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Formulation and Evaluation of Poly Herbal Antioxidant Cream: With Special Emphasis on Prevention of Premature Aging of Skin.

Anviksha Tiwari, Aishwarya Tyagi, and Savita Chaurasia*.

IMS Engineering College, Adhyatmik Nagar, Ghaziabad APJ Abdul Kalam Technical University, India.

ABSTRACT

Antioxidants are well known to slow the process of aging. Also it plays a significant role in slowing down skin related problems like wrinkles and pigmentation. In present study a polyherbal antioxidant cream is formulated using ethanolic extracts of Amla (*Phyllanthus emblica*), Cinnamon (*Cinnamomum verum*), and Jatamansi (*Nardostachys jatamansi*). Antioxidant activity of plant extracts was assessed by FRAP assay & TAC assay. Plant extracts were evaluated for total phenol and flavonoid content which is responsible for antioxidant activity. Antibacterial activity of these extracts was also studied using disc diffusion method. O/W emulsion based cream was formulated using all the three plant extracts. Formulated cream was tested for antioxidant activity, and it was found that cream showed better antioxidant potential in comparison to individual extracts. This effect may be due to synergistic effect. Formulated cream was also tested for its stability and other physical properties. The cream showed good stability at room temperature with a pH that is suitable for the skin & showed no irritancy. Plant extracts used in cream also possess antibacterial activity; hence it can be beneficial for prevention of skin infection also.

Keywords: Poly Herbal cream, Herbal Extracts, Anti-ageing, Anti-microbial, Anti-oxidants

Corresponding author

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INTRODUCTION

Human skin is constantly exposed to the air, solar radiation, other environmental pollutants or other mechanical and chemical insults, which are capable of inducing the generation of free radicals. The free radical theory of aging (FRTA) states that organisms age because cells accumulate free radical damage over time. Aging of facial skin can be noticed by seven key signs like fine lines and wrinkles, changes in skin tone and texture, skin surface and skin dullness. Among all these signs appearance of fine lines and wrinkles on the skin is common and most prominent sign of aging [1]. Aging of the skin is the result of continuous "wear and tear" processes, which damage cellular DNA and proteins. Aging is of two types: chronological aging, intrinsic aging and photo aging which is also called as the premature aging is caused by continuous exposure of the skin to the solar UV radiations. It results in the several skin symptoms such as leathery texture, mottled pigmentation and wrinkles. Apart from the intrinsic and photo aging the other aging is the stochastic aging which is damaged by the metabolic processes from free radicals and cosmic irradiations. Hence reducing oxidative stress has become the major focus on anti-aging research and the antioxidant supplements. Oxidative stress play major role in ageing process, varieties of antioxidants are used to reduce the ageing [2].

Use of plants and plant products as medicines has been documented in the history since centuries. Herbal cosmetics are the preparations used to enhance the human appearance. Volumes of literatures have been written describing the use of various herbs, shrubs and plants. The increasing use of traditional therapies demands more scientifically sound evidence for the principles behind therapies and for effectiveness of medicines. Natural products have the properties to rejuvenate and protect the skin from environmental pollution, chemicals, atmospheric temperature [3]. Conventional drug treatments irritate the skin, causing itching, redness, drying or allergic reactions. It is realize that chemical medicines may carry serious side effects. Masses are drifting towards nature therefore herbalism and ancient medicines are coming back to the daily practices. Hence it is of great interest to search new anti-aging skin care leads from natural resources so as to ensure the desire anti-aging effect of herbal products.

Cosmeceuticals are cosmetic-pharmaceutical hybrid products intended to improve the health and beauty of the skin by providing a specific result, ranging from acne-control and antiwrinkle effects, to sun protection. Cosmeceuticals have medicinal benefits which affect the biological functioning of skin depending upon type of functional ingredients they contain. These are cosmetic products that are not just used for beautification but for different skin ailments. These products improve the functioning/texture of the skin by boosting collagen growth by eradicating harmful effects of free radicals, maintains keratin structure in good condition and making the skin healthier [4].

Thus present study was carried out to formulate anti-aging polyherbal cream based on antioxidant potential of ethanolic extracts of Amla (*Phyllanthus emblica*), Cinnamon (*Cinnamomum verum*), and Jatamansi (*Nardostachys jatamans*). Formulation was made by incorporating specific concentrations of stearic acid and acetyl alcohol. Type of base used is water in oil (W/O).

