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"Formulation and Evaluation of Anti-Aging Cream Containing Liquorice"

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ABSTRACT
Aging is one of the common disorder caused due to various factors like altered metabolism, lack of nutrients and antioxidants, pollution, exposure to sun rays, dust, age, sleep, general health condition, emotional well-being, physical impairment, disease, etc. ageing leads to lack of confidence and negative impact of mental health of a person. This research focuses on various anti-ageing creams for the prevention of ageing.
Creams are semisolid preparation that contains one or more medicaments usually in a base with refreshing fragrances and are intended to spread on skin easily. The anti-ageing creams are the one of widely used dosage form, anti-ageing creams often are moisturizer with active ingredient that offer additional benefits. The effectiveness of these products depends in part on your skin type and the active ingredient. The materials, ingredient required for the preparation of anti-ageing cream, properties of active pharmaceutical ingredient used for, different storage conditions. The present review covers more or less all aspects associated with anti-ageing cream and also throws light on the development criteria for anti-ageing cream.
KEYWORDS : Antioxidants, sun rays, ageing, anti-aging.

I. INTRODUCTION

SKIN[6]
The skin is the largest organ of the body, with a total area of about 20 square feet.

Structure of the skin
The skin consists of three main layers:
Epidermis (the outer layer)
Dermis (the middle layer)
Subcutaneous or hypodermic

Functions
Skin performs the following functions:

- Protection: An anatomical barrier from pathogens and damage between the internal and external environment in bodily defense. Langerhans cells in the skin are part of the adaptive immune system.
- Sensation: Contains a variety of nerve endings that jump to heat and cold, touch, pressure, vibration, and tissue injury
- Thermoregulation: Excrine (sweat) glands and dilated blood vessels (increased superficial perfusion) aid heat loss, while constricted vessels greatly reduce cutaneous blood flow and conserve heat. Erector pili muscles in mammals adjust the angle of hair shafts to change the degree of insulation provided by hair or fur.
- Control of evaporation: The skin provides a relatively dry and semi-impermeable barrier to reduce fluid loss.
- Storage and synthesis: Acts as a storage center for lipids and water

SKIN AGEING[13]

- Skin ageing is the result of continual deterioration process because of damage of cellular DNA and protein.
- Ageing process is classified into two distinct type, i.e. " sequential skin ageing" and "photo-ageing".
- Sequential skin ageing is universal and predictable process characterized by physiological alteration in skin function. In the ageing process keratinocytes are unable to form a functional

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- stratum corneum and rate of formation from neutral lipids slows down, resulting in dry pale skin with wrinkle.
- In contrast, photo ageing is caused by caused by over exposure to UV rays from sunlight. It is characterized by dry, pale and shallow skin, displaying fine wrinkles as well as deep furrows caused by the disorganization of epidermal and dermal components associated with elastosis and heliodermatitis.
- Skin ageing is influenced by many factors including ultraviolet radiation (UV), excess alcohol consumption, tobacco abuse and environmental pollution. Combined, these factors lead to cumulative deterioration in skin appearance and function.
- Tissue homeostasis generally declines with age, in part because stem/progenitor cells fail to self-renew or differentiate. In the skin of mice, mitochondrial oxidative stress can promote cellular senescence and aging phenotypes. Ordinarily mitochondrial superoxide dismutase (SOD2) protects against oxidative stress. Using a mouse model of genetic SOD2 deficiency, it was shown that failure to express this important antioxidant enzyme in epidermal cells caused cellular senescence, nuclear DNA damage, and irreversible arrest of proliferation of a fraction of keratinocytes.[13,14]
- Skin aging is caused in part by TGF-β, which reduces the subcutaneous fat that gives skin a pleasant appearance and texture. TGF-β does this by blocking the conversion of dermal fibroblasts into fat cells; with fewer fat cells underneath to provide support, the skin becomes saggy and wrinkled. Subcutaneous fat also produces cathelicidin, which is a peptide that fights bacterial infections
- Anti-aging creams are predominantly moisturizer-based cosmoceutical skin care products marketed with the promise of making the consumer look younger by reducing, masking or preventing signs of skin aging. These signs are laxity (sagging), rhytids (wrinkles), and photoaging, which includes erythema (redness), dyspigmentation (brown discolorations), solar elastosis (yellowing), keratoses (abnormal growths), and poor texture.

