	100	100	100	100	100
Perfume	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

After Feel: After applying the cream on the skin, sleepiness and emolliency were noted.

2.5.2 Physicochemical parameters

Determination of pH: For measuring the pH level, 50 ml of distilled water has been used for weighed 0.5 g of the cream and the average of triplicates were determined.

Determination of Viscosity: Viscosity was evaluated in Brookfield viscometer (LV-64 spindle). The spindle was immersed in formulated cream and the 25 rpm rotation rate was adjusted, as described elsewhere [16].

Spreadability Test: The Spreadability of cream was determined with the parallel plate method. The one gram of placed between two glass slides of 20/20 cm and 125 gm weight was placed upon the upper glass plate so that cream between the two slides was pressed uniformly to form the thin layer. The weight was removed and the spread diameter was measured [16].

Determination of Type of Emulsion (Dye Method): A scarlet red dye was mixed with the cream. A drop of the cream was placed on a glass slide and examined under a microscope. In the case of the water-in-oil (w/o) type of emulsion, the dispersed globules appear red and the continuous phase colorless. In the case of the oil-in-water (o/w) type of emulsion, the dispersed globules appear colorless and the continuous phase red [17].

Homogeneity: The prepared cream formulation was evaluated by visual appearance and touch for its homogeneity.

Type of Smear: After the application of the cream, the type of film or smear formed on the skin was checked.

On Removal: It was examined by washing hands (cream applied part) with tap water after applying the cream [17].

Irritancy test: An area (1sq.cm) was marked on the left-hand dorsal surface of human volunteers. The cream was applied to the specified area and time is noted. The presence of irritancy, erythema, and edema was checked at regular intervals up to 24 hrs and reported [18].

Acid Value: The mixture of an equal proportion of alcohol and solvent ether was prepared in a

flask. To this weighed 10g of cream was added and the flask was connected to a reflux condenser and started heating slowly until the sample dissolves completely. After completion of this 1 ml of phenolphthalein was added and titrated with 0.1N NaOH, until a faintly pink color appears after shaking for 30 sec [18].

$$\text{Acid value} = n \times 5.61/w$$

n = The number of ml of NaOH required; w = The weight of cream

Saponification value: The 25ml of 0.5 N alcoholic KOH was prepared and to this 2 g of the cream was refluxed for 30 min. After that, 1 ml of phenolphthalein is added and titrated immediately, with 0.5 N HCL [18].

$$\text{Saponification value} = (b-a) \times 28.05/w$$

a = the volume in ml of titrant; b = the volume in ml of titrate; w = the weight of cream

Microbial Limit Test: Petri dishes having 9-10 cm in diameter are used for microbial limit test detection. Soybean-casein digest agar medium was used for bacteria detection and Sabouraud glucose agar was used for fungi detection. Firstly the agar medium was sterilized and then 20 ml of sterilized agar medium was added and mixed evenly. Then the 10 g of cream was dissolved in phosphate buffer (7.2) and made up to 100 ml. The spread plate method was preferred for the microbial limit tests. Firstly the agar medium was poured into the Petri dish, cooled till settled down. 100 µl of the dissolved cream was spread on the solidified and dried surface of the agar medium and spread uniformly using a spreader. After the agar solidified, it was incubated for 5 days at 20–25°C for fungi detection and at 30–35°C for bacteria detection. The number of developed colonies per plate was calculated and recorded [19].

2.6 Accelerated Stability Testing

The Stability testing of drug products is an imperative part of the New Product development. The stable formulation or product is the key success of design the product as per the ICH guidelines. The stability studies were carried out according to ICH guidelines. The cream-filled with a bottle and kept in a humidity chamber maintained at 8°C ± 0.1°C in the refrigerator and at 25°C ± 1°C, 30°C ± 1°C and 40°C ± 1°C in an incubator with 75% relative humidity (RH), and

the above parameters were observed for 8 weeks at weekly intervals. At the end of the studies, samples were analyzed for the physical properties and viscosity of the cream formulation [20].

2.7 Pharmacological Screening Methods

2.7.1 In vitro study for antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) ASSAY:

The 200 μL of the analytical sample were mixed with 800 μL of 0.1 M Tris-HCl buffer (pH-7.4) were added into a test tube. Then 1 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was added. Immediately the mixture was mixed in a test tube for a few seconds and all solutions were placed in a dark place. After 30 min the absorbance of the solution was measured at 517 nm. And finally, the blank solution was measured as it was prepared by mixing 1.2ml ethanol and 800 μL of 0.1 M Tris-HCl buffer. Ascorbic acid was used as a standard compound. All measured reading was done in triplicate. The % inhibition of the sample was calculated using the following formula:

$$\% \text{Inhibition} = [(AB - AA)/AB] \times 100,$$

Where AB is the absorption of the blank sample at $t = 0$ min; and AA is the absorption of tested extract solution at $t = 15$ mins [21].

