



PRODUCTION OF ANTI-AGING COSMETICS CONTAINING AMLA FRUIT AND BETEL LEAF EXTRACT

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Abstract: Tannins are polyphenolic biomolecules having an astringent property which is useful for the anti-aging effect on skin by protein precipitation mechanism. That is how the tannin shows anti-bacterial activity by the same astringent property which induces the cell aggregation of cell membranes. In our present study, the tannin is collected from two natural sources – Betel leaf (*Piper betle*) and amla fruit (*Phyllanthus emblica*). The aqueous extracts of both are evaluated by phytochemical screening and microbiologically tested through the disc-diffusion method. The combined effect of both extracts shows better results and with that three cosmetic formulations – Toner, Gel (for oily skin), and Cream (for dry skin) are prepared. As the extracts are positively showed antimicrobial activity so that the formulations can be claimed as anti-aging cosmetics.

Key-words: - Amla extract, Anti-aging, Betel leaf extract, Cosmetics, Cream, Gel, Tannin, Toner.

I. INTRODUCTION

According to EU Cosmetic Directory, Article 7a; 6th Amendment (1993) a cosmetic can be defined as any substance or preparation intended to be placed in contact with the various parts of human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view to exclusively or mainly to cleaning them, perfuming them or changing their appearances and/or correcting body odour and/or protecting them or keeping them in good condition. The following study encompasses the formulation of three anti-aging products- toner, gel and cream.

Here the preparation of these cosmetics encompasses for providing an anti-aging treatment via proper skin care regime catered with some herbal cosmetics. Being a voluminous organ, our skin is susceptible to aging process and from the nascent stage of life but the exhibition of aging symptoms increases by the time. The cutaneous aging is augmented by some intrinsic and extrinsic factors. Thinned stratum corneum, dry skin, fine wrinkles, pigmentation and gradual dermal atrophy epitomize the intrinsic aging process or cellular senescence. Whether the extrinsic aging yields rough, dry skin, scaling (Xerosis) , freckles, thickened stratum corneum, solar elastosis (elastic fibers are increased in number, clumped and disoriented), degradation of collagen(specifically type VII collagen in keratinocytes) and lesions can also be found there like precancerous (Seborrhoeic and solar keratoses) or cancerous (melanomas and carcinomas). Mainly this extrinsic factor induced aging is caused by long-term exposure to solar ultraviolet radiation (about 80% of facial aging). It may also indulged by air pollution, smoking or poor nutrition.

The reactive oxygen species (ROS) plays a crucial role in this aging process. Which can be produced by mitochondrial electron transport change, peroxisomal and endoplasmic ER localized proteins, Fenton reaction and enzymes like cyclooxygenases, lipoxygenases, xanthin oxidases and nicotinamide adenine dinucleotide phosphate (NADP)oxidizes. The cellular chromophores absorb the energy from UV rays and produced ROS which inhibits the activity of receptor protein tyrosine phosphatases (RPTPs) by attacking the cystein residue of the active site of RPTP. This elevates the level of phosphorylated RTKs and ignites the downstream signaling pathways including the activation of mitogen-activated protein kinase (MAPK) and subsequent nuclear factor and transcription factor activator protein repress collagen production which yields an aged skin.

Young and beautiful appearance of skin has a positive impact over people's sociological and reproductive (romantic attraction from psychological aspect) status. To care this type of skin someone needs to be careful about the choice of cosmetics and their proper application. A balanced diet, abstinence from smoking and alcohol consumption give an excellent kick start to the skin care regime. Firstly, the cleansing of skin is very important which removes the dirt, dust, loose corneocytes and microorganisms. The cleansers must have mild surfactants with refatting properties (for the prevention of dryness). Then comes toners, which exerts deep cleansing, removes remnants of dirt and cleanser. An astringent toner is extremely beneficial for the aged skin and oily skin (helps to remove excess sebum). After that application of moisturizer or protective product is required which will show the desired anti-ageing activity. For dry skin an oil-based product is required such as cream and for the dry skin gel preparation is needed which will not make the skin oily further.

Following study encompasses that the aqueous extracts of betel leaf (*Piper betle*) and amla fruit (*Phyllanthus emblica*) show a good enough activity for anti-ageing treatment.