Benefits of anti-ageing cream

- ✓ Improves complexion
- ✓ Anti aging cream keeps wrinkles at bay and adds a natural glow to your skin, and reduces fine lines
- ✓ Smoothens wrinkles
- ✓ Rich with natural oils, herbs and fruit extracts, and the cream works gently on the skin.
- ✓ Moisturizers and hydrates ageing and sun damaged skin
- ✓ Neutralizes environmental damage
- ✓ The cream visibly reduces wrinkles and neutralizes environmental damages to give back that youthful suppleness.

Composition of anti-aging cream formulation containing liquorice extract[2,3,4]

Sr no.	Ingredients	F1	F2	F3	F4	F5
1	Liquorice	1	1.5	0.5	2.5	2
Phase A:-Oil phase						
2	Stearic acid	25	25	25	25	25
3	Cetyl alcohol	5	5	5	5	5
4	Orange oil	12.5	12.5	12.5	12.5	12.5
Phase B:- Aqueous phase						
5	Triethanolamine	5	5	5	5	5
6	Glycerin	10	10	10	10	10
7	Tween 80	10	10	10	10	10
8	Polyethylene glycols	10	10	10	10	10
9	Sodium benzoate	1.25	1.25	1.25	1.25	1.25
10	EDTA	0.1	0.1	0.1	0.1	0.1
11	Rose water	15	15	15	15	15
12	Distilled water	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100

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LIQOURICE:

Synonym-

Mulaithi, Madhuka, Yastimadhu, Sweet wood, Glycirrhiza

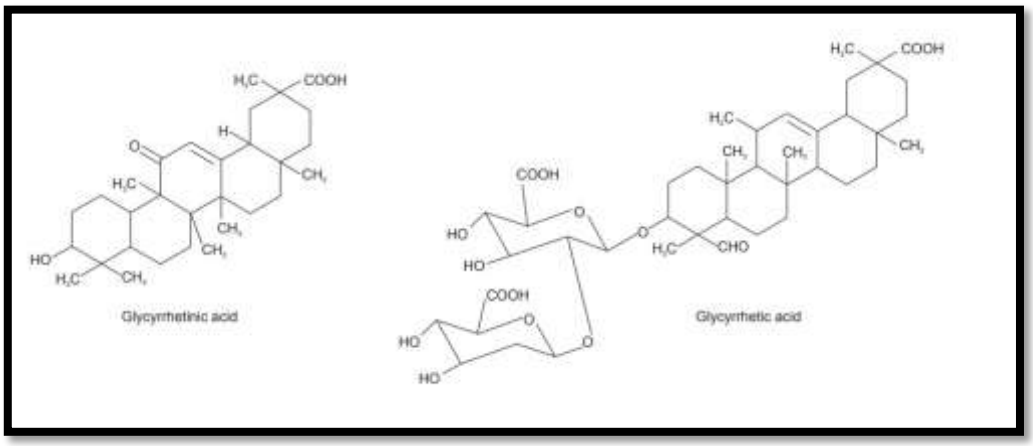
Biological source-

Liquorice consists of subterranean peeled and unpeeled stolons, roots and subterranean stems of Glycyrrhizaglabra Linn, and other species of Glycytrhiza, belonging to family Leguminosae.

Chemical Constituents-

The chief constituent of liquorice root is Glycyrrhizin (6–8%), obtainable in the form of a sweet, which is 50 times sweeter than sucrose, white crystalline powder, con-sisting of the calcium and potassium salts of glycynhizic acid. Glycyrrhizic acid on hydrolysis yields glycyrrhetic or glycyrrhetinic acid.

