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Hemant Kumar Sharma
School of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India

Abhinay Kumar Dwivedi
School of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India

Corresponding Author:
Hemant Kumar Sharma
School of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India

Evaluation of antioxidant and antibacterial potential poly herbal skin care cream

Hemant Kumar Sharma and Abhinay Kumar Dwivedi

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Abstract
Plants has all remedy to alleviate from suffering of human were recognized from ancient times and human were used various plant parts, plant material and plant products to restore the health as well as to get rid of distress and suffering. Skin is the largest protective organ that came in to direct contact with outer harmful agents that might get affects the skin health and functions. UV radiation, pollution, diseases causing organism and certain intrinsic factors are the major causes to defile the skin health and functions, so the protection of skin is utmost important to maintain the proper skin health. Cosmetics are the agents used to beautify, clean, alter, nourish and protect the skin from intrinsic as well as from extraneous factors. Numerous cosmetic agents are available in the market to ensure the protection against these harmful agents. Safety and biocompatibility are the major concern with the synthetic cosmetics inclined people towards herbal or natural cosmetics agents. Herbal or natural cosmetic have got more attention in the recent years due to lesser side effect, biocompatibility to skin, cost effectiveness, traditional experience of uses and availability. Increasing demand of herbal cosmetics incited to explore the new herbal substance from plant origin that could give better alternative in the cluster of existing formulation In the present study we have tried to formulate a skin care preparation that might be give better alternative for the protection of skin as well as favors to maintain the skin health.

Keywords: Antioxidants, antibacterial, skin care, UV radiations, herbal cream

Introduction
Skin is one of the largest and prominent organ that came in to direct contact with outer environment. Every individual aspire to make their skin healthy and beautiful. From the ancient time human were using different kinds of natural substances to protect the skin from harmful agents of the outer environments. Antioxidants and antibacterial are the compound obtained from plants source could protect the skin from free radicals, UV radiation and microbial attack. These agents has found as most crucial agent to defile the skin function and appearance. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species while antibacterial are the agents acts against diseases or infection causing bacterial species. In the present study we tried to made a topical skin care formulation using plant extracts that offers protection against malignancies, ageing, skin infections and other damaging effects on the skin [1, 2, 3].

Materials and Methods
Analytical grade chemicals have been used for the purpose of study, all the chemicals procured from Central Drug House (P) LTD. New Delhi, The glass wares used in the study have borosilicate and ASGI mark. UV-VIS Spectrophotometer model UV-1700 Pharmaspec Shimadzu, Japan.

Collection and Processing of Plant Material
The leaves of *Azadirachta indica*, *Ocimum sanctum*, *Centella asiatica and Hibiscus rosa sinensis* has been collected from the medicinal herbal garden Bansal College of Pharmacy, Bhopal M.P. India, in the month of February march. Plants sample has been authenticated by Dr. Suman Mishra, Botany Scientist, Vindhya Herbal Testing & Research Laboratory, A unit of Minor Forest Produce Processing & Research Center (MFP-PARC) Van Parisar, Barkheda

Pathani, Bhopal, MP, India. All collected plant materials were washed with tap water to remove dust and dirt then shade dried for seven days, The shade dried plant material then pulverized using electric grinder the powdered plant material the sieved through sieve no 40 to get fine powder, The fine powder has been used for extraction with suitable solvents

Extraction of Plant Material

The hydro alcoholic extract of plant material has been prepared by maceration method. 100 g of each powdered plant material has been macerated with 80% ethanol for seven days with occasional stirring, the extract were collected and filtered using whatman filter paper, the filtrate has been collected and concentrated under reduced pressure at the temperature 40 °C by rotary evaporator. The concentrated extracts were placed in the desiccators to remove residual solvent.

Formulation of Cream

The plant extracts collected above have been used to formulate the cream by using suitable base the formulation and selection of base has been done on the basis of preliminary evaluation parameter then selected base has been used to formulate the cream, varying concentration of extract were used to formulate F1 to F4 cream. All ingredients were weighed accurately. The soya lecithin, stearic acid, cetyl alcohol were melted in a beaker and heated up to 75 °C. The plant extract were dissolved in water then filtered. To the filtrate humectant glycerol was added and heated to 75 °C. When the temperature of both oil and water phases reached to 75 °C. The aqueous phase was added slowly into oily phase with continuous stirring until the mixture get cooled then preservative sodium benzoate and left at room temperature to obtain the required product. The flavoring agent was added at the last to cream base to get desired flavor to herbal cream [4, 5, 6, 7]. The compositions of the herbal cream are given in table1.

Table 1: Ingredients and concentration used in formulations

Ingredients	Concentration % W/W			
	F1	F2	F3	F4
Stearic acid	11	11	11	11
Cetyl alcohol	8	8	8	8
Soya lecithin	2.5	2.5	2.5	2.5
Glycerol	5	5	5	5
A. indica	1	0.5	2	1.5
O. sanctum	0.5	1	1.5	2
C. asiatica	1.5	2	0.5	1
H. sinensis	2	1.5	1	0.5
Sodium Benzoate	0.20	0.20	0.20	0.20
Rose water	7	7	7	7
Water, qs, 100	qs	qs	qs	qs

Evaluation of cream

The evaluation of herbal cream has been done to check the quality of prepared cream; the cream has been tested for homogeneity, appearance, spreadability, after feel, type of smear, $_{p}$H, viscosity, type of emulsion, antioxidant, and antibacterial and sun protection effect. The physical parameters of herbal creams were studied on room temperature and accelerated temperature.

Homogeneity

All formulations were tested for homogeneity by touch for texture and by visual appearance. Result obtained of each formulation is given in Table 3.

Appearance

The appearance of the cream was judged by its color, texture and roughness and graded accordingly [8].

Spreadability

One gram of cream formulation was placed on the lower plate and the upper plate which weighs 45 g exerts forces to the sample in the lower plate was place on the top of the sample, A constant force was generated by adding known weight on the upper plate exerted weight and the mean values of spread surface area on the lower plate were calculated all samples have been tested thrice at constant temperature and result indicated in the table [9, 10].

After feel

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Type of smear

It was determined by applying the cream on the surface of the skin of a human volunteer (Self, no ethical permission need as it is non-toxic, natural, and safe components which makes it exceptional 65. After applying the cream, the type of smear or film formed on the skin was checked [11, 12]. Result obtained of each formulation is given in Table 3.

Removal

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

pH measurement

One gram accurately weighed cream sample has been dispersed in 100 mL of water. The pH of the suspension was measured at 27 °C using digital pH meter. Result obtained of each formulation shown in Table No.3 [12, 13].

Viscosity measurement

The viscosity of each herbal cream was measured and compared before and after freeze thaw cycling by Brookfield Viscometer at 100 rpm, using spindle number 7 [12, 13].

Dilution test

The herbal cream has been taken in the beaker and diluted with water, it get easily mixed with water and no separation of oil and water phase takes place. The cream were found stable on addition of water the results revealed that water in the formulation was the dispersion medium and herbal cream was o/w type of emulsion [13].

Freeze-thaw determination

All formulations were tested physical stability by accelerated method: freeze-thaw cycling. Each formulation was packed in tight containers and stored for 48 hours at 4 °C in the refrigerator and then 48 hours at 45 °C in the hot air oven. The freeze-thaw cycling was continued for 6 cycles**.**

***In-vitro* Anti-oxidant Activity**

Antioxidant potential of different plant extract has been determined by DPPH Radical Scavenging Activity using ascorbic acid as standard

Preparation of DPPH reagent

0.1 mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

Preparation of Sample/Standard

One mg dried extract of *Ocimum sanctum, Hibiscus rosa sinensis, Centella asiatica and Azadirachta indica* have been taken to make 1 mg/mL stock solution with methanol.1 mg/mL methanolic solution of standard stock solution has been prepared with ascorbic acid. Different volumes of extracts/standard (20–100 µl) have been taken from stock solution in a set of test tubes and methanol will be added to make the volume to 1 ml. To this, 2 ml of 0.1 mM DPPH reagent has been added mixed thoroughly and absorbance will be recorded at 517 nm after 30 minutes incubation in dark at room temperature [14, 15].

Preparation of control

3 ml of 0.1 mM DPPH solution has been prepared and incubated for 30 min at room temperature in dark condition. Absorbance of the control will be taken against methanol (as blank) at 517 nm. Percentage antioxidant activity of sample/ standard were calculated by using formula [16, 17].

% Inhibition = [(Abs of control- Abs of sample/ Abs of control x 100]

Antibacterial Activity

Antibacterial activity of the prepared and selected formulation F4 has been tested by well diffusion assay against gram positive (*S. aureus* MTCC 10787) and gram negative (*E. coli* MTCC 42) the microorganism culture for the test has been procured from PBRI Bhopal.

Preparation of Nutrient Media

28 g of nutrient agar media was dissolved in one litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121 °C at 15 lbs pressure for 15 minutes. After sterilization, media was allowed to be cool but not solidify. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified [18, 19].

Well diffusion assay

Cultures of bacterial strains were spread on the nutrient agar media (NAM). Then 0.1 g and 0.2 g of test formulation F3 and F4 has been taken and mixed with 10 mL solvent (distilled water) to made 2% solution. One mg of standard (Ofloxacin and Gentamycin) was taken in 1 mL solvent (distilled water) to make 1 mg/1 mL standard solution. The inoculums of *S. aureus* MTCC 10787) and *E. coli* MTCC 42 was prepared; test organisms were inoculated in 10 mL of Nutrient broth. The bacterial suspension was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100 µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate**.** The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different concentration 2% of sample and another well were filled with 50 µL of standard drug respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 °C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well [20, 21].

Results

Table 2: Parameter evaluated at room temperature

F	Parameter studied at room temperature								
	H	A	S	AF	TS	R	pH	V	TE
F1	**	NCC	**	E	NG	ES	6.2	16092.72±0.03	o/w
F2	***	NCC	***	E	NG	ES	6.4	16023.18±0.06	o/w
F3	***	NCC	***	E	NG	ES	6.3	16037.61±0.02	o/w
F4	***	NCC	***	E	NG	ES	5.9	16041.47±0.05	o/w

***: Excellent **: Good *: NCC: No change in colour, Satisfactory, E: Emollient NG: Non greasy ES: Easy colour H-Homogeneity, A-Appearance, S-Spredibility, AF-After feel, TS-Type of smear, R-Removal, V-Viscosity, TE-Type of emulsion, F-Formulation

Table 3: Parameter evaluated after freeze thaw cycling

F	Parameter after freeze thaw determination								
	H	A	S	AF	TS	R	pH	V	TE
F1	**	NCC	**	E	NG	ES	5.9	16142.32±0.03	o/w
F2	***	NCC	**	E	NG	ES	6.8	16011.08±0.06	o/w
F3	***	NCC	**	E	NG	ES	6.1	16027.31±0.02	o/w
F4	***	NCC	***	E	NG	ES	5.9	16039.07±0.05	o/w

***: Excellent **: Good *: Satisfactory E: Emollient NG: Non greasy ES: Easy, NCC: No change in colour H-Homogeneity, A-Appearance, S-Spredibility, AF-After feel, TS-Type of smear, R-Removal, V-Viscosity, TE-Type of emulsion,F-Formulation

Antioxidant activities of different extract used in formulation

Table 4: DPPH radical scavenging activity of ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.447	52.74
40	0.34	64.05
60	0.261	72.41
80	0.187	80.23
100	0.119	87.42
Control	0.946	0
IC_{50}		10.04

Table 5: DPPH radical scavenging activity of *ocimum* extract

Concentration(µg/ml)	Absorbance	% inhibition
20	0.321	42.67
40	0.28	50
60	0.262	53.21
80	0.241	56.96
100	0.21	62.5
control	0.56	0
IC_{50}		46.86

Table 5: DPPH radical scavenging activity of *hibiscus* extract

Concentration (µg/ml)	Absorbance	% inhibition
20	0.324	42.14
40	0.3	46.42
60	0.273	51.25
80	0.232	58.57
100	0.191	65.89
control	0.56	0
IC_{50}		50.46

Table 6: DPPH radical scavenging activity of *Centella* extract

Concentration (µg/ml)	Absorbance	% inhibition
20	0.312	44.28
40	0.273	51.25
60	0.225	59.82
80	0.172	69.28
100	0.128	77.14
control	0.56	0
IC_{50}		35.33

Table 7: DPPH radical scavenging activity of *Azadirachta* extract

Concentration (µg/ml)	Absorbance	% inhibition
20	0.300	46.42
40	0.271	51.60
60	0.244	56.42
80	0.198	64.64
100	0.132	76.42
control	0.56	0
IC_{50}		35.95

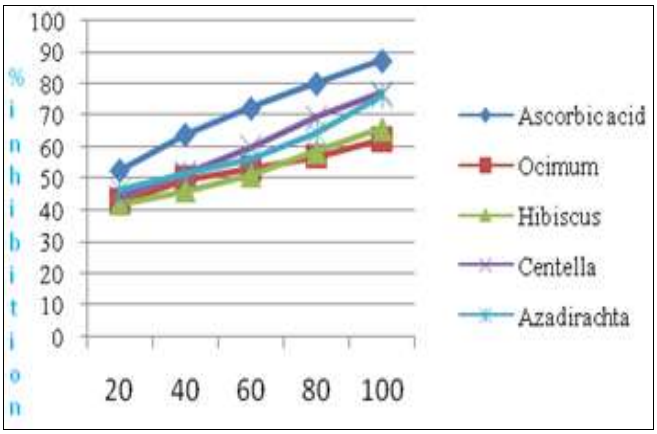

Fig 1: Graph represents the percentage inhibition *vs* concentration of sample extracts

Table 8: Antimicrobial activity of f4 sample against *s. aureus*

Sample	Plate 1	Plate 2	Plate 3	Mean±SD
Control	0 mm	0 mm	0mm	0±0
F4	10 mm	10 mm	9 mm	9.66±0.577
Ofloxacin (1 mg/ml)	22 mm	20 mm	28 mm	23.33±4.163

Table 9: Antimicrobial activity of f4 sample against *e. coli*

Sample	Plate 1	Plate 2	Plate 3	Mean±SD
Control	0 mm	0 mm	0 mm	0±0
F4	14 mm	14 mm	16 mm	14.66±1.154
Gentamycin (1 mg/ml)	25 mm	27 mm	24 mm	25.33±1.527

Fig 2: Antimicrobial activity of f4 sample against *s. aureus*

Fig 3: Antimicrobial activity of f4 against *e. coli*

Discussion

DPPH radical scavenging activity of *Centella asiatica*, *Azadirachta indica*, *Ocimum sactum* and *Hibiscus rosa sinensis* extracts exhibited percent inhibition 77.14%, 76.42%, 65.89% and 62.5%, and the IC_{50} value was found to be 35.33 µg/mL, 35.95 µg/mL 46.86 µg/mL, 50.46 µg/mL. Ascorbic acid was used as a reference compound which exhibited percent inhibition 87.42% and showed IC_{50} value of 10.04 µg/mL. As the results of IC_{50} value calculated the *Centella asiatica* extract has greater antioxidant potential campared to other extract and The antioxidant potential of *Azadirachta indica* was found nearer to *Centella asiatica*. The four formulations (F1 to F4) were prepared and evaluated using different parameter and it was found that formulation F4 showed better stability profile after freeze thaw determination then other formulations. The antibacterial potential of F4 has been evaluated against *S. aureus* and *E. coli* and the results revealed the maximum zone of inhibition of 9.66 mm against *S. aureus* and 14.66 mm against *E. coli* were found at 2% concentration. Ofloxacin used as a standard drug for gram positive bacteria showed the maximum zones of inhibition 23.33 mm in diameters and Gentamycin used as a standard drug for gram negative bacteria exhibited the maximum zones of inhibition 25.33 mm in diameters in 1mg/mL concentration. The formulation F4 showed antibacterial activity against both tested gram positive and gram negative bacteria.

Conclusion

Increasing demand of herbal cosmetics incited to explore the new herbal substance from plant origin that could give better alternative in the cluster of existing formulation. In this study we have tested the efficiency of newer combination of herbal ingredients and the results indicated that the formulation F4 has given satisfactory results on the parameter tested. The present formulation could be used as anti-ageing, antibacterial and antioxidant for better protection of skin and might be a better alternative for skin care. More study needed to refine the formulation by using isolated components and some more effect need to be tested that could broader the effect of formulation.

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