H₂O₂ scavenging inhibition activity: The protocol of this assay was based on the ability of the sample to scavenge the H₂O₂ to confirm *in vitro* the antioxidant activity. The C7 formulation was diluted with 500 μL of 0.1 M phosphate buffer (pH 7.0). Then to this sample, H₂O₂ (100 μL), 0.1 M NaCl (100 μL), and 0.1 M phosphate buffer (700 μL , pH, 7.0) were uniformly mixed. In addition, a 1 mL mixture of a solution containing 0.2 g/mL phenol red and 0.1 mg/mL horseradish peroxidase was added and mixed thoroughly and then incubated at 37°C for 15 minutes. The absorbance was taken for all dilutions at 610 nm against a blank after the addition of 1 M NaOH (100 μL) to the incubated solution. The percentage of inhibition was calculated according to the following formula:

$$\text{Percentage Inhibition (H}_2\text{O}_2) = \frac{A_{\text{control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Where, A_{control} - The control solution without a sample (only water); A_{sample} - The absorbance with the sample [22].

2.7.2 In vitro study for anti-aging cream

Determination of anti-elastase activity:

Antielastase assay was performed by spectrophotometrical method to determine the ability of the polyherbal formulations to inhibit the elastase. An Elastase is a protease enzyme that degrades elastin. So, the Inhibition of elastase can prevent skin aging. The assay was performed by using 0.2 M Tris-HCl buffer (pH 8.0). Porcine pancreatic elastase (EC.3.4.21.36) was dissolved in this buffer to prepare 100 $\mu\text{g}/\text{mL}$ of enzyme stock solution and then diluted to get 10 $\mu\text{g}/\text{mL}$. The substrate, 0.22 mM NSANA (N-succinyl-Ala-Ala-Ala-p-nitroanilide) was dissolved in the buffer to prepare a 1 mg/mL substrate solution. These Different polyherbal formulations were incubated with the enzyme for 20 min at room temperature before adding the substrate to begin the experiment. The final reaction mixture consisted of 25 μL of various concentrations of plant extracts, 25 μL of the substrate, 25 μL of the enzyme, and 175 μL of Tris-HCl buffer. Copper sulfate solution (100 mM) was used as a positive control and the negative control consisted of Tris-HCl buffer. The absorbance was measured immediately at 405 nm and then continuously for 20 min. The anti-elastase activity of the plant extracts was calculated using the following formula:

$$\% \text{ inhibition} = \frac{[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}}] \times 100}$$

Where, Absorbance_{control} is the absorbance of control drug; Absorbance_{sample} is the absorbance of sample drug [23].

Anti-collagenase activity: The Anti-collagenase activity of the sample is the ability of the polyherbal formulations to inhibit the proteolytic enzyme - collagenase. In this method, 50 μL trypsin buffer solution (pH 7.5), 50 μL of sample and 50 μL of the enzyme (125 U/ml ChC, type IA) were added into 96-well microplate. To start the reaction was added 50 μL N- (3- [2-Furyl] - acryloyl)-Leu-Gly-Pro-Ala (FALGPA) 0.5 mM. After the reaction started, the solution was then incubated for 15 min. With the help of a microplate reader, the reacted microplate was monitored at 340 nm at a constant temperature of 25°C. Positive controls were used gallic acid and percentage inhibition was calculated by:

$$\text{Percentage inhibition (\%)} = 1 - \frac{O_s}{O_c} \times 100$$

Where, OS = the corrected absorbance enzyme in the presence of samples; OC = the corrected absorbance of the enzyme without samples [24].

2.7.3 Ex vivo skin diffusion studies

The study was performed using Franz diffusion cell using dialysis membrane. Comparative studies were carried out between C4 and C7 cream and Quercetin (Standards: Quercetin was solubilized with ethanol 50% (v/v), resulting in solutions with concentrations of 0.05 mg/mL). The skin on both sides of the ear was cut open and was cleaned of underlying tissues, fat, and hair. The membrane was then stored in the refrigerator until use. Before the study, the membrane was allowed to attain room temperature in phosphate buffer (pH 7.4) for 12 hours. The flow of warm water (37°C maintained) started by switching the knob, so the warm water circulating throughout the six-station diffusion cell. The acceptor compartment with a volume of approximately 12 ml was filled with phosphate buffer. The skin membrane (dermal side down) was clamped between the donor and acceptor compartments which had a diffusion area of 1.9 cm². 1gm of each formulation was weighed and placed on the membrane and the donor chamber was covered to prevent evaporation. The receptor solution was stirred automatically by adding magnetic bead continuously at 100 rpm with the help of a magnetic stirrer. 2 ml aliquots were withdrawn through the sampling port at 2hr, 4hr, 6hr, 8hr, 10hr, 12 hr, and 14 hr, and the cells were replaced with the same quantity of fresh buffer each time to maintain sink condition. Quercetin content in each aliquot was then measured spectrophotometrically at 370 nm and calculated using quercetin standard curve taken in phosphate buffer. The quercetin retained in the skin was determined at the end of 12 hours after the *ex-vivo* study. The skin was removed from the diffusion cell, the excess formulation was washed off and the rat skin was cut into pieces and soaked in methanol (10 ml). It was then homogenized at 24,000 rpm for 5 minutes. The solution was filtered and centrifuged at 10,000

rpm for 20 minutes at 4°C. The amount of quercetin retained was measured spectrophotometrically at 370 nm and calculated from the quercetin standard curve [20].

