Formulation of facial cosmetic cream containing fruit extract of *Carica papaya* Linn. (သင်္ဘော်) and its antioxidant activity

Shwe Yi Win Hlyan Min[1], Cho Yi Myint[1], Nway Nway Aung[2], Ei Ei Khine[3], Aye Win Oo[4]
[1]University of Pharmacy (Mandalay)
[2]Pharmaceutical Factory (Kyauk-se)
[3]Central Research and Development Centre (Yangon)
[4]Departmant of Medical Research (Yangon)

Abstract

Skin aging	-the continuous deterioration process
	-caused by damage of cellular DNA and protein.
Free radicals	-causes oxidative alterations
	-encourage polymerization reactions
	-causes permanent deterioration of the skin cell.

- Antioxidants -play an important role in preventing premature skin aging.

  -the development of safer antioxidants from natural

- Plants have been used as a good source of antioxidant to fight against oxidative damage of the skin.
- The market share for herbal beauty products has been increased regarding to the harmful effect of synthetic chemicals

- Aim ⟶ to formulate antioxidant facial cosmetic cream from 50% ethanolic extract of *C.papaya* L. fruit.

- botanical investigation was done by
  - morphological study
  - microscopic examination

- Antioxidant cream ⟹ fusion method followed by levigation with 50% ethanolic extract of *C.papaya* L. fruit

- Evaluated for physicochemical parameters such as
  - pH
  - viscosity
  - spreadability
  - tube extrudability

- The in-vitro free radical scavenging activity of all formulated creams was examined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method.

- skin irritation test on the formulation F3b ⟹ Draize's method according to OECD test guidelines 404.

- good rheological characteristics and acceptable pH range

- formulation F3b showed better antioxidant activity than the other five different formulations and it was not much different compared to the extract which IC_{50} value was 0.74 mg/mL.

- The skin irritation study of formulation F3b showed that there is no irritation potential in rabbit skin.

- Therefore, it can be concluded that antioxidant facial cosmetic cream containing 50% ethanolic extract of *C.papaya* L. fruit is safe and useful to treat premature skin aging problems.

INTRODUCTION

- Skin aging ⟹ older people (a variety of oxidative damage as well as photo degradation by UV rays from the sun)

- To reduce premature aging of the skin, development of skincare products containing antioxidant compound is important.

- Antioxidants can neutralize free radicals and play an important in battling aging and skin cancer.

- Synthetic products cause human health hazards with several side effects leading to numerous diseases in extensive use.

- Herbal products are safe to use, suitable for all skin types, easily available

Carica papaya L. natural sources of antioxidants

- Vitamin A
- Vitamin C
- Vitamin E
- phenolic compounds
- flavonoids
- **papain** in mature unripe fruit acts as a powerful exfoliator and dissolves inactive proