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Original Article

Formulation and stability evaluation of Natural Preservatives in Poly-Herbal Skin Care Cream

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ABSTRACT: The aim of this study is to formulate and evaluate the stability of polyherbal cream based on natural products namely, *Carica papaya* leaf, *Psidium guajava* leaf, *Vitis vinifera* seed applied as natural preservatives. *Carica papaya* leaf and *Psidium guajava* leaves were shade dried and size reduced into coarse powder and then the powdered leaf material was stored in containers. They were extracted using ethanol and the extract was stored in refrigerator. A polyherbal O/W skin care cream was formulated using PLEE & GLEE and GSE. The formulated cream was evaluated for its pH, Viscosity, Dye test, Homogeneity, appearance, after feel, type of smear, Removal, Acid value, Saponification value, Irritancy test, Accelerated stability testing, UV spectrophotometric analysis, centrifuge test. Stability of the formulation was achieved for 2 weeks. The formulation also evaluated for its resistance and activity against a variety of microorganisms. The microbiological stability of the formulations was evaluated through agar diffusion assays using cultures of *Escherichia coli*, Bacteria and Yeast. The pH of cream formulations was found in range of 5.9 to 6.9. The cosmetic formulation prepared presented a pH value that seems adequate for topical application, which is frequently observed in cosmetic products, besides evidencing no phase separation after centrifuge. The viscosity of the cream formulation prepared was found to be satisfactory which was suitable for stability study. The cream was found to be of the o/w type emulsion by dye solubility test. In addition, the findings suggested that the cream formulations with higher concentration of grape seed extract have a promising antibacterial effect against the proliferation of various microorganisms.

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INTRODUCTION

The herbal creams containing synthetic preservatives are used in skin care cosmetics have side effects. Herbal cosmetics are used in crude form and powdered form mixed with various ingredients and directly applied on the skin for immediate relief but cannot be preserved and stored for long periods. Herbal extracts can be prepared from plant parts and prepared into different skin care cosmetic creams, lotions and ointments. Herbal cosmetics can prevent the skin from different skin conditions, skin allergic reactions and skin diseases. Herbal cosmetics are preferred more than synthetic cosmetic, they have fewer side effects, safe on skin, efficacy and quality and the cost of these products are also economic and affordable to customers.

Since time immemorial herbal medicine have been used from ancient times in Ayurveda, Siddha and Unani, these herbal cosmetics like aloe vera, amla, henna, neem, tulsi, turmeric, bitter orange peel, soap nut, cypress and sandal wood in the form of fixed oils and volatile oils are used in many cosmetic creams.

Carica papaya Linn belonging to the family *Caricaceae* is a well-known medicinal plant in the world. It is used as a traditional medicine for the treatment of various diseases like cancer, malaria, dengue fever, viral infection such as common cold, eczema, warts etc. Papaya as well the leaf is a good source of Vitamin A (Catotene), Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin C (Ascorbic acid), Vitamin E, Niacin, Minerals such as Calcium, Iron, Phosphorous, Potassium,

Proteins, Fats, Calories, Carbohydrates, β -carotene, Fibers and Folate that helps to boost the number of platelets present on the blood. The leaf contains beta-carotene, calcium, carpaine, fats, flavonols, niacin, papain, tannins, and vitamin C [1, 2].

Guava consists of the dried leaves of *Psidium guajava* L. Medium sized tree with thin smooth, patchy, peeling whitish brown bark but under high moisture conditions, grows to 6-9 m in height. Guava leaf contains flavonoids, tannins and Isoprenoids [3, 4].

They are used as anticough, [5] spasmolytic, [6, 7] antibacterial, [8] antiameobic, [9] antifungal [10] antidiarrheal, [11, 12, 13] antidiabetic, hypoglycemic and anti-hyperlipidemic, [14] Antioxidant, [15, 16, 17] cardioprotective, [18] antimutagenic, [19] antiulcer, [20] hepatoprotective [21] and anti-depressant activity on central nervous system, [22] analgesic, anti-inflammatory [23] and other activities.

MATERIALS AND METHODS

Collection of plant material

Papaya *Carica papaya* leaves and *Psidium guajava* guava leaves were collected from local area from Tirupati.

Extraction of plant material

Papaya *Carica papaya* leaf and guava *Psidium guajava* leaves were dried in room temperature for 1 week and then powdered using mixer grinder. Then the powdered leaf material was stored in containers. The extraction of plant material was carried out using ethanol. The powdered drug of papaya leaf powder and guava leaf powder were transferred into a stoppered flask with 200ml ethanol (Maceration) and then filtered using watt man filter paper. The prepared extract was evaporated until a semisolid mass was obtained. The prepared extract was transferred to glass container and stored in refrigerator.

Extraction of Grape fruit seed extract

Grape fruits were purchased from local fruit market. The seeds were separated from fruits and the seeds were dried at room temperature for 24 hrs. The dried seeds were size reduced using mixer grinder and passed through sieve no 80. A fine powder was obtained, grape fruit seed powder was extracted using 100 ml ethanol.

Preparation and formulation of poly-herbal cream

The poly-herbal cream containing *Carica papaya* leaf ethanol extract PLE and *Psidium guajava* leaf ethanol extract GLE were formulated. Oil in water O/W cream was formulated using, the oily phase (Part A) and the aqueous phase (Part B). Oil in water (O/W) emulsion - based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Bees wax, cetyl alcohol, Almond oil were dissolved in the oil phase (Part A) and heated to 75°C. The preservatives and other water-soluble components and fraction of ethanolic extract of *Carica papaya* leaf and *Psidium guajava* leaf ethanol extract (grape fruit seed extract, triethanolamine, propylene glycol, glycerol, rosemary oil and water) were dissolved in the aqueous phase (Part B) and heated to 75°C.

After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place. [24, 25] the formula for the cream is given in Table 1.

Table 1: Formula for cream

Ingredients % w/w	C1% w/w	C2 % w/w
Beeswax	--	1.5%
Stearic acid	4%	6%
Cetyl alcohol	3%	5%
Almond oil	4%	4%
CPLLE	2%	5%
PGLEE	2%	5%
Propylene glycol	2%	5%
Glycerol	3%	5%
Triethanol amine	0.75%	1%
Grape Seed extract	0.18%	0.18%
Rosemary oil	0.02%	0.02%
Water q s to 100%	q s to 100%	q s to 100%

The herbal formulation was prepared using cream base incorporating all necessary ingredients along with the extracts of *Carica papaya* and *Psidium guajava*. Formulation was then evaluated for its physical properties.



Figure 1: Cream Formulation

Evaluation of the cream

The cream was evaluated for its physiochemical parameters pH, Viscosity, Dye test, Homogeneity, appearance, after feel, Type of smear, Removal, Acid value, Irritancy test, Accelerated stability testing.

pH of the Cream

The pH meter (EUTECH) was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.



Fig. 2: pH Meter

Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer (Servewell Pvt. Ltd. Model Number. LVDVE) at different rpm, using spindle number-64. The spindle was rotated at 10, 50, 60, 100 RPM.



Fig. 3: Brookfield Viscometer

Dye test

The ruthenium red dye is mixed with the cream. Place a drop of the cream on a microscopic slide covers it with a cover slip and examined under a microscope. If the disperse globules appear red the ground colorless. The cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appear colourless in the red ground [26].

Homogeneity

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

Appearance

The appearance of the cream was judged by its color, pearlscence and roughness and graded.

After feel

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

Type of smear

After application of cream, the type of film or smear formed on the skin were checked.

Removal

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

Spreadability

Spreadability denotes the extent of area to which the formulation readily spreads on application to skin or hair. The bioavailability efficiency of a formulation also depends on its spreading value [26].

Spectrophotometric test

The cosmetic formulations were diluted in ultra-pure water at ratio of 1/100 (w/v) and then submitted to scanning analysis by spectrophotometry in the UV-VIS region (244 nm). Variations in the intensity or wavelength of the absorption bands indicate alterations in the intensity of the color or even modification of the coloring material which is considered as formulation instability [27].

Centrifugation test

To perform the centrifugation test, 5 g of sample was subjected to a cycle of 3000 rpm for 30 minutes at room temperature. At the end of the centrifugation period, the cosmetic formulations were examined for phase separation which is an indication of cosmetic formulation instability.

Irritancy test

Mark an area (1sq.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 up to 24 hrs and reported.

Accelerated stability testing

Stability testing was carried out for Formula no. 2 by keeping 50 g of cream at 45°C and another 50 g at room temperature. It was checked for any visual disturbances and phase separation from time to time over a period of 2 weeks.

To determine the viscosity, the cream was kept at room temperature and at an elevated temperature of 45°C. For this, 50 g of the cream base was kept at 45°C in stability chamber. Stability of this cream was measured after regular intervals of time for 2 weeks.

Microbial stability

The microbial stability of the cosmetic formulations was evaluated through the microbial contamination test.

After being prepared the culture media were autoclaved at 125°C for 20 minutes and then 20 mL of the culture medium was poured into a sterile petri dish. Then 0.2g of the formulation was placed in the center of each petri dish, and then the plates were incubated at 37°C or at 25°C for 3 days according to the inoculated microorganisms. After the incubation period, plates were taken out and checked for microbial growth, which is an indication of contamination.

Microbial enumeration test and absence of specified microorganism

Total count

Test for *E. coli*: To the specimen of the sample add fluid lactose medium and make up to 100 ml, incubated for 24 hrs at 37 °C.

Bacterial count: Sample dissolved in phosphate buffer pH 7.2, shaken well and made upto 100ml. pipette out 1 ml of dilution into a sterile petridish, pour 30ml of the soyabean casein digest agar, and mix well. Solidify the agar and invert and incubate at 30° to 35° for 48 to 72 hrs.

Yeast and Mould count: Similarly pipette out 1 ml of dilution into a sterile petridish and pour 30 ml of the sabour dextrose agar mix solidify invert and incubated at 20° to 25° for 5 days.

RESULTS & DISCUSSION

pH of the Cream: The pH of the cream was found to be in range of 5.9 to 6.9 which is good for skin pH. The cream has shown pH nearer to skin required i.e pH of C2 was found to be 6.9.

Viscosity: The viscosity of cream was in the range of 11910 – 11900 cps which indicates that the cream is easily spreadable by small amounts of shear. But the C2 shows good spreadable property than other formulations. The results are given in Table 2 & Fig. 4

Acid value and Saponification value: The results of acid value and saponification value of the formulation of cream are presented in Table 3 and showed satisfactorily values.

Spectrophotometric test: Analytical technologies Limited, Double Beam UV Spectrophotometer Model 212R RI.

The results are given in Table no 4 & Fig no 5 & 6

Irritancy test: The formulation C2 shows no redness, edema, inflammation and irritation during irritancy studies. These formulations are safe to use for skin. The results are given in table no 5

Dye test: This dye confirms that the formulation was o/w type emulsion cream. But formulation (C2) shows more stable in o/w type emulsion.

Homogeneity: The formulations produced uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch. (Table 5)

Appearance: When formulation was kept for long time, it found that no change in colour of cream. (Table 5)

After feel: Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was found (Table 5).

Type of smear: After application of cream of C2, the type of smear formed on the skin were non-greasy. (Table 5)

Removal: The cream of C2 applied on skin was easily removed by washing with tap water. (Table 5)

Table 2: Viscosity of the cream

S. No	RPM	Viscosity	%
1	10	54650 cps	98.5%
2	50	11910 cps	98.7%
3	60	5788 cps	97.8%
4	100	5861 cps	99.5%

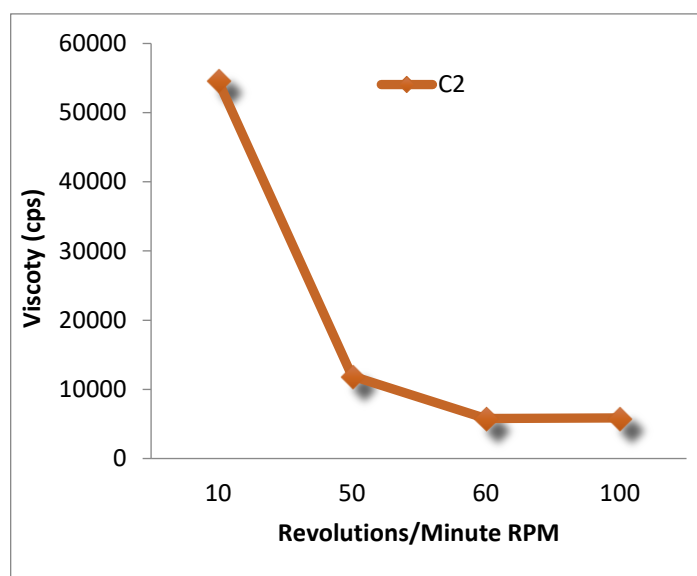


Fig. 4: Viscosity cps

Table 3: Acid Value and Saponification value

S. No	Parameter	C2
1	Acid value	12.56
2	Saponification value	22.3

Table 4: Spectrophotometric test

Concentration		Absorbance
1 ml	20 ml	0.060
2 ml	20 ml	0.090
3 ml	20 ml	0.135
4 ml	20 ml	0.169
5 ml	20 ml	0.233

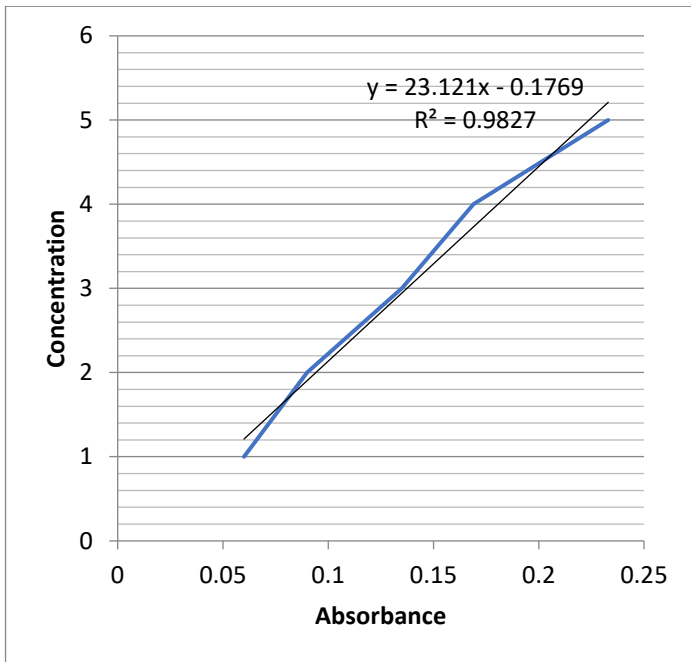


Fig. 5: Spectrophotometric test

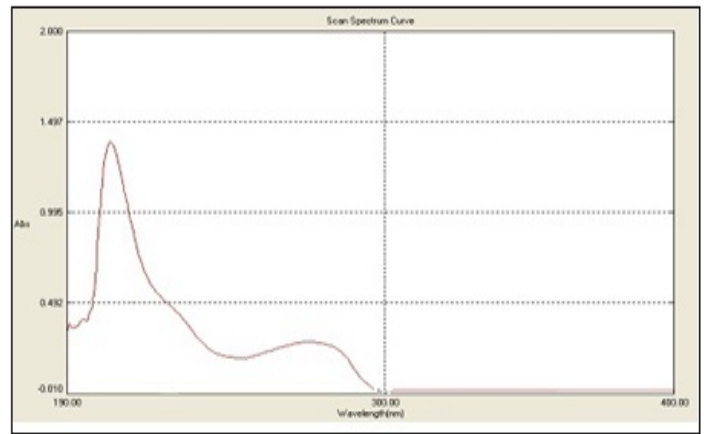


Fig. 6: Spectrophotometric test

Table 5: Skin Irritation Test

Formulation	Irritant	Erythema	Edema
C2	NIL	NIL	NIL

Table 6: Physical parameter of cream on room and accelerated temperature

Formulation	Days	Temperature	pH	Homogeneity	Appearance	Spreadability	After feel	Type of smear	Removal	
C2	7	20-25°C	40-45°C	6.5	Good	NCC	Good	Easy	Non greasy	Easy
	14	20-25°C	40-45°C	6.5	Good	NCC	Good	Easy	Non greasy	Easy

NCC- No Colour Change

Contamination test

The microbial stability of formulations was evaluated through contamination test using a variety of microorganisms namely *Escherichia coli*, *Bacteria* and *Yeast*. After the incubation period, the plates were taken out and checked for microbial growth by comparing it with the control. No microbial growth was observed in the formulation incorporated concentrations of natural preservatives (Figures 7, 8 & 9). The obtained results had confirmed the microbial stability of our formulations.

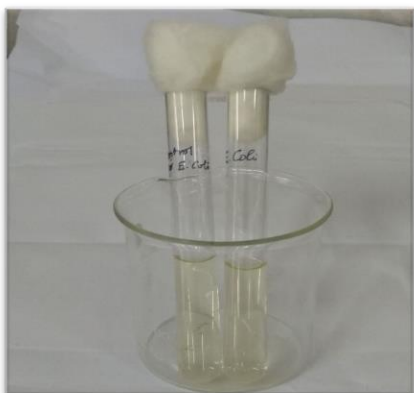


Fig. 7: *E. coli*

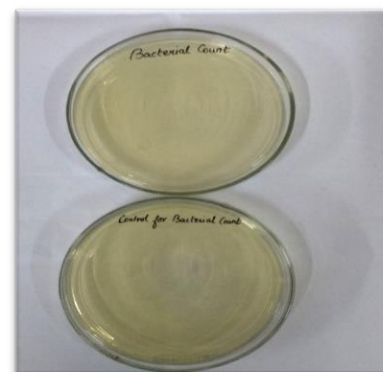


Fig. 8: Bacterial count

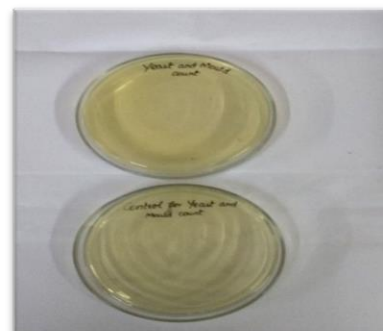


Fig. 9: Yeast & Mould count

DISCUSSION

Carica papaya, *Psidium guajava* and *Vitis vinifera* are well known for its medicinal and cosmeceuticals value in Indian traditional system of medicine. In the present work, it was decided to extract and formulate polyherbal cosmetic cream. The papain enzyme present in papaya fruit and leaves aids in cleansing blood naturally and digestion. The leaf contains beta-carotene, calcium, carpaine, fats, flavonols, niacin, papain, tannins, and vitamin C [28]. Exfoliative action on skin due to its keratolytic action, papain degrades dead cells in the outermost skin layer, thus improving skin health, hygiene and brightness [29]. Topical chymopapain and papain are efficiently used to treat children's burns and recurrent plantar ulcers in leprosy patients, because they prevent infection and accelerate wound healing [30]. Papaya also contains α -hydroxyacids (AHAs). These active compounds also have moisturizing action due to their hydroxyl groups [31].

The combination of chemicals such as carotenoids, tannins, polyphenols and flavonoids present in guava leaves promote a range of health benefits. The leaves of guava are used in various skin and hair treatments. Guava leaves are effective in eliminating acne and black spots from the skin. They contain an antiseptic that can kill acne causing bacteria, for the treatment of blackheads. Guava leaves contain antioxidants which destroy the free radicals that damage your skin, thus protecting your skin from ageing as well as improving skin tone and texture. A decoction of mature guava leaves can be applied on the skin for tightening it. Guava leaves are an instant cure for getting rid of itchiness as they contain allergy blocking compounds [32].

Phenolic compounds are among the most studied natural antioxidant compounds, they also present antimicrobial, anti-inflammatory or antiaging actions and can permeate through the skin barrier. Grapes contain valuable phenolic components and grape byproducts are widely available low cost raw materials. The application of phenolic compounds from grape products and byproducts as sources of natural ingredients for cosmetics. Acne fighting, skin tightening and healing, reduction of dark circles eye, hydration, protection of the skin from aging, skin protection against UVB radiation [33].

The antimicrobial activity of the cream was improved using natural preservatives, respectively. Grape seed extract showed remarkable preservative capabilities could therefore be considered as alternative preservatives. Where no contamination occurred in the formulation stored for a long time at room temperature or during the elaboration of tests, whereas a decrease of contamination in formulations containing Grape seed extract as a natural preservative. From above it concluded that this plant extracts produce excellent skin protection. Hence both extracts of plants are good choice to use as ingredient in face cream.

CONCLUSION

Formulations C1 to C2 were prepared with the same ingredients but with different compositions of emulsifiers and thickeners. Two of the cream formulations were observed to have similarity in consistency. No apparent change in the physical appearance was observed with these two compositions.

Therefore, C2 was chosen as the final formulation to prepare the cream for this thesis. The prepared polyherbal face cream was O/W type emulsion, hence can be easily washed with plane water that gives better customer compliance. There is a growing demand for herbal cosmetics in the world market and they are invaluable gifts of nature. Therefore, we tried to make a polyherbal face cream containing the extract of *Carica papaya*, *Psidium guajava* and *Vitis vinifera*. Our study indicated that the formulation C2 was found to be more stable, while remaining formulation was not stable and resulted in breakdown of the emulsion when stored for long time. The formulation C2 had almost constant pH, homogeneous, emollient, non-greasy and easily removed after the application. The stable formulations were safe in respect to skin irritation and allergic sensitization. Therefore, to confirm the microbial stability of the formulation the cream was tested against various microorganisms the results revealed that no microbial growth was observed in the formulation incorporated with natural preservative. The obtained results confirmed the microbial stability of our formulations.

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