2.8 Statistical Analysis

The purpose of using 3² full factorial designs was to conduct the comprehensive study of the effect of the emulsifier Stearic acid and Tween 60 and their emulsification action to prepare the stable cream. The results obtained from *in-vitro* studies were evaluated by a One-way analysis of variance (ANOVA) test were applied to determine eventual variation between different percent inhibitions. Statistically, a significant difference was considered at a p-value of less than 5% ($p < 0.05$). Data were expressed as mean \pm standard error.

3. RESULT AND DISCUSSION

All the formulations were seen to be semisolid in the state. The preliminary observation showed that the formulations were no typically changed in appearance, odor, and texture at all. All the formulations were producing a uniform distribution of extracts in the polyherbal cream. This was defined by visual examination and by touch. The pH of all formulations was found to be in the range of 6.0 ± 0.18 to 6.9 ± 0.15 that is within the range. All recorded pH values are presented in Table 3. Although alkaline, this pH is typical of creams containing liquid paraffin, beeswax and acceptable by the ISI standards. Viscosities of all the formulations (C1-C9) were noted and found in the range of 1100 ± 5.27 to 3789 ± 8.32 CPS at 15 rpm. All the formulations were showed pseudoplastic flow. The results from the patch test conclude that there is no irritation, erythema and edema-like condition was found on the skin of healthy volunteers as shown in Fig.1. The patch was passed by all formulations with none of any adverse and hypersensitive reactions.



Fig. 1. Result of Patch Test for polyherbal formulation

Table 2. Test applied for acid value and saponification value

Sr. No.	Parameter	Formula								
		C1	C2	C3	C4	C5	C6	C7	C8	C9
1	Acid value (mg NaOH/g of cream)	6.2	6.5	6.9	6.4	6.6	6.8	6.5	6.6	6.7
2	Saponification value (mg KOH/g of cream)	26.89	28.21	26.40	25.08	26.13	25.06	27.48	27.77	24.89

Table 3. Physical parameter of C1 to C9 creams on room and accelerated temperature

Days	Temperature	C1		C2		C3		C4		C5		C6		C7		C8		C9	
		pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity	pH	Homogeneity
First Week	RT	6.2	G	6.2	G	5.9	G	6.3	G	6.4	G	5.9	G	6.4	G	6.1	G	6.0	G
	40°C ± 1°C	6.8	G	6.5	G	6.5	G	6.5	G	6.7	G	6.4	G	6.6	G	6.3	G	6.3	G
Third Week	RT	6.2	G	6.3	G	6.1	G	6.3	G	6.4	G	6.2	G	6.5	G	6.2	G	6.2	G
	40°C ± 1°C	6.8	G	6.5	G	6.5	S	6.6	G	6.7	G	6.6	S	6.7	G	6.4	S	6.5	S
Fifth Week	RT	6.4	G	6.3	G	6.3	G	6.5	G	6.4	G	6.3	G	6.5	G	6.4	G	6.4	G
	40°C ± 1°C	7.0	S	6.7	S	6.8	S	6.6	G	6.7	S	6.7	S	6.8	G	6.5	S	6.6	S
Eighth Week	RT	6.5	G	6.4	G	6.5	G	6.6	G	6.5	G	6.5	G	6.7	G	6.5	G	6.5	G
	40°C ± 1°C	7.0	S	7.0	S	7.0	P	6.7	S	6.9	S	6.9	P	6.8	G	6.8	S	6.8	S

*Note – G = Good, S= Satisfactory, P = Poor

The dye test was confirming that all the formulations were o/w type of emulsion cream. The acid value of all formulations was found to be between 5.5 - 6.9 and the saponification value in between 20.5 to 29.8 which is shown in Table 2.

3.1 Microbial Limit Test

The number of colonies developed per plate was calculated and recorded. The Total Microbial Count was shown in all petri plates coded with C1 to C9 in the range of 62 to 78. All the formulations passed the antimicrobial limit test < 10^3 colonies.

3.2 Accelerated Stability Testing

When formulation was kept for an extended time period, it was found that there were no changes in the color of the cream. Subsequently, the C1 to C9 formulations were screened out for the Spreadability test, after-feel, homogeneity, type of smear, and the removal of the cream. When the cream was spreaded between two glass slides, the thin uniform layer was formed. So all the formulations found were passed the Spreadability test. Then the same formulations showed the emollient feel and the smear was found nongreasy but washability from the skin was found easy for all formulations. By performing an accelerated stability study, the formulations were exposed to different temperature conditions. The homogeneity was found to be an irregular pattern in all formulations as reported in Table 3. The C3, C6, C9 formulation showed satisfactory homogeneity for accelerated temperature while C1, C2, C8 formulation showed better homogeneity than the C3, C6, C9. However, C4 and C7 formulations showed more stable and better homogeneity among other all formulations.

3.3 Pharmacological Screening Methods

The preliminary and phytochemical examination of the formulations from C1 to C9 was done and reaches the point of conclusion that C7 formulation was the better stable formulation among others. All the results of the preliminary screening and accelerated stability study revealed that the C7 showed a good response regarding the effect of temperature, spreadability, film-forming ability, etc., and have non-irritant, not developing the erythema and edema. So, C7

formulation was prepared as per the optimized formula reported in Table 1. The study of pharmacological screening through *In vitro* study for antioxidant activity was taken to consideration.

3.4 In vitro Study for Antioxidant Activity

The free radical continuously scavenges the tissues which are produced during routine metabolic reactions and that are implicating in the aging process. The antioxidants which were covered the injurious effect caused by free radicals. Moreover, many studies attempt to show the alteration of the activity of different enzymes in the aging process. The DPPH assay is based on scavenging the DPPH (1, 1-diphenyl-2-picrylhydrazyl). The Antioxidant activity was tested using this method. IC₅₀ of the extract for DPPH assay was 56 ± 0.04 $\mu\text{g/ml}$ when compared to ascorbic acid 52 ± 0.15 $\mu\text{g/ml}$. (Fig. 2) The H₂O₂ scavenging assay was also based on somewhat principle to DPPH assay where the % Inhibition H₂O₂ was measured and evaluating the sample for antioxidant and antiaging cream. The IC₅₀ of extract in H₂O₂ scavenging assay was 67 ± 0.68 $\mu\text{g/ml}$ in comparison to standard ascorbic acid 64 ± 0.49 $\mu\text{g/ml}$. (Fig. 3) The DPPH assay and H₂O₂ Assay were assessed for C7 formulation, the % inhibition activity was found to be dose dependant on significant antioxidant activity. The C7 showed the better active polyherbal combination against free radical scavenging activity as compared with the standard substance, ascorbic acid.

3.5 In Vitro Study for Anti-Aging Cream

3.5.1 Determination of Anti-elastase activity

The C7 were again attested with the *In-vitro* Anti elastase activity and anti-collagenase activity. Fig. 4 shows the C7 proven the antiaging and antiwrinkle action when compared with the same *in vitro* activities performed with standard Gallic acid. A concentration of 100 $\mu\text{g/mL}$ of C7 formulation showed 89.5% (IC₅₀ = 54.62 $\mu\text{g/mL}$) while concentration of 100 $\mu\text{g/mL}$ of Gallic acid is 74.6% (IC₅₀ = 67.83 $\mu\text{g/mL}$) collagenase enzyme inhibition. (Fig. 4) Whereas lower concentrations of 20, 40, 60 and 80 $\mu\text{g/mL}$ shown 20.6%, 36.9%, 50.1% and 76.8% collagenase inhibition respectively for C7 formulation while 11.8%, 23.2%, 46.6%, and 62.7% respectively for gallic acid.

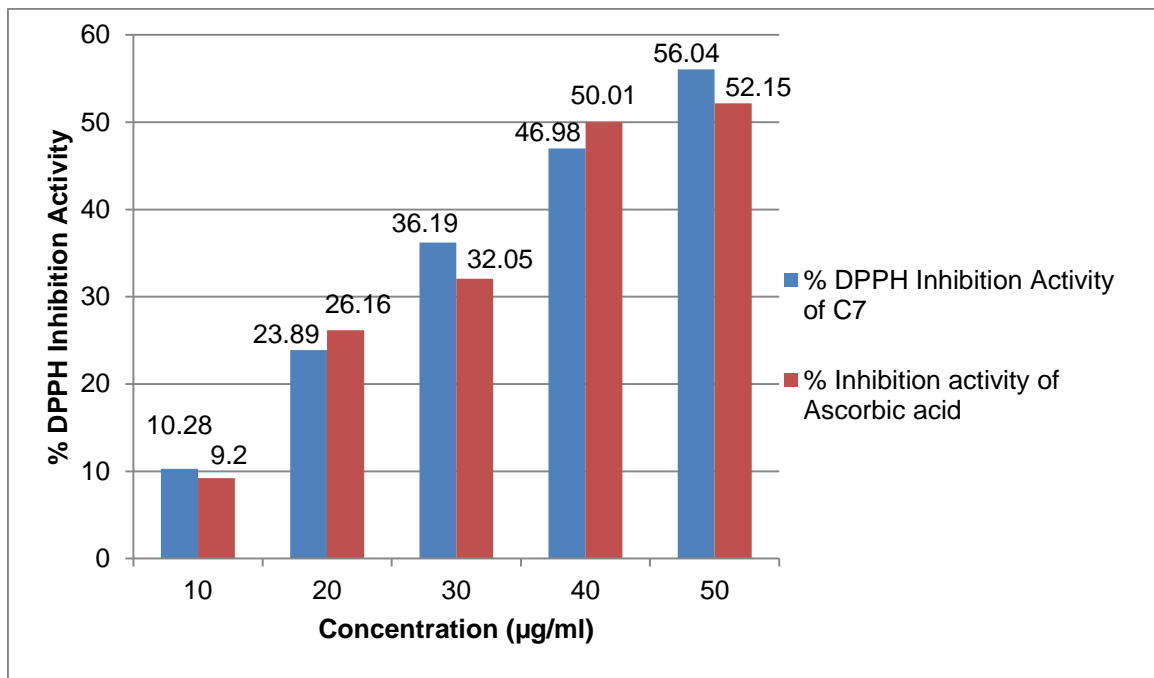


Fig. 2. DPPH radical scavenging activity of Polyherbal Antiaging cream

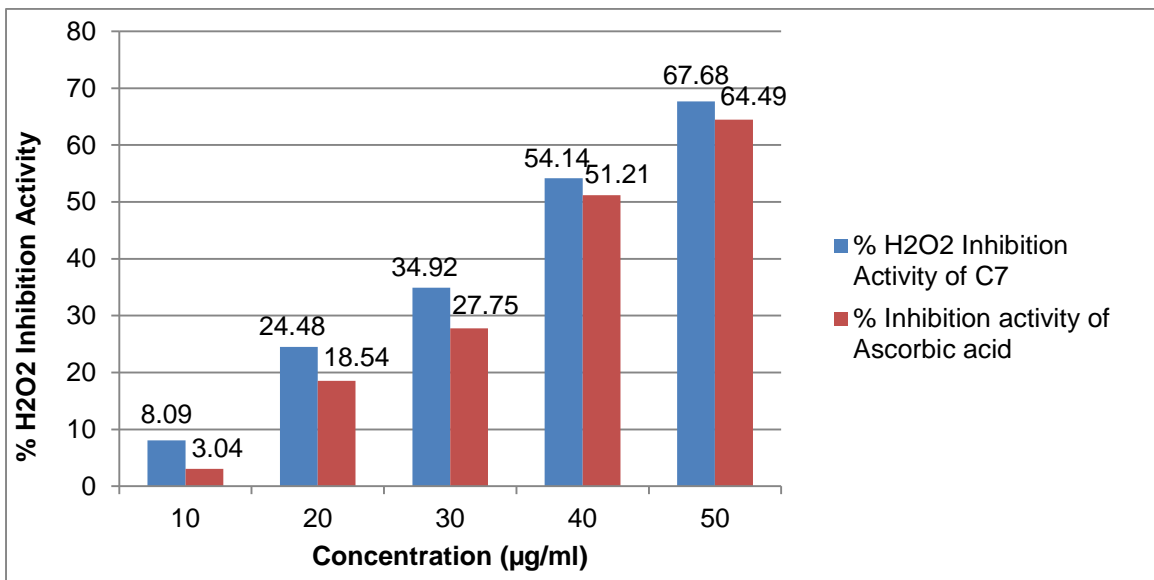


Fig. 3. Hydrogen peroxide free radical scavenging activity of Polyherbal Antiaging cream

3.5.2 Determination of Anti-collagenase activity

A concentration of 250 µg/mL of C7 formulation showed 67.5% (IC50 = 193.65 µg/mL) (Fig. 5) while Copper sulphate solution showed 70.6% (IC50 = 772.42 µg/mL) elastase enzyme inhibition (Fig. 6). Whereas lower concentrations of 50, 100, 150, 200 and 250 µg/mL shown

19.8%, 26.3%, 35.7% and 49.1% elastase inhibition respectively for C7 formulation while 12.4%, 26.8%, 32.6% and 48.2% respectively for copper sulphate solution.

3.6 Ex vivo Diffusion Studies

Percent of Quercetin released at each time point was calculated by a Quercetin standard with a

curve as shown in Fig. 7 with a regression equation of $y = 13.53x - 19.28$ and $R^2 = 0.9888$. Where Y is the yields of anti-collagenase, and X is the concentration of extract. The cumulative percentage of quercetin in the polyherbal antiaging cream ($78.32 \pm 0.28\%$) over 12 hours was higher than with Standard Quercetin ($49.51 \pm 0.42\%$). Polyherbal antiaging cream showed higher retention of Quercetin in the skin ($22.82 \pm 0.45\%$) compared to the standard

quercetin ($5.3 \pm 0.16\%$) (Fig. 8). Thus polyherbal antiaging cream showed higher localization of quercetin in the skin as compared to standard quercetin because all extracts are having a great source of quercetin is one of the parts of flavonol and flavonoids content. This property can be attracted for getting antiaging property to polyherbal extract; they can transport drugs to target skin cells.

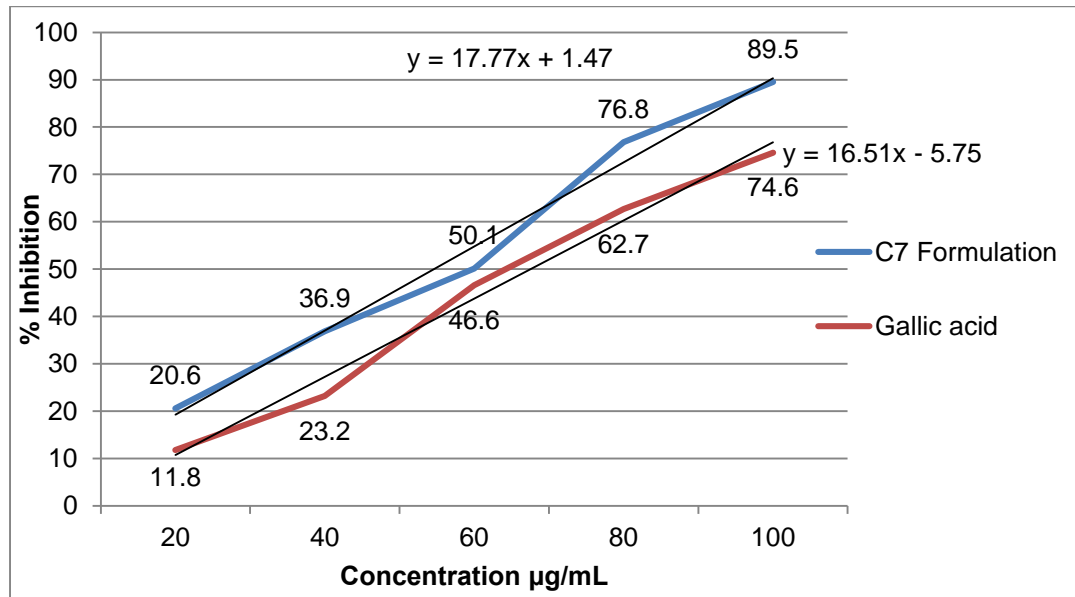


Fig. 4. The graph of % Inhibition of Collagenase enzyme vs. Concentration (µg/mL) of Cream formulation (IC₅₀ = 54.62 µg/mL)

The Gallic acid is used as standard enzyme inhibitor (IC₅₀= 67.83 µg/mL). Data were representative of three different experiments with similar results. (*P < 0.05 when compared with Gallic Acid)

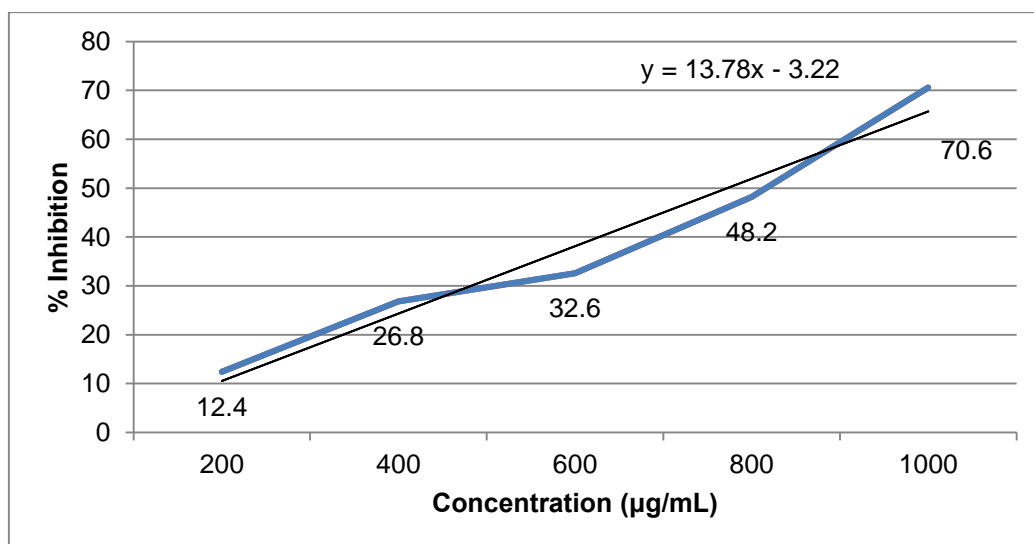


Fig. 5. The graph of % Inhibition of Elastase enzyme vs. Concentration (µg/mL) of Copper Sulphate (IC₅₀= 772.42 µg/mL).

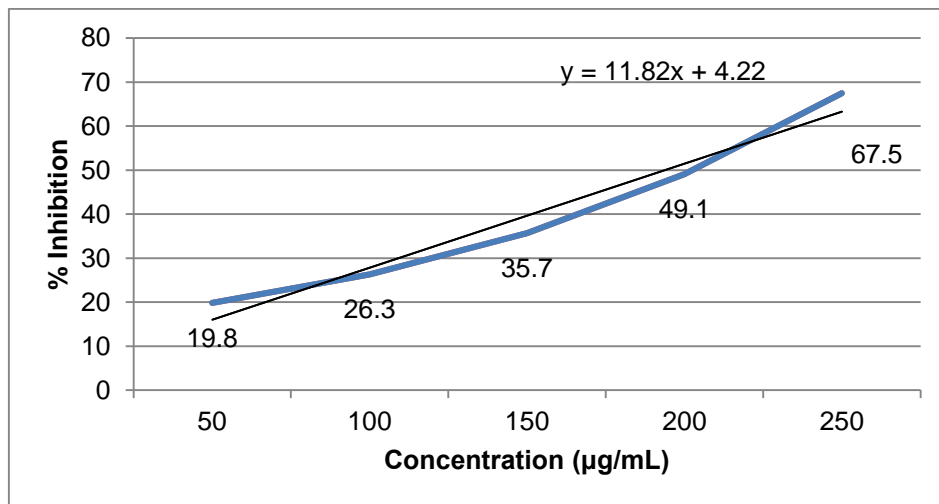


Fig. 6. The graph of % Inhibition of Elastase enzyme Vs Concentration (µg/mL) of Cream formulation (IC₅₀=193.65µg/mL)

The data are representative of three different experiments with similar results. (*P < 0.05 when compared with Standard solution of Copper sulphate)

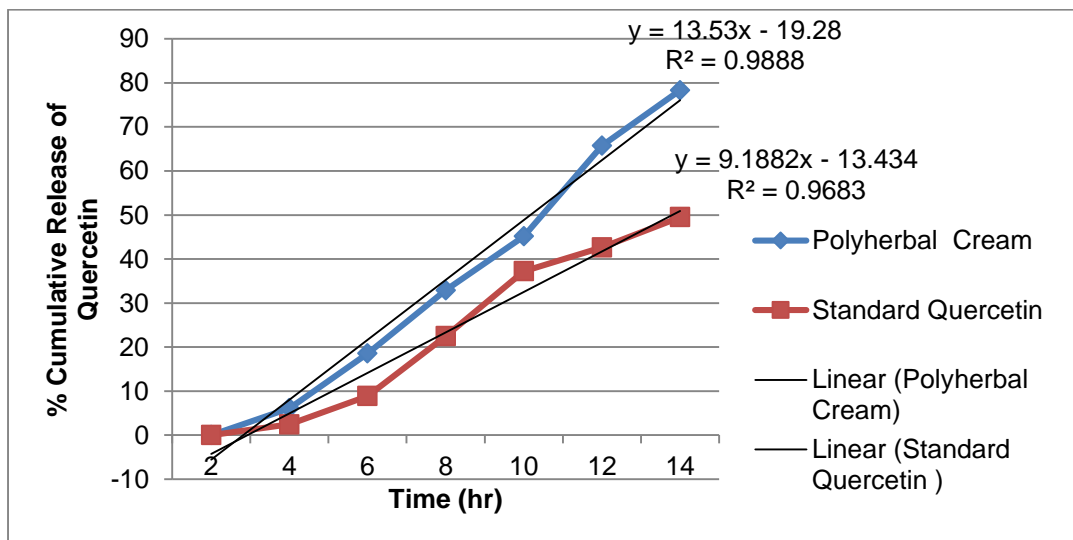


Fig. 7. Ex vivo diffusion studies

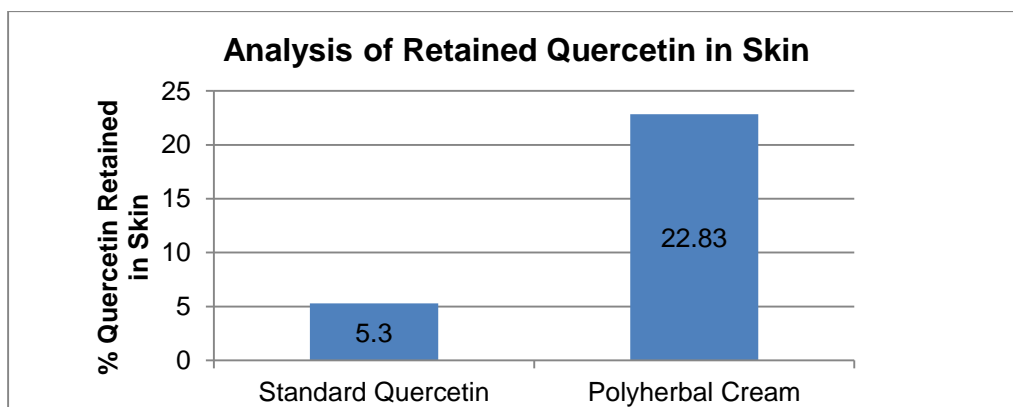


Fig. 8. Analysis of Quercetin retained in skin

4. CONCLUSION

In conclusion, the combination of *Moringa oleifera* hydroalcoholic extract (2%), *Juglans regia* Aqueous extract (1%), *Vitis vinifera* Ethanolic extract (1.5%), *Camellia sinensis* Coldwater extract (1.8%), *Punica granatum* Aqueous extract (2%) provide excellent antioxidant, anti-elastase activity, and Anti-collagenase activity. Among the Optimized formulations from C1 to C9, formulation C7 shows better stability and antiaging property prepared using stearic acid and tween 60 (9:1). The promising anti-aging effect could be an outcome of individual skincare effects of plant extracts. The research work suggests that the wide variety of Polyphenolic and Flavonoids content in different plant extracts may also cause skin rejuvenating effects. The Ex-vivo diffusion study on C7 confirmed the good permeability through the skin and good for collagen production in the skin and strengthens elasticity and structure, so it will reduce fine lines, wrinkles, and sagging. At last, this study again proved the concept of the polyherbal formulation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