II. MATERIALS AND METHOD:

Preparation of amla fruit extract:

Fresh amla fruits were collected from the market. Then they were subjected to be washed to remove dirt and sliced. Finally, sliced amla fruits were shed-dried. 100gm of dried amla fruits were taken into a beaker containing 500ml distilled water. The mixture was left for 24hours with occasional stirring at room temperature. After 24hours the mixture was strained out with a fine sieve. The crude extract was then air evaporated for 3 days. The final product used as an aqueous extract of amla fruit.

Preparation of betel leaf extract:

Fresh betel leaves were purchased from local market. The leaves were washed and sliced into pieces. After that 100gm of leaves were taken into a beaker containing 300ml of distilled water. The mixture was heated at 60o c for 2hours with continuous agitation. Then the mixture is screened with a fine sieve. The final product is the aqueous extract of betel leaf.

Preparation of cosmetics:

Toner:

Prepared toner or astringent lotion follows a non-alcoholic formula-Polypropylene glycol- 3ml

Sodium pyrrolidone- 2ml

Amla fruit extract- 10ml

Betel leaf extract- 10ml

Methyl paraben- 0.5gm

Distilled water - up to 30ml. All the ingredients expect water were mixed with stirring and finally volume made up with water.

Gel:

A sophisticated gel for oily skin was prepared with following formula-

Carbopol 940- 1gm

Glycerin- 3ml

Sorbitol- 0.5gm

Methyl paraben- 1 gm

Amla extract -10ml

Betel leaf extract- 10ml

Triethanolamine – q.s. Carbopol 940 was hydrated with extracts. Preservative was dissolved into the glycerin then added into the Carbopol solution. Remaining ingredients were added to the mixture. Triethanolamine was added adequately to form a gel like consistency.

Cream:

An oil-in-water emulsion is designed to spread easily and rub into the skin quickly.

Vegetable oil- 6ml

Cetyl alcohol- 3gm

Glyceryl monostearate- 7gm

Triethanolamine- 1.4ml

Glycerol- 6ml

Amla fruit extract- 10ml

Betel leaf extract- 10ml

Methyl paraben- 0.5gm

FD&C color- 0.05gm

Rose oil- 0.5ml

Oil, Cetyl alcohol, monostearate and color were taken in a beaker(A). Extracts, glycerol, triethanolamine and methyl paraben were taken in another beaker(B). both beakers were placed over a water bath at 75oc temperature. Mixture of beaker A was added into beaker B with continuous agitation. When the cream cooled to 35oc rose oil is added. The resulting product intended for dry skin care regime.

III. PHYTOCHEMICAL SCREENING OF PREPARED EXTRACTS:

Table: 3.1: Descriptive results.

Tests	Amla fruit extract	Betel leaf extract	Remarks
Alkaloids 1. 2ml of Mayer's reagent (Potassium mercuric iodide solution) added to the 3ml of sample. 2. 2ml of Wagner's reagent (iodine- potassium iodide solution) added to the 3ml of sample. 3. 2ml of Dragendroff's reagent (potassium bismuth iodide solution) added to the 3ml of sample.	No cream-colored precipitate observed. No reddish-brown colored precipitate observed. No reddish-brown colored precipitate observed.	No cream-colored precipitate observed. No reddish-brown colored precipitate observed. Brown colored precipitate observed.	Amla fruit extract may not contain any alkaloid. Betel leaf extract may contain alkaloids.
Amino acid 1. 2ml of Ninhydrin solution added to the 3ml of sample then boiled.	No violet color has seen.	No violet color has seen.	Both the extracts may not contain any amino acid.
Flavonoid 1. A small strip of magnesium turning added to the sample then concentrated hydrochloric acid added to that dropwise.	No pink, red, blue or green color have seen.	No pink, red, blue or green color have seen.	Both the extracts may not contain any flavonoids.
Tannin 1. 2ml of ferric chloride solution to the 3ml of sample. 2. 1ml of 1% gelatin solution added and followed by addition of 10% sodium chloride solution.	Blue color has observed. Milk white precipitate has formed.	Blue color has observed. Milk white precipitate has formed.	condensed tannin may present.
Saponin glycoside 1. 2ml of sample added to the test tube. The sample has shaken.	No Froth is formed.	Froth is formed.	Betel leaf extract may contain saponin glycosides.
Starch 1. 2ml of aqueous solution of iodine added to the 3ml of sample.	Violet color has seen.	No violet or blue color have seen.	Amla fruit extract may contain starch (Amylum).

Table 3.1: displayed the detailed experiments to identify the chemical constituents present at the extracts.

Chemical constituents:

Betel leaves contain tannins (ellagitannin, gallic acid), sugar and diastases and a vital oil, conjointly contain phenols chavibetol, chavicol that have powerful antiseptic properties. in conjunction with an alkaloid arakene.

Amla contains Emblicanin-A: twenty seventh percent, Emblicanin-B: twenty third percent, Punigluconin, Pedunculagin: fourteenth percent, Gallo-ellagitannoids: eighteenth percent, Rutin: 10%. The primary four ingredients listed are polyphenols (tannins); consecutive constituent may be a combination, primarily of acid and ellagic acid. Rutin is another phenolic resin compound, a typical flavonoid found in several plants.

IV. EVALUATION:**Disc Diffusion Method:**

- I. A culture of *Escherichia coli* is taken from the library.
- II. Then a sterile cotton swab dipped into the standardized bacterial suspension.
- III. The agar plate is inoculated by streaking with the swab containing the inoculum.
- IV. The plate is rotated by 60° and repeat the rubbing procedure. Repeated two times. This will ensure an even distribution of the inoculum. The surface of the medium is allowed to dry for 3-5 minutes but not longer than 15 minutes (It may absorb excess moisture).
- V. Disks are taken and dipped into the extracts.
- VI. Disks are placed on the surface of the inoculated and dried plate, using sterile forceps.
- VII. Plates are incubated in an inverted position at 30°C to get an optimum growth.
- VIII. The zones of inhibition are measured after 24 to 48 hours.

V. RESULTS AND DISCUSSION

Both the aqueous extracts contain tannin. Now, the tannins have each anti-microbial and anti-ageing property. The antimicrobial mechanism of tannic acid will be explained as follows i) complexation of enzymes ii) condensed tannins inhibit the synthesis of beta-lactamase produced by the bacteria iii) They are capable of binding metal ions involved in the metabolism of bacteria, iv) condensed tannins affect the permeability of the bacterial cell wall ; disrupting the absorption of trace elements essential for bacterial growth, v) microbial cell membrane is the primary site of inhibitory action by tannins through cell aggregation and disruption of cell membranes and functions by protein precipitation mechanism.

Our extracts conjointly shown marked antimicrobial property: The process has done by disc-diffusion method.

Table 5.1: Descriptive Results

Name of the bacteria	Amla fruit extract	Betel leaf extract	Mixture (1:1) of extracts
<i>Escherichia coli</i>	2	1.5	2.1

Table 5.1 results are expressed as the diameter of inhibition circle. The diameter is in millimeter (mm).

With the same protein precipitation mechanism tannin prevents the ageing process of skin. As previously mentioned, the aged skin comprises of open pores; the protein precipitation mechanism of tannins helps to reduce the pore sizes and makes the skin smoother and more youthful. And on further effect this property also exerts a skin tightening effect which will eliminate the exacerbation of skin loosening.

The unifying pathogenic agents responsible for photo-damage are UV-generated Reactive Oxygen Species (ROS) that deplete and damage the enzymatic and non-enzymatic antioxidant defense systems of the skin, and the release of matrix metalloproteases (MMPs) such as MMP-1 and MMP-3, that damage the extracellular matrix proteins. The antioxidants present in these extracts have Singlet oxygen quenching ability and Superoxide anion quenching ability. And as both the antimicrobial and anti- ageing property shown by the same mechanism that is protein precipitation so that we can come into the conclusion that the amla fruit extract and betel leaf extract both are capable of showing anti-ageing property as they have shown marked anti-microbial activity.

But it has some paradoxical effects, that tannins reduce the oil (sebum) secretion which may make the skin further dry and dull. Though it has some good aspect that, for oily and acne prone skin it will show a desirable effect. In case of acne vulgaris disorder there is some microorganisms which are generally accumulated on the stagnant sebum at the clogged (by hyper keratinization) pores. Due to the above-mentioned properties the prepared product can also be useful for this disorder by reducing the sebum secretion, reducing pore sizes and by bacteriostatic property.

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