MATERIALS AND METHODS

Plant collection

Matured fruit of Amla and the Cinnamon bark were obtained from the local market of Ghaziabad and the roots of Jatamansi were obtained from a Ravi Nursery in Kanpur, Uttar Pradesh. Plant materials were authenticated by Department of Botany, M.M. College, Modinagar,U.P.

Preparation of ethanolic extract

Shade dried plant material was grinded coarsely in a mechanical blender and was subjected to Soxhlet extractor, using ethanol for 24 hours**.** The ethanol extracts were concentrated under reduced pressure by Buchi type rotator evaporator and kept in a vacuum dessicator for complete removal of solvent. Extracts were preserved at 4º C for further use [5].

Qualitative phytochemical analysis of extracts

Phytochemical testing was carried out for all the extracts as per the standard methods. [6,7]

Terpenoids: 2 ml Chloroform was added to 5 ml aqueous sample along with 3 ml conc.H2SO4. Greyish colour development indicates the presence of terpenoids.

Saponins: 1ml sample was taken and added to 4ml distilled water . The solution was vigorously shaken for a minute and then the system was left to rest for 15 minutes. Persistence of froth indicates the saponins presence.

Philobatannins: 2ml aqueous sample was taken and 1% HCL was added to it followed by the boiling of the resultant solution. A red precipitate marks the presence of philobatannins.

Carbohydrates: 1ml aqueous sample was taken and a few drops of Fehling's solution were added to it. The solution was then incubated at 4℃ for 5 minutes. Appearance of a red colour precipitate indicates the presence of carbohydrates.

Tannins: 0.25 g of various solvent extract was dissolved in 10ml distilled water and filtered. 1% aqueous Iron chloride(FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test sample.

Phenols: To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Alkaloids: Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

Proteins and Amino Acids: Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Flavonoids: Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of Flavonoids.

Quantitative Estimation of phytochemicals

Total flavanoid content: Aluminum chloride method was used for flavonoid determination. 0.1ml of each extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415nm. Quercitin was used as a standard. Total flavonoid content was expressed as µg QE g^{-1} of extract [8].

Total phenolic content: Total phenolic content was determined by the Folin-Ciocalteau method. 0.1 ml of each extract was mixed with 4.9 ml distilled water, 0.5 ml of Folin Ciocalteu reagent was added to the mixture. After 5min of incubation, 5 ml of 7% of aqueous Na_2CO_3 solution was added. The mixture was allowed to stand for 30 minutes and the absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Systronics, model no. 2202). The standard curve was prepared by gallic acid (0.1mg/ml) in methanol: water (50:50, v/v). Total phenolic content was expressed as of gallic acid equivalent µg GAE g^{-1} of extract [9,10,11].

Determination antioxidant properties

Ferric reducing antioxidant power assay (FRAP): Varying concentration of each extract was added to solution containing 2.5ml of 50 mM phosphate buffer and 2.5 ml of 1% potassium ferricynide in different test tubes the reaction setup was then kept for incubation at 50^{O} C in water bath for 20 minutes. Then 2.5 ml of 10% tri chloro acetic acid was added to each test tube. The reaction mixture was then centrifuged at 3000rpm for

10 minutes. Carefully, 1.25 ml supernatant was withdrawn. The supernatant was mixed with 1.25 ml distilled water and 0.25 ml of 0.1 % FeCl3. Finally, absorbance was recorded at 700nm in a spectrophotometer [12].

Total antioxidant capacity (TAC): The parts of the plant were dissolved in tween 20 to make the concentration of 1mg/ml. Different concentration of plant extracts were placed in test tubes 0.3ml of the reagent solution(0.6M of sulphuric acid,28mM of sodium phosphate,4mM ammonium molybdate) was the added to the resulting mixture and was incubated at 95 ^{0} C for 90minutes. After the mixture was cooled to room temperature the absorbance of both solution was measured by using the UV-Visible spectrophotometer at 695nm. The experiment was performed in three sets. The antioxidant properties of the plant were compared to that of ascorbic acid [13].

Determination of antibacterial properties

Antibacterial activities of plant extract were investigated by the disc diffusion method. 2 ml of bacterial suspension were used for seeded Nutrient agar plates. Sterilised Whatmann filter paper No .1 disc were impregnated with plant extract (10mg/ml) followed by air drying. The air dried disc were placed upon different agar plates seeded with *Escherichia coli* and *Bacillus subtilis*. The plates were then incubated at 37 ^{O}C for 24 hours, the zone of growth inhibition around the disc were measured after 18 to 24 hours of incubation.The sensitivity of the microbes species to the plant extract were determined by measuring the size of zone of inhibition (including diameter of disc), on the agar surface around the disc [14].

Cream Formulation

Oil in Water (O/W) emulsion-based semisolid cream was formulated (Figure 1). The emulsifier (steric acid) and other oil soluble components (cetyl alcohol, light paraffin liquid) were dissolved in oil phase (part A) and heated to 75^{0}C for 30 seconds in microwave. The water soluble components (triethanolamine, ethanol extract) were dissolved in aqueous phase Part B) and heated to 75^{0}C in microwave. After heating the aqueous phase was added in portion to the oil phase along with continuous stirring [15, 16]. Table 1 shows the composition of the cream.

Table 1: Composition of Polyherbal Cream

INGREDIENTS	(mg)
OIL PHASE	
Steric acid	1.5
Cetyl alcohol	1.0
Almond oil	1.0
Light paraffin liquid	0.3
AQUEOUS PHASE	
Plant extracts	0.6 (150 each)
Triethanolamine	0.160
EDTA	0.01
Moisturizer	1.2
Rose water	20.44(ml)

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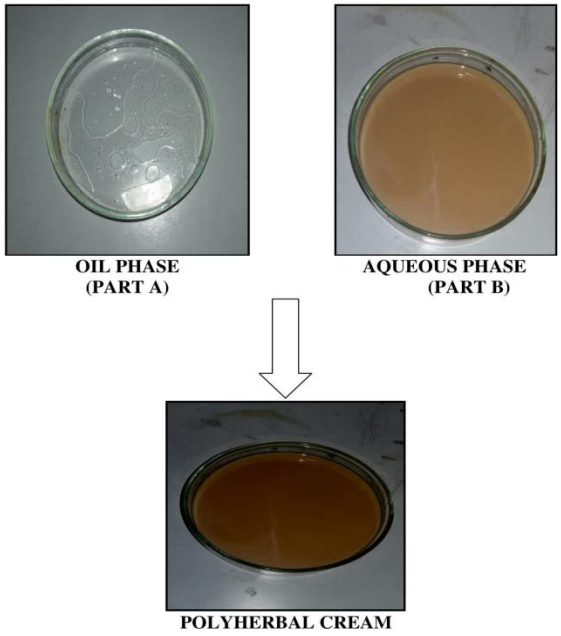

Figure 1: Formulation of Polyherbal Cream

Evaluation of cream

Organoleptic evaluation: The cream thus obtained was evaluated for its organoleptic properties like color, odour, and state. The appearance of the cream was judged by its color and roughness and graded.

pH of the Cream: The pH meter was calibrated using standard buffer solution about 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Test for Thermal Stability: Thermal stability of the formulation will be determined by the humidity chamber controlled at 60- 70% RH and 37±1^{o}C

Test for microbial growth in formulated creams: All the extracts under study showed 32 antibacterial activity. Results indicate antibacterial activity of ethanolic extract of plants used in the experiment.

Homogeneity: The formulations were tested for the homogeneity by visual appearance and by touch.

After feel: Slipperiness of the cream and amount of residue left after the application was checked.

Removal: The removal of the cream applied was examined by washing the applied part with tap water.

Patch test: Mark an area on any sensitive area of the body (behind the ear). The cream was applied for 24 hours. Control patches were also applied. The site of patch is inspected after 24 hrs. No sign of Irritancy, erythematic, edema, was observed.

Type of smear: After application of cream, the type of film or smear formed on the skin were checked [17,18]

Evaluation of Antioxidant properties of cream: Antioxidant potential of formulated cream was determined with following two assays: FRAP assay(Section 5.1) & TAC assay (Section 5.2)

Statistical Analysis

The results are expressed as the mean of three measurements ±standard deviation.

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RESULTS AND DISCUSSION

Percentage Yield

Percent yield of all the ethanolic extracts was calculated. Highest yield was of Amla (27.2%) followed by Jatamansi (9.12%) and Cinnamon(4.58%).

Qualitative phytochemical analysis of extracts

Results in Table 2 indicate presence of terpenoids, saponin, alkaloids, proteins and amino acids and flavanoids . Cinnamon extract showed high contents of terpenoids and also contains philobatannins, saponin, alkaloids and proteins and amino acids in significant amount. Cinnamon extract showed high contents of terpenoids and significant amount of saponin and alkaloids. Amla showed high amount of alkaloids and saponin. Jatamansi extract contains proteins and amino acids in high quantity along with flavanoids and alkaloids in significant amount.

Table 2: Qualitative phytochemical analysis of extracts

PHYTOCHEMICAL	AMLA	JATAMANSI	CINNAMON
Terpenoids	++	++	+++
Saponins	+	+	+
Philobatannins	×	×	×
Carbohydrates	×	×	×
Tannins	++	+	+
Alkaloids (wagner's test)	++	++	+++
Phenols	×	++	×
Proteins and amino acids	++	++	×
Flavanoids (alkaline reagent test)	+++	+++	+

Note: (+): shows presence of compounds; (x): shows absence of compound; Very high: [++++]; High: [+++]; Medium: [+++]; Low: [+]

Quantitative estimation of phytochemicals

The ethanolic extracts of plant material under study was studied for their contents of total phenols and flavonoids. Results in Table 3, show that all plant extracts posses phenol & flavonoid content. Highest content of both phenol and flavonoids was found in Amla (341±21.15 µg GAE g^{-1} extract; 280±10.87 µg QE g^{-1} extract) followed by Cinnamon and lowest was observed in Jatamansi.

Table 3: Quantitative estimation of phytochemicals

S. No.	Plant Extract	Total Phenol (µgGAE/gm Extract)	Flavonoid Content (µgQE/gm Extract)
1	Amla	341±21.15	280±10.87
2	Cinnamon	320±15.55	212±15.22
3	Jatamansi	172±07.19	329±15.18

Values are Mean±SD (n=3)

Determination of Antioxidant Properties

Ferric reducing antioxidant power assay: Table 4, shows the reducing power of the extracts. An increase in absorbance indicated the possession of reducing property. Higher absorbance is indicative of high reducing power. All the extracts showed dose dependent response for reducing ability. At a dose of 0.6 mg/ml, highest reducing power was observed with Jatamansi (0.31±0.06) followed by Cinnamon (0.24±0.37) and Amla (0.09±0.01) respectively. Results were compared with ascorbic acid.

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Table 4: Ferric reducing antioxidant power assay of plant extracts & polyherbal cream

S. No.	Conc. (mg/ml)	Absorbance at 700 nm				
		Amla	Jatamansi	Cinnamon	Ascorbic	Cream
1	0.1	0.04±0.05	0.04±0.01	0.03±0.002	0.11±0.02	0.17±0.02
2	0.2	0.05±0.04	0.05±0.03	0.11±0.07	0.24±0.27	0.20±0.05
3	0.4	0.06±0.12	0.12±0.01	0.17±0.13	0.33±0.03	0.22±0.01
4	0.6	0.09±0.01	0.31±0.06	0.24±0.37	0.29±0.06	0.26±0.03
5	0.8	0.12±0.004	0.47±0.07	0.31±0.13	0.43±0.003	0.33±0.04

Values are Mean±SD (n=3)

Total anti oxidant capacity: Table 5 presents the total antioxidant capacity obtained through the phosphomolybdenum assay for each extract in comparison with that of ascorbic acid (standard antioxidant). An increase in absorbance indicated the possession of antioxidant property. All the extracts showed dose dependent response for antioxidant capacity. The highest activity was found in amla (0.21±0.04) followed by jatamansi (0.18±0.02) and cinnamon (0.12±0.13) respectively at the concentration of 0.2mg/ml. Results were compared with ascorbic acid.

Table 5: Total antioxidant capacity assay of different plant extracts & polyherbal cream

S. No.	Conc. (mg/ml)	Absorbance at 700nm				
		Amla	Jatamansi	Cinnamon	Ascorbic	Cream
1	0.1	0.09±0.02	0.10±0.01	0.02±0.02	0.11±0.03	0.19±0.03
2	0.2	0.21±0.04	0.18±0.02	0.12±0.13	0.24±0.01	0.23±0.01
3	0.4	0.37±0.07	0.31±0.11	0.26±0.07	0.33±0.02	0.33±0.02
4	0.6	0.49±0.03	0.41±0.03	0.39±0.03	0.29±0.06	0.46±0.07
5	0.8	0.61±0.04	0.55±0.02	0.50±0.003	0.43±0.02	0.70±0.03

Values are Mean±SD (n=3)

Determination of Antibacterial Activity

Antibacterial activity of plant extract was investigated by the disc diffusion method (Figure 2). The sensitivity of the microbes species to the plant extract were determined by measuring the size of zone of inhibition (including diameter of disc), on the agar surface around the disc. All the plant extracts under study showed antibacterial activity against *B. subtilis* and *E. coli.* which was found to be maximum for jatamansi on both bacterial strains.

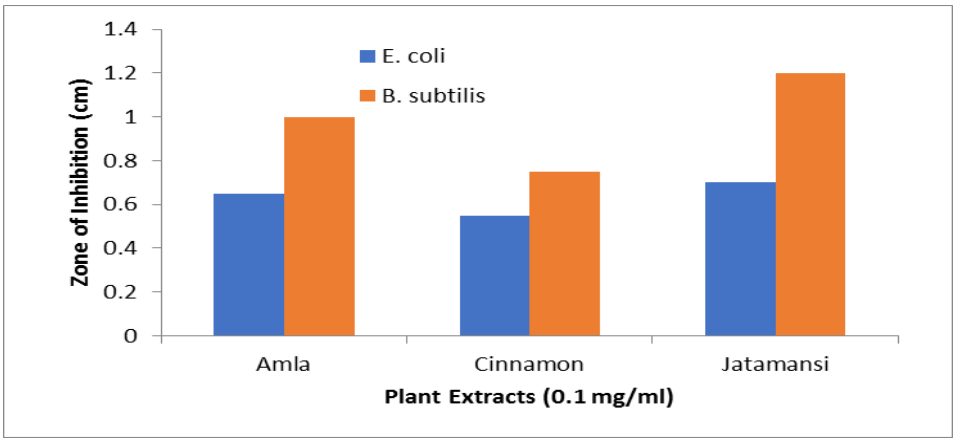

Figure 2: Antibacterial activity of plant extracts by disc diffusion method

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Polyherbal cream formulation

Organoleptic evaluation: Polyherbal cream was kept at room temperature. It was tested on ten human volunteers for 7 days. Cream did not show any sign of irritation, erythema or oedema. Homogeneity, spreadibility, stability of cream was good, smear was non greasy, easy to remove, no change in colour of skin was observed after applying cream. Cream was tested on following parameters and results were tabulated in Table 6.

Table 6: Evaluation of physical parameters of cream

DAYS	pH	P_1	P_2	P_3	P_4	P_5	P_6	P_7
1	6.5	++	NCC	++	E	NG	ES	G
2	6.5	+	NCC	++	E	NG	ES	G
3	6.7	++	NCC	++	E	NG	ES	G
4	6.7	++	NCC	++	E	NG	ES	G
5	6.8	++	NCC	++	E	NG	ES	G
6	6.8	+	NCC	++	E	NG	ES	G
7	6.8	+	NCC	++	E	NG	ES	G

P_1-homogenity, P_2-appearance,P_3-spreadibility,P_4-After feel, P_5-type of smear,P_6-removal,P_7-stability
+: satisfactory, ++: good, NCC: no change in colour, E: emollient, NG: non greasy, ES: easy, G: good

pH of the Cream: The pH of cream found to be in the range of 6.5-6.8 which is very good for skin pH.

Homogeneity: The homogeneity of cream is good that is checked by visual appearance and touch.

Appearance: When formulation were kept for long time, it found that no change in colour of cream.

Spreadibility: The spreadibility of cream is good that is checked by patch test.

After feel: The cream is emollient.

Type of smear: After application of cream, the type of smear formed on the skin were non greasy.

Removal: The cream applied on skin was easily removed with water.

Stability: The stability of cream is found to be good.

Evaluation of Antioxidant properties of cream

Antioxidant potential of formulated cream assayed on FRAY assay & TAC assay. Results of formulated cream were compared with the individual plant extracts used for formulation. It is clearly evident from the results shown in Table 4 & Table 5 that the formulated cream showed enhanced reducing property as well as antioxidant capacity. Enhanced antioxidant effect may be due to the synergistic effect of all three plant extracts used for formulation.

CONCLUSION

Free radicals are involved in ageing process and anti-oxidant slow down the process of ageing by preventing free radical damage. A poly-herbal cream formulated by using extracts of amla, jatamansi and cinnamon and have been found to be a good source of antioxidants due to presence of total phenolic and flavonoid content. A significant correlation was observed between antioxidant activity and phenolic content indicating that phenols and flavonoids contribute significantly to antioxidant activities. Efficacy of different plant extracts can be increased by combining the different plant extracts. Therefore, cream prepared by mixing the extracts of amla, cinnamon and jatamansi showed synergistic effect in comparison to individual extracts. Regular application of this cream will be beneficial in preventing premature aging of skin due to accumulation of free radicals. The prepared poly-herbal face cream was O/W type emulsion, hence can be easily washed with plane water.

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