STUDY SIGNIFICANCE

The study highlights the efficacy of "Polyherbalism" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pandey S, Meshya N, Viral D. Herbs play an important role in the field of cosmetics. International Journal of Pharm Tech Research. 2010;2:632-639.
2. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: Basic mechanisms, functional effects, and pathological considerations. American Journal of Physiology. 2007;292(1):18–36.
3. Joshi LS, Pawar HA. Herbal Cosmetics and Cosmeceuticals: An Overview. Natural Products Chemistry & Research. 2015; 3:2. DOI: 10.4172/2329-6836.1000170.
4. Mulkah OA, Osaretin ATE. Evaluation of the anti-aging and antioxidant action of Ananas sativa and Moringa oleifera in a fruit fly model organism. J Food Biochem. 2020;00:e13426. DOI: 10.1111/jfbc.13426.
5. Muzaffera U, Paula VI, Agilanb B, Prasad NR. Protective effect of Juglans regia L., against ultraviolet-B induced photoaging in human epidermal keratinocytes Biomedicine & Pharmacotherapy. 2019; 111:724–732.
6. Kim J, Oh J, Averilla JN, Jung Kim HJ, Kim JS. Grape Peel Extract and Resveratrol Inhibit Wrinkle Formation in Mice Model Through Activation of Nrf2/HO-1 Signaling Pathway. Journal of Food Science. 2019; 84(6). DOI: 10.1111/1750-3841.14643
7. Carloni P. Antioxidant activity of white, green and black tea obtained from the same tea cultivar. Food Research International; 2012. DOI.org/10.1016/j.foodres.2012.07.057.
8. Jadoon S, Karim S, Asad MHH, Akram MR, Khan AK, Malik A, Chen C, Murtaza G. Anti-Aging Potential of Phytoextract Loaded-Pharmaceutical Creams for Human Skin Cell Longevity. Hindawi Publishing Corporation: Oxidative Medicine and Cellular Longevity; 2015. Article ID 709628. DOI:org/10.1155/2015/709628
9. Gyawali R, Gupta RK, Shrestha S, Joshi R, Paudel PN. Formulation And Evaluation Of Polyherbal Cream Containing Cinnamomum Zeylanicum Blume, Glycyrrhiza Glabra L And Azadirachta Indica A. Juss Extracts To Topical Use. Journal of Institute of Science and Technology. 2020;25(2):61-71.

10. Wahyu SW, Wahyu W, Chrismis NG, Nyoman EL, Adek A, Ermi G. Comparison of Antioxidant and Anti-collagenase Activity of Genistein and Epicatechin Geeta1. *Pharmaceutical Sciences and Research*. 2019;6(2):111-117.
11. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Dubey SP, Khan N, Jain DK. Polyherbal Formulation Concept for Synergic Action: A Review, *Journal of Drug Delivery and Therapeutics*. 2019;9(1-s):453-466.
12. Jadoon S, Karim S, Asad MHHB, Akram MR, Khan K, Malik A. Anti-Aging Potential of Phytoextract Loaded-Pharmaceutical Creams for Human Skin Cell Longevity. *Oxidative Medicine and Cellular Longevity*. 2015;1-17. DOI:10.1155/2015/709628.
13. Doshi P, Adsule P, Banerjee K, Oulkar D. Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera* L) byproducts. *Journal of Food Science and Technology*. 2013;52(1):181-190. DOI:10.1007/s13197-013-0991-1.
14. Tamsyn SA, Pauline H, Declan PN. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine*. 2009;9(27):1-11.
15. Ghimeray A, Jung U, Lee H, Kim Y, Ryu E, Chang M. In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing Punica granatum, Ginkgo biloba, Ficus carica, and Morus alba fruits extract. *Clinical, Cosmetic and Investigational Dermatology*. 2015; 389. DOI:10.2147/ccid.s80906.
16. Kumar A, Naguib YW, Shi YC, Cui Z. A method to improve the efficacy of topical eflornithine hydrochloride Cream. *Drug Delivery*. 2016b; 23(5): 1495-501.
17. Duraivel S, Shaheda A, Basha R, Pasha E, Jilani S. Formulation and evaluation of Antiwrinkle activity of Cream and Nano emulsion of Moringa oleifera seed oil. *IOSR-Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 2014; 9(4): 58-73.
18. Aswal A, Kalra M, Rout A. Preparation and evaluation of polyherbal cosmetic cream. *Der Pharmacia Lettre*. 2013; 5(1) : 83-88.
19. Sekar M, Sivalingam P, Mahmad A. Formulation and Evaluation of Novel Antiaging Cream Containing Rambutan Fruits Extract. *International Journal of Pharmaceutical Sciences and Research*. 2017; 8(3): 1056-1065.
20. Pereira A, Mallya R. Formulation and evaluation of a photoprotectant cream containing Phyllanthus emblica extract phospholipid complex. *Journal of Pharmacognosy and Phytochemistry*. 2015; 4(2): 232-240.
21. Tomoko S, Yoshihiro S, Takeshi Y, Atsuko T, Yoshihiro K. Applicability of DPPH assay for evaluating the antioxidant capacity of food additives, *Analytical sciences*. 2014; 30:717-721.
22. Ghimeray A, Jung U, Lee H, Kim Y, Ryu E, Chang M. In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing Punica granatum, Ginkgo biloba, Ficus carica, and Morus alba fruits extract. *Clin Cosmet Investig Dermatol*. 2015; 8:389-396.
23. Sundaram K, Sarangi I, Sundararajan DD, George VS, Mohideen S. Poly herbal formulation with anti-elastase and anti-oxidant properties for skin anti-aging. *BMC Complementary and Alternative Medicine*. 2018; 18(1). doi:10.1186/s12906-018-2097-9.
24. Mansauda KLR, Anwar E, Nurhayati T. Antioxidant and Anti-Collagenase Activity of Sargassum plagyophyllum Extract as an Anti-Wrinkle Cosmetic Ingredient. *Pharmacog J*. 2018; 10(5):932-6.

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