Formulation And Evaluation of Polyherbal Anti-aging Cream Containing Extracts of *Moringa oleifera* & *Withania somnifera*

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***Abstract*—Moringa oleifera and *Withania somnifera* has been reported to possess antioxidant and anti-inflammatory properties. *Moringa oleifera* and *Withania somnifera* extracts have been used to treat antimicrobial infections. The aim of this present study is to formulate and evaluate of polyherbal anti-aging cream by combining the extract of *Moringa oleifera* with *Withania somnifera* to achieve multipurpose skin effects such as anti-aging, fairness, softening and antiseptic effects. The polyherbal anti-aging cream formulations comprising of hydroalcoholic extract of *Moringa oleifera* and *Withania somnifera*, Carbopol 940, xanthan gum, stearic acid, glycerol monostearate and Acetyl alcohol were prepared and evaluated for physicochemical parameters and the results showed the production of stable polyherbal anti-aging cream. The formulated polyherbal anti-aging cream (O/W) was subjected to characterize like visual inspection, pH, viscosity, good spread ability, good consistency, homogeneity, no evidence of phase separation and ease to washable from skin. Formulation (F4) exhibited fulfilled the objectives of the current research and % drug release is 98.78 and exhibit good anti-microbial activity.**

***Index Terms*—antioxidant, anti-inflammatory, antimicrobial, polyherbal, anti-aging.**

I. INTRODUCTION

Skin aging is the result of a continual deterioration process because of damage to cellular DNA and protein. The ageing process is classified into two distinct types i.e. “Sequential Skin Aging” and “Photo Aging”. Both types have distinct Clinical and Historical features. Sequential Skin Aging is the universal and predictable process characterized by physiological alteration in skin function. In the aging process keratinocytes are unable to form a functional stratum corneumand rate of formation of neutral lipids slows down, resulting in dry and pale skin with wrinkle.In contrast, Photo Aging is caused by over exposure to UV rays from sunlight. It is characterized by dry, pale and sallow skin, displaying fine wrinkles as well as deep furrows caused by the disorganization of epidermal and dermal components associated with elastosisand heliodermatis. Herbs and plants have already proved useful as tool in complementary medicine. [1,2] Cosmetic products are used to protect against exogenous and endogenous harmful agents, and enhance the beauty and attractiveness of skin. The use of cosmetics not only developing an attractive external appearance, but towards achieving longevity of good health by reducing skin disorders. The synthetic or natural ingredients present in a skin care formulation that supports the health, texture, integrity of skin, moisturizing, maintaining the elasticity of skin by reduction of type I collagen, photo protection etc. This property of cosmetics is due to presence of ingredients in skin care formulations, because it helps to reduce the production offree radicals in the skin and manage the skin properties for a long time. The cosmetic productsare the best choice to reduce skin disorders such as hyperpigmentation, skin ageing, skin wrinkling, rough skin texture etc. Between 2010 and 2050 the number of older individuals werein a smaller amount in the developed countries which expected to extend upto 250% comparedto a 71% increase in developing countries. The world's population of people of 60 years of age and older is expected to increase by 22% from 10%

(from 800 million to 2 billion) by 2050. Almost, one-fourth person would be older than the age of 60. It has been estimated that over 8%of the population of the Southeast Asian regions are above the age of 60 years [2].

Skin ageing is a process in which skin quality deteriorates with age due to the synergistic effects of chronological ageing, photo-ageing, hormonal deficiency and environmental factors [3]. In skin ageing, there is a reduction in the number of fibroblasts that synthesize collagen and vessels that supply the skin which leads to an increase in laxity and hence forms wrinkles [4]. The sun- exposed skin gradually leads to sagging of skin. This leads to the loss of fibril and collagen type VII (Col-7) that retards the bond between epidermis and dermis leading to extrinsically aged skin [5]. Many theories of ageing have been proposed including DNA or genetic theory, free radical theory, neuroendocrine theory, membrane theory, Hayflick limit theory, telomerase theory and mitochondrial decline theory. In intrinsically aged skin, the histological changes occur within the basal cell layer due to the internal influences which produce skin sagging, thinning of skin [6], while in extrinsically sun-exposed aged skin due to the accumulation of abnormal elastic tissue in the mid and deep dermis, the solar elastosis or UV-irradiation, increase in elastin promoter activity which induces the elastin gene transcriptional activity and decreases the fibrillin-1 expression resulting in heavy deposition of elastic fibres, which are dystrophic and shorten. If the lysine derived cross-linked compound increases, then the photoaged skin is confirmed [7].
Generally, there are four types of skin ageing that occurs which include intrinsic ageing and characterized by unblemished, smooth, pale(r), drier, less elastic skin with fine wrinkles [8, 9] and occurs within the tissue itself *via* reductions in dermal mast cells, fibroblasts, collagen production and extrinsic ageing which can be caused due to extreme exposure to sun (*i.e*., photo-ageing) and various exogenous factors such as pro-oxidant and antioxidant influences on cell turnover via neuro-endocrine-immune biological response modifiers [10] which affects mainly the face and neck. The third one is photo-ageing which is caused by sunlight which comprises mostly infrared (52-55%), visible (44%) and 3% UV light, which is harmful to the skin and is completely absorbed by the ozone layer [11]. Hormonal ageing is caused by decreased collagen synthesis, skin thickness, skin hydration and epidermal barrier function [12, 13]. Scientific literature suggests various molecular basis that fully justifies the mechanismof skin pathophysiology for skin ageing. This process may be explained by the theory of cellular senescence or deprive the cellular DNA repair capacity and loss of telomeres, point mutations of extranuclear mitochondrial DNA, oxidative stress, or the abnormalities exist in chromosomes, single-gene mutations, reduced sugar, chronic inflammation, and many more. Research predicts that generally extrinsic factors are the main cause of skin ageing and only 3% of ageing factors contributed by intrinsic factors.

1.1 Mechanism of Ageing at the Cellular and Molecular Level

The cell culture technique and molecular biological technique are modern analytical tools. The analysis of ageing at molecular level of cell culture was undertaken in 1920 by Careel, who performed an experiment on the basis of chick heart fibroblast in which cells could divide indefinitely [17]. When they were removed from *in vivo* mechanism and then placed *in vitro* environment, they became immortal. In 1960, Hayflick demonstrated that normal human fibroblast has limited capacity to proliferate *in vitro* [18]. He showed that normal cells could become double at the infinite number of times and after the phase of exponential growth, theystop dividing which is known as Hayflick limit. Ageing is associated with two overlapping processes which led to death one is progressive degeneration of cell and another one is Loss of regeneration capacity.

The muscle regenerative potential decline due to changes in satellite cells which further leads to depletion of proliferative and myogenic capacities. As the skin ageing phenomenon starts, the regenerative ability of skin muscles lessens down due to the gradual decrease in satellite cells, muscle stem cells, which undergo age-related declines in proliferative and myogenic capacities. Indeed, satellite cell numbers decline gradually in mammalian muscles with advancing age [19].

The proliferative degeneration and regeneration of

cells are constant under normal conditions at each stage of life. However, in mitotic homeostasis there occurs replacement of damaged cells and preservation of functional integrity of cells as well as tissues. The mechanism of degeneration is due to the generation of Reactive Oxygen Species (ROS) and non- enzymatic glycosylation of proteins with loss of proliferative and regenerative telomerase shortening and apoptosis. This simplifies the role of both exogenous and endogenous factors in terms of theageing process [20].

1.2. Glycation in Ageing

Age-related cell degeneration involves the accumulation of advanced glycosylation endproducts *i.e.*, Advanced Glycation End-products (AGE) [21]. Production of AGEs on collagen leads to cross-linking such as the expansion of molecular packing, abnormalities in the extracellular matrix, impaired cell-matrix interaction. AGEs also bind to specific receptors on immune cells which triggers the release of inflammatory mediators and generation of ROS leads to increase in the production of AGEs damage.