Structure :



Morphology[12]

Color: Unpeeled Liquorice-Externally, yellowish brown or dark brown; and internally, yellowish color.

Odour: Faint and characteristic.

Taste: Sweet.

Size: Length = 20 to 50 cm; Diameter = 2 cm.

Shape : Unpeeled drug--Straight and nearly cylindrical.

Peeled drug- Mostly angular.

Fracture: Fibrous in bark; and splintery in the wood.

fig. liquorice roots and its extract powder.

Procedure for preparation of anti-aging cream containing liquorice.[3,14,15]

An anti-aging cream contains an aqueous phase and oil phase. Oil phase like (stearic acid an emulsifier and other oil miscible components orange oil and cetyl alcohol) was mixed and heated upto 75℃. Components of aqueous phase (phase B) mixed together and warmed to about the same

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temperature of an oil phase. After heating, the aqueous phase and oil phase were mixed by continuous stirring in a magnetic stir. The therapeutically active ingredient (liquorice extract) and preservative sodium benzoate were added after cooling to 40℃. perfume was added.

EVALUATION OF ACTIVE BASE FORMULATION[3,14,15]

1. Determination of physical parameters of prepared cream[

Determine the parameter like color, odour, state, texture, etc of the prepared cream containing active ingredient liquorice**.**

2.Ph of the cream[3]

The ph meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50ml of distilled water and its pH was measured.

3.Viscosity[3,14]

A 100gm of each formulation was weighed and transpired to beaker and the viscosity of formulation was determined with the help of Brookfield viscometer (DV ll+ Pro model) using spindle number S64 at a 20rpm at a temperature of 25□C. The determination was carried out in triplicate and the average of three readings was recorded. Viscosity of formulation was determine using the formula-

Viscosity (cp) = digital reading * factor

4.Acid value[3,4,7]

Take 10gm of substance dissolved in accurately weighed, in 50ml mixture of equal volume of alcohol and solvent ether, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely, to this 1ml of phenolphthalein added and titrated with 0.1N NaOH, until faintly pink color appears after shaking for 30seconds.

Acid value = n*5.61/w

n = the number of ml of NaOH required , w = the weight of substance

5.Saponification value[3]

Introduce about 2gm of substance refluxed with 25ml of 0.5 N alcoholic KOH for 30minutes, to this 1ml of phenolphthalein added and titrated immediately, with 0.5N HCL.

Saponification value =(b-a)*28.05/w

The volume in ml of titrant = a

The volume in ml of titrate = b

The weight of substance in gm = w

6.Irritancy test[3]

Mark an area (1 sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals upto 24hrs and reported.

7.Accelerated stability testing[3]

Accelerated stability testing of prepared formulation was conducted at room temperature, studied for 7days. And then the formulation studied at 45℃ ± 1℃ for 20days. The formulations was kept both at room and elevated temperarure and observed on 0^{th}, 5^{th}, 10^{th}, 15^{th} and 20^{th} day for the all evaluation parameters.

8.Spreadability test[3,4]

Sample was applied between two glass slides and was compressed to uniform thickness by placing 100gm weight for 5minutes. Weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower slide was taken as measure of spreadability. It was calculated using the formula :

Spreadability = m*l/t

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken

9.Microbial growth test[3,13,14]

The formulated cream was inoculated on the plates of Muller Hinton agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed into the incubator and are incubated at 37□C for 24hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

10.Total antioxidants capacity[3]

Total antioxidant activity was estimated by phosphomolybdenum assay

Preparation of molybdate reagent solution

1ml each of 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate were added in 20ml of distilled water and made up volume to 50ml by adding distilled water

Procedure :-

Hydro alcoholic extract of sample formulation in different concentration ranging from 100µl to 500µl were added to each test tube individually containing 3ml of distilled water and 1ml of molybdate reagent solution. These tubes were kept incubated at 95□C for 90min. after incubation, these tubes were normalized to room temperature for 20-30min and the absorbance of

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the reaction mixture was measured at 695nm. Ascorbic acid was used as positive reference standard.

II. RESULT

Table.no.1 Determination of physical parameters of prepared cream

Sr.no	Properties	Observations
1	Color	Brown in color
2	Odour	Characteristic
3	State	Semi-solid
4	Texture	Smooth
5	Grittiness	No grittiness

Table no.2 Determination of pH

Sr.no	Days	Formulations				
		F1	F2	F3	F4	F5
1	Initial day	6.4	6.2	6.2	6.3	6.2
2	7 days	6.2	6.3	6.5	6.2	6.4
3	15 days	6.3	6.4	6.5	6.2	6.1
4	21 days	6.3	6.3	6.5	6.3	6.3
5	30 days	6.2	6.4	6.5	6.2	6.3

Table no.3 Viscosity

Sr. no	Days	Formulations				
		F1	F2	F3	F4	F5
1	Initial day	21530	22500	23550	25590	25534
2	7 days	23520	25580	25580	27050	27059
3	15 days	24550	27540	27590	27062	27070
4	21 days	25533	28540	28550	27075	27068
5	30 days	25580	29550	29590	27080	27085

Table no.4 Acid value and saponification value

	AF1	AF2	AF3	AF4	AF5
Acid value	6.1	5.7	5.3	6.2	5.4
Saponification value	27.1	27.3	26.3	25.8	26.8

Table no..5 Irritancy test

Formulation	Irritant effect	Erythema	Edema
AF1	NIL	NIL	NIL
AF2	NIL	NIL	NIL
AF3	NIL	NIL	NIL
AF4	NIL	NIL	NIL
AF5	NIL	NIL	NIL

Table no.6 Accelerated stability testing

Sr. no	Evaluation parameters	AF4	AF
		Room temperature	Accelerated conditions (45℃)
1	Appearance	Good	Good
2	Colour	Light brown	Light brown
3	pH	6.4	6.4

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4	Consistency	Semisolid & soft	Semisolid & soft
5	Viscosity	25590 cps	25595 cps
6	Spreadability	Excellent	Excellent
7	Washability	Excellent	Excellent
8	Irritancy test	No irritation was observed	No irritation was observed

Table no.7 Spreadability test

Parameters	**Formulations**				
	F1	**F2**	**F3**	**F4**	**F5**
Spreadability	26.33± 0.3	24.47 0.4	22.35 0.5	21.83 0.6	23.76 0.2

Table no.8 Antimicrobial evaluation of clindamycine, anti-aging cream and Normal saline

Sr.no	**Zone of Inhibition**		
	Marketed formulation (Clindamycine)	**Herbal Preparation (anti-aging cream)**	**Control (Normal saline)**
F1	35	36	No Zone of Inhibition
F2	36	35	No Zone of Inhibition
F3	34	33	No Zone of Inhibition
F4	35	32	No Zone of Inhibition

Table no. 9 Total antioxidants capacity

PM Assay	**Absorbance at 695nm**	
	AF4	**AF5**
100	0.362 ±0.02	0.143±0.01
200	0.753±0.05	0.333±0.04
300	1.124±0.03	0.529±0.02
400	1.451±0.03	0.871±0.02
500	1.764±0.07	1.135±0.01

III. CONCLUSION

From this research, it is clear that anti-ageing cream plays a vital role in prevention of ageing and also reduces wrinkles and dark spots. The anti-ageing factor is probably due to glycyrrhizin present in liquorice root extract (Powder) which is a very potent antioxidant. When used topically, the antioxidants protect skin against damage from the sun's UV rays and free radicals. This in turn, prevents premature ageing.

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