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Formulation and *In vitro* evaluation of poly-herbal anti-ageing face cream of extracts kiwi fruit and coffee beans

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Abstract
Objective: The goal of this study was to create an anti-aging face cream by combining the ethanolic extract of *Actinidia chinensis/Actinidia deliciosa* with *Coffea arabica* to provide multipurpose skin effects such as fairness, softening, and antibacterial properties.
Methods: The herbs were collected and extracted with Water. These prepared extracts were further used in preparation of the formulation which were evaluated on the standard parameters such as Ph, After Feel, Acid value, Smear effect, Irritancy test, *In vitro* Antioxidant model DPPH activity etc.
Result: Our study indicated that, the cream was stable with no signs of breakdown of emulsion and change in colour of the product. The cream was also maintained constant pH, homogeneity emollient properties; they were not greasy and easily Removable after the application. The Cream also showed significant anti-oxidant activity with an IC50 value is 28.39 mg/ml whereas ascorbic acid had showed 18.40 mg/ml. This indicates that the formulated creams were safe for the consumers.
Conclusion: It can be concluded that herbal creams with antioxidant properties that have no negative effects can be used as a barrier to protect the skin and prevent ageing.

Keywords: anti ageing cream, kiwi fruits, coffee beans, DPPH model

Introduction
The exterior organ that defends against mechanical stress, UV light, and infection is the skin, also known as the integument. The skin also plays a role in thermoregulation, fluid conservation and excretion, sensory perception, and aesthetics [1, 2].
Human skin is a specially designed organ that allows for terrestrial existence by controlling heat and dehydration from the body while also blocking the invasion of harmful chemicals and bacteria. It is the largest organ in the human body, accounting for roughly 10% of a person's total mass and covering an approximate diameter of 1.7 m2. Despite the fact that such a huge and easily accessible organ appears to provide optimal and many locations for administering therapeutic chemicals for both local and systemic activities, human skin is a highly efficient self-repairing barrier designed to keep 'the inside in and the outside out [3, 4].
Skin ageing is the outcome of a gradual deterioration caused by cellular DNA and protein damage. The two types of skin ageing are "sequential skin ageing" and "photo ageing." Keratinocytes are unable to generate a functional Stratum corneum as they age, and the pace of production of neutral lipids slows, resulting in dry skin and wrinkles. Many factors influence skin ageing, including ultraviolet (UV) radiation, excessive alcohol intake, cigarette usage, and pollution. Uneven pigmentation, increased wrinkles, loss of elasticity, dryness, and roughness are all signs of skin ageing. The use of natural substances in the skin to protect particular topical antioxidant formulations used to reduce skin ageing. ROS (reactive oxygen species), a type of free radical that leads to skin ageing, is also linked to skin ageing. Although ROS is produced naturally during cellular metabolism, too much of it can lead to OS (oxidative stress). OS can be a risk factor for a variety of health problems, including skin ageing [5, 6].
Kiwi is scientifically known as *Actinidia chinensis/Actinidia deliciosa* which is belonging to family Actinidiaceae. It is characterized as oblong fruit with ambiguous, tan-brown coating and a corpulent innerside which is stereotypically green with a white central core and black-brown seeds. Fruits of kiwi comprises of high levels of vitamin C (ascorbic acid), Vit E, serotonin, potassium. It also contains catechins, liteinand zeaxanthin. Kiwi possess free radical scavenging activity and thereby try to protect the skin against oxidative damage by neutralizing the free radicals while amino acids treat UV damage [7, 8]. Coffee bean is another important ingredient in the formulation It is obtained from the dried ripe seed of *Coffea arabica* which is belonging to Family Rubiaceae. It has a Characteristic Odor and bitter taste. The main constituents of coffee bean are caffeine, tannin, fixed oil, proteins, 13% proteins, 10-15% fixed oils, chlorogenic or Caffe tannic acid and sugars in the form of dextrin, glucose, etc. in the seeds, caffeine is present as a salt of chlorogenic acid. Coffee's antioxidant compounds include caffeic acid, caffeine, the chlorogenic acids, eugenol, Gamma-tocopherol, isoeugenol, p-coumaric acid, scopoline and tannic acid [9].

Material and Method
Collection of plant material
Fruits of kiwi were collected from local market of Dewas and coffee beans were collected from ICH restaurant of Dewas.

Preparation of Extracts
500 gm of kiwi fruits and 20 gm of coffee beans powder extracted with water for 72 hrs. The extracts thus obtained were dried in hot air oven and kept in desiccators for further use [10].

Formulation of anti-ageing herbal cream
The oily phase and aqueous phase were heated up to 70ºC and mixed using homogenizer by addition of methyl of methyl paraben and fragrance. With constant mixing, remaining distilled water was added and stirring was continued until mixture cooled down [11]. Cream was formed when the consistency of the mixture was viscous and opaque (Table:1).

Table 1: Composition of formulation

S. no	Composition	Percentage
Aqueous phase		
1	Glycerin	5%
2	Methyl paraben	0.05%
3	Propylene glycol	30%
4	Deionised water q.s	100%
Oily phase		
5	Stearic acid	10%
6	Cetyl alcohol	6%
7	Liquid paraffin	6.6%

Anti-aging cream evaluation
The antiaging cream was evaluated using the following factors. To examine all of the parameters, the conventional process was followed.

PH of the cream
A standard buffer solution was used to calibrate the pH metre. The pH of 0.5 g of cream was determined after it was weighed and dissolved in 50 ml of distilled water [12].

Homogeneity
Visual appearance and touch were used to check for homogeneity in the formulation [10].

Appearance
Color, pearlescence, and roughness were used to assess the cream's appearance, which was scored [13].

After feel
Emolliency, slipperiness, and the amount of residue left after applying a certain amount of cream were all evaluated [14].

Smear type
The type of film or smear that developed on the skin after applying the cream was investigated [15].

Removability
Washing the afflicted region with tap water was used to see how easily the cream could be removed [12].

Acid value
In a flask, 10 g of cream was dissolved in a 50 ml combination of equal parts alcohol and solvent ether. After connecting the flask to a reflux condenser and slowly heating it until the sample dissolves completely, 1 ml of phenolphthalein was added and titrated with 0.1N NaOH until a faint pink tint develops after 30 seconds of shaking [17].

Acid value= n*5.61/W where N=the number of ml of NAOH required and W=the weight of cream

Irritancy test
A 1sq.cm region was marked on the left-hand dorsal surface of human participants. The cream was applied to the specified area, and the time it took to dry was recorded. For up to 24 hours, irritation, erythema, and edoema were checked and reported at regular intervals [17].

Viscosity
The brook field viscometer was used to determine the viscosity [17].
*The result for physiocochemical parameters is shown in Table:II

Table 2: Result of physiocochemical parameters

S.No	Parameter	Result
1	pH	4.57
2	Acid Value	16.1
3	Homogenity	Good
4	Appearance	No Change in color
5	After Feel	Emollient and slipperiness
6	Type of Smear	Non- Greasy
7	Viscosity	48.40 cP

Accelerated stability testing
Creams were divided into four parts and stability test was performed at 8°C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40°C ± 1°C and 40°C ± 1°C in incubator with 75% relative humidity (RH), and the above parameters were observed for 8 weeks at weekly intervals (Table:3) [18].

Table 3: Accelerated stability test of the formulation

S No.	Test parameter	0 Days	15 Days	30 Days
1	pH	4.57	4.57	4.57
2	Homogeneity	Good	Good	Good
3	Appearance	No Change In Color	No Change In Color	No Change In Color
4	After Feel	Emollient And Slipperiness	Emollient And Slipperiness	Emollient And Slipperiness
5	Type Of Smear	Non-Greasy	Non-Greasy	Non-Greasy
6	Acid Value	16.1	16.1	16.5

***In vitro* Antioxidant activity DPPH Model [19-20]**
The inhibition-based *in vitro* approaches are used. Samples are added to a free radical-generating system, free radical inhibition is evaluated, and this inhibition is connected to the sample's antioxidant activity. The generated radical, the reproducibility of the creation procedure, and the endpoint employed for the determination all differ significantly.

Principle
The antioxidant combines with the stable free radical DPPH to produce 1,1- Diphenyl-2- Picryl Hydrazine. The ability of

DPPH to scavenge free radicals was determined by measuring the absorbance at 517 nm.
Different concentrations (20, 40, 60, 80, 100µg/mL) of test sample and 0.1mM DPPH solution was prepared in methanol. Ascorbic acid (100µg/mL) was used as standard. 2mL of DPPH solution and 1 mL of methanol was used as control. 2 mL of sample of various concentrations was mixed with 2 mL of DPPH solution. The mixture was incubated for 10 minutes in the dark and absorbance was measured at 515 nm by spectrophotometer using methanol as blank. The percentage inhibition of DPPH radical was calculated by following formula

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the test sample. IC_{50} was calculated by plotting % inhibition as a function of sample concentration. IC_{50} is defined as sample concentration necessary to inhibit 50% of DPPH solution (Table 4.

Table 4: *In vitro* antioxidant activity using DPPH model

S. No.	Concentration in mg/ml	% Inhibition Ascorbic Acid	% inhibition Cream
1	20	48.80202	48.1715
2	40	54.72888	52.33291
3	60	58.76419	57.62926
4	80	61.03405	63.43001
5	100	63.17781	66.96091
6	IC_{50}	18.40	28.39

Result

pH
The pH of the formulated cream was found to be in range of 4.30 to 5.20 which is good and recommended pH for the skin.

Type of formulation
Dye test showed that formulation formulated was o/w type.

Acid value
Acid value of the formulated cream was found to be in within the limit

Type of smear
The type of smear formed on the skin was not greasy after the application of formulated cream.

Homogenecity
All the formulations were producing a uniform distribution of extracts in the cream.

Appearance
This was confirmed by visual examination and by touch. When formulation kept for a long time, it was found that there were no changes in the colour of the cream.

After feel
After feel test showed that the formulated creams were emollient and slipperiness.

Viscosity
The T- shaped spindle was used to determine the viscosity of cream at 100 rpm and spindle no.02 and result was 48.40 $_{C}$P.

Accelerated stability studies
All the physiochemical parameters were maintained during the accelerated stability studies at temperatures 8°C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40°C ± 1°C and 40°C ± 1°C in incubator for 8 weeks. The results of accelerated stability test showed that there were not any particular changes in the colour of the cream.

Antioxidant studies
The formulation showed encouraging response in quenching DPPH radical with an IC_{50} value of 18.40 & 28.39µg/ml of standard ascorbic acid and formulated cream.

Discussion
The formulated cream was o/w type emulsion, hence can easily washed with water and gives better consumer compliance. Our study indicated that, the cream was stable with no signs of breakdown of emulsion and change in color of the product. The cream was also maintained constant pH, homogeneity emollient properties; they were not greasy and easily removable after the application. Formulated cream showed significant anti-oxidant activity with an IC50 value is 28.39 µg/ml, while for ascorbic acid the IC_{50} value was 18.04 µg/ml. This indicates that the formulated cream was safe for the consumers.
The research work suggests that the herbal formulation and its ingredients were studied to be consistent in quality and purity and can be easily used as a anti-ageing cream. The validation of the cream was done and was found in limits. From above discussion, it is concluded that the formulation is safe usable for the skin. This study can be helpful for upcoming researchers to select these herbs for the formulation and evaluation of other cosmetic applications which can be claimed for their efficacy with scientific data.

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Conflict of Intereset
None

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