1.3 Free Radical in Ageing

In the Free Radical Theory of Ageing (FRTA), the involvement of free radicals in endogenous metabolic reactions, was proposed in 1954 [22]. The FRTA suggests that the common ageing process is the initiation of free Radical Reactions (RRs). After that some suggestions were included such as (a) Most free radical reactions were initiated by the mitochondria at an increasing rate with age, (b) The life span is determined by the rate of free radical damage to the mitochondria [23]. It became clear that improvements in general living conditions increasedAverage Life Expectancies at Birth (ALE-B) by decreasing the free radical reactions associated with suboptimal living conditions or by decreasing the free radical reactions associated with suboptimal living conditions or decreasing (a) The chain lengths of free radical reactions *e.g.,* with antioxidants such as vitamin E (b) Their rates of initiation may be depressedby minimizing copper, iron, and other oxidant catalysts, can lower the rate of formation of ageing changes, and decrease the rate of ageing and of disease pathogenesis [24].

Any molecule that possesses a free electron and is highly reactive is known as “free radical”. The reduced components of the ancient oxygen-free atmosphere by free radical reactions, initiated mainly by ionizing radiation from the sun. The products of these reactions, including simple self-replicating progenitors of DNA, reflected the innate chemical properties of the atoms and molecules. The free radical reactions also provide compounds in the environment necessary for the survival and growth of the first protocells, and to produce more or less random free radical reactions mediated changes throughout the cells. Some changes were inheritable, improving the ability of the offspring to survive and function, *i.e.,* to evolve, whereas others were ageing changes that led to cell death (Fig. 22). The intrinsic ageing process due to age- inducing free radical reactions going on continuously throughout the cells and tissues. In the beginning, the reactions were largely initiated by UV radiation from the sun and, to a lesser extent, by volcanic activity, and now almost all arise endogenously from enzymatic and non-enzymatic free radical reaction [25].

1.4 Cell Cycle and Ageing

Atrophy of organ or tissue is a the well-recognized feature of ageing which can destroy genes to build up organ due to poor circulation, loss of hormonal support, decrease inthe physical activity and less active lifestyle. In recent years, the mechanism of ageing is due to reduced regenerative capacity which involved two processes to cell cycle control which include Regulation of cell proliferation and inductionof proliferation and programmed cell death [26]. Thus, the death of cell initiated mitotic cycle in which cell division produces two daughter cells whose fate will be different in which cells, they are sometimes called stem cells which are tightly controlled by specific regulatory protein. Other alteration in Rb (retinoblastoma) pathway involves the systemof cyclin, (CDK), and CDK inhibitors (CDI) whereas the process of phosphorylation depends on the activity of kinase CDK4 and CDK6 and these kinases are controlled by CDK inhibitors exfoliated by p21WAF1 protein and p21WAF1 is regulated by p53 which is a protein that guards the integrity of genome and promotes cell cycle in arrest to response DNA damage [27]. The replication of M1 barrier can be overcome by viral oncoproteins, such as SV -40T antigenor adenovirus E1A. By

neutralizing the function of p53 and pRb, these stimuli cells are continuously dividing until they reach a state of crisis (M2). At this stage, chromosomal fusion breaks lead to cell death.Telomerase is DNA fragments that form ends of chromosomes extending up-to 12,000 base pair which guards chromosome stability and protect them from degradation and rearrangement. In normal somatic cells, telomerase is shortened during DNA replication at 100 base pairs per cell division due to DNA polymerase cannot fully replicate 3'end of linear DNA called 'end –replication problem. The shortening of telomerase decreases as human fibroblast divides, leading to wrinkle formation. The shortened telomerase damages DNA that initiates p53 andpRB pathways [28]. The shortening of telomerase in ageing leads to the contribution of apoptosis, which in the aged closely related to cell cycle regulation with a link provided by p53 protein which has capacity of inducing apoptosis when DNA damages and these damages accumulate during ageing [29].

Cream formulation was semisolid formulations intended for topical application. The cream formulations were prepared by using various herbal extracts, herbal oils, and various excipients. There are two main types of cream formulation, such as oil in water (O/W) type of emulsion and water in oil (W/O) type of emulsion. The present formulation was oil in water (O/W) type of emulsion. The cream formulation was various other classes like foundation cream, cleansing cream, cold cream, pain-relieving cream, night and massage cream, head and body cream, vanishing cream and shaving cream. [2,3]

II. MATERIALS & METHODS

Leaves of *Moringa oleifera* and roots of *Withania somnifera* were collected and herbarium specimen was authenticated by Dr. Vijay Kumar Mastakar, Scientist in charge, Acharya Jagdish Chandra Bose Indian Botanic Garden Botanical Survey of India, P.O. Botanic Garden, Howrah: 711103. Leaves of *Moringa oleifera*, family Moringaceae and roots of *Withania somnifera*, family Solanaceae were washed with tap water and shade dried. Shade dried material was placed into a blender to be groundedinto powder and stored in air tight container for further use. The powder was macerateused ethanol and water for ratio 70:30v/v. Filtered with muslin cloth and filtrate was allowed to centrifuge at 2000rpm at 25◦C. The supernatant was collected and incorporated in the cream base formula Table
(1). Carbopol 940, Xanthan Gum, Cetyl alcohol, Glycerol monostearate, Stearic acid, ethylalcohol, methyl paraben, was purchased from Loba chemicals. [3,4,5]

Method of preparation Polyherbal antiaging cream [6]
In China dish Carbopol 980 and Xanthan gum was dissolved in water phase. This liquid dispersion shows slightly sweeling. Then add extract of *Moringa oleifera* and *Withania somnifera* in above liquid dispersion. In another China dish melt oil phase Cetyl alcohol, Glycerol monostearate and Stearic acid by using heating mantle. To this mixture (oil phase) above liquid dispersion (water phase) was added. Then methyl paraben was added. Triturate all above ingredients for 30 min. Finally, poly herbal anti-aging creamwas formed.

Characterization & Phytochemical Evaluation in Hydroalcoholic Extract
Various tests were performed, as mentioned in Table (2) to identify the phytoconstituents present in the products and their effect is shown on the body. Every plant exhibits certain phytochemical properties which show a number of beneficial effects. [4,7,10,14]

Characterization of Formulated Polyherbal Antiaging Cream [8,9,11]
Visual appearance: The physical appearance of prepared formulation was evaluatedfor their state, colour, odour and texture. All evaluations were reported in table (6).
pH: The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0ml of distilled water and its pH was measured by using pH meter and results were noted in table (6).
Homogenicity: The formulation was tested for the Homogeneity by visual appearance and by touch and results were noted in table (6).
Viscosity: The viscosity of the cream was determined by using Brookfield Viscometer LVDV. The viscosity of cream was measured at room temperature.

Extrudability: Extrudability became based totally upon the quantity of the cream extruded from collapsible tube on software of positive load. More the amount of cream extruded suggests better extrudability. It became determine by way of making use of the weight on cream stuffed collapsible tube and recorded the burden on which cream turned into extruded from tube and results were noted in table (6).

Spreadibilty: Spreadibilty of system is essential to provide sufficient dose to be take in from pores and skin to get properly healing response. An apparatus where in as lid fixed on wooded block and top slide has movable and one cease of movable slide tied with weight pan. The formulation of creams result was noted in table (6).

Washability: The removal of the cream applied on skin was done by washing under tap water with minimal force to remove the cream and results were noted in table (6).

Irritation test: Cream shows no redness, irritation, edema, inflammation during irritation studies. These formulations are safe to use for skin and results were noted in table (6).

III. RESULTS

Table (1): Composition of formulations of Polyherbal anti-aging cream**.**

Composition	F1% w/v	F2 % w/v	F3% w/v	F4 % w/v	F5 % w/v	F6 % w/v
Moringa oleifera	0.25	0.25	0.25	0.25	0.25	0.25
Withania somnifera	0.25	0.25	0.25	0.25	0.25	0.25
Carbopol 940	0.25	0.5	1	0.25	0.5	1
Xanthan Gum	0.25	0.25	0.25	0.5	0.5	0.5
Cetyl alcohol	1	1	1	1.5	1.5	1.5
Glycerol monostearate	1	1	1	1	1	1
Stearic acid	3	3.5	4	3	3.5	4
Methyl paraben	0.21	0.21	0.21	0.21	0.21	0.21
Distilled Water	q.s	q.s	q.s	q.s	q.s	q.s

Table (2): Phytochemical screening of *Moringa Oleifera* leaves and *Withania somnifera* Roots.

S. No.	Test	Hydroalcoholic extract of *Moringa oleifera*	Hydroalcoholic extract of *Withania somnifera*
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	+Ve +Ve	+Ve -Ve
2.	Glycosides A) Legal's Test:	+Ve	-Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	+Ve +Ve	+Ve -Ve
4.	Saponins A) Froth Test:	+Ve	-Ve
5.	Phenolics A) Ferric Chloride Test:	+Ve	+Ve
6.	Proteins A) Xanthoproteic Test:	+Ve	+Ve
7.	Carbohydrate A) Fehling's Test:	+Ve	+Ve

8.	Diterpenes A) Copper acetate Test:	+Ve	-Ve
9.	Tannins A) Gelatin Test	-Ve	+Ve

Table (3): Media preparation (broth and agar media).

Agar	1.5 gm
Extract of beef	0.3 gm
Peptone	0.5 gm
Sodium chloride	0.55 gm
Distilled water	100 ml
pH	7

Table (4): Antimicrobial activity of standard and optimized polyherbal antiaging cream against *S. aureus* and *E. coli.*

S. No.	Drug/ Formulation	*S. aureus*	*E. coli*
		Zone of inhibition (mm)	
1.	Standard	13±0.47	13±0.47
2.	Cream 0.5%	10±0.47	11±0.47
3.	Cream 0.25%	8±0.47	7±0.47

Figure (1): Antimicrobial activity of standard and optimized formulation polyherbal antiaging cream against *S. aureus* and *E. coli.*

Table (5): *In-vitro* drug release study of optimized batch (F4).

S. No.	Time (hrs)	Cumulative % drug release
1	0.5	16.65
2	1	35.56
3	1.5	45.65
4	2	55.89
5	2.5	68.78
6	3	73.32
7	3.5	82.23
8	4.0	98.78

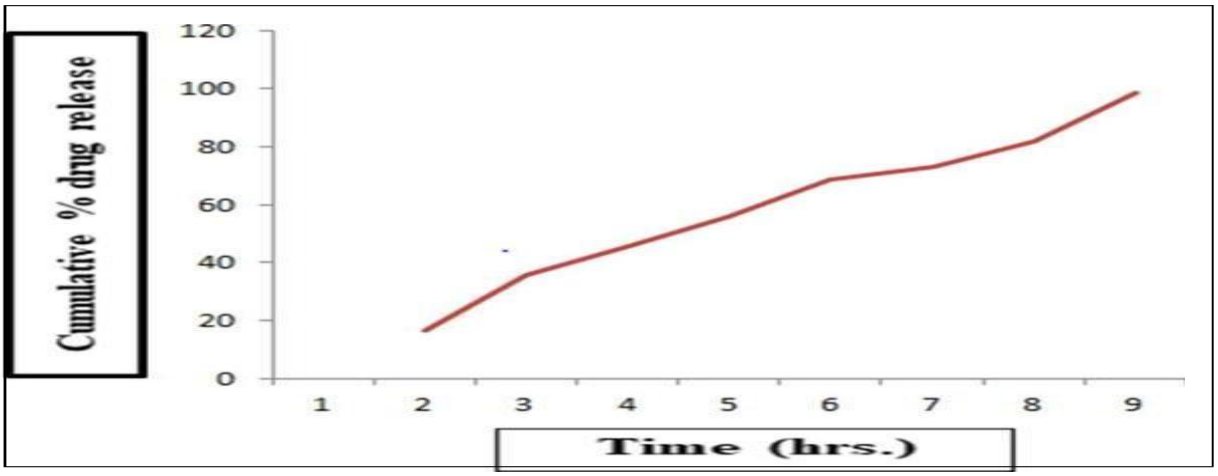

Figure (2): In-vitro drug release study of optimized batch (F4).

Table (6): Characterziation of (F4) Polyherbal Antiaging Cream.

S. No.	Parameters	Observations
1.	State	Semisolid
2.	Color	White
3.	Texture	Smooth
4.	pH	7.0±0.2
5.	Homogenicity	Good
6.	Viscosity	35200±41 cp`
7.	Extrudability	135±4 g
8.	Spreadibility	10.23±0.32 (g.cm/sec)
9.	Washability	Easily and readily washable
10.	Irritation test	No harmful effects on skinand eyes
12	*In vitro* % drug release	98.78

Figure (3): Polyherbal Antiaging Cream.

IV. DISCUSSION

Antimicrobial activity of optimized batch F4 of cream [8,12,13]

The flask containing medium become cotton plugged and turned into placed in autoclave for sterilization at 15 lbs /inch2 (121°C) for 15 min. After sterilization, the media in flask turned into immediately poured (20 ml/ plate) into sterile Petri disheson aircraft surface. The poured plates had been left at room temperature to solidifyand incubate at 37°C in a single day to test the sterility of plates. The plates had been dried at 50°C for half-hour earlier than use. The microbial cultures used within the take a look at had been obtained in lyophilized shape. With the help aseptic techniques, the lyophilized cultures are inoculated in sterile potato dextrose broth than incubated for 48 hours at 28°C. After incubation the increase is observed in the shape of turbidity. These broth cultures had been similarly inoculated on to the nutrient and potato dextrose agar plates with loop full of microbes and similarly incubated for subsequent 24 hours at 37°C to gain the pure way of life and stored as stocks whichcan be for use in addition

research paintings. The properly diffusion method changed into used to determine the antimicrobial activity of poly herbal antiaging cream organized from *Moringa oleifera*, and *Withania somnifera* using popular procedure. There were 2 concentrations used that are 0.5 and 0.25 % poly herbal antiaging creamin antibiogram studies. Its essential feature is the setting of wells with the antibiotics at the surfaces of agar without delay after inoculation with the organism tested. Undiluted overnight broth cultures need to in no way be used as an inoculum. The plates were incubated at 28°C for 48 hr. After which tested for clear zones of inhibition around the wells impregnated with particular attention of drug.

In-vitro drug release study [8,15]

In vitro release of the drug can be performed by diffusion flask method. Here egg membrane is used as a biological membrane. The franz diffusion mobile has receptor compartment with an effective extent about 60 ml and effective floor area of permeation 3.14 cm^2. The egg membrane is set up among the donor and the receptor compartment. A 2 cm^2 length patch taken and weighed then positioned one side of membrane dealing with donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is rounded by using water jacket with a view to preserve the temperature at 32 ±0.5°C. Heat is furnished using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by means of teflon coated magnetic bead that's positioned within the diffusion cell. During every sampling programming language, samples are withdrawn and replaced with the add of same volumes of clean receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 298 nm.

V. CONCLUSION

The main purpose behind this investigation was to develop polyherbal antiaging cream using extracts of *Moringa oleifera* leaf and *Withania somnifera* root was formulated based upon traditional knowledge and emphasis was to formulate a stable and functionally effective polyherbal antiaging cream by excluding all types of synthetic additives. As seen from the results, it is possible a natural herbal cream by using *Moringa oleifera* leaf and *Withania somnifera* root which has antimicrobial activity that is better approach with respect to various cream having synthetic chemicals as antimicrobial agents. The results showed that the formulation F4 of polyherbal antiaging cream contains all good characters of an ideal creamit was found to be harmless, more effective, ease to manufacturing and economical comparedto synthetic antiaging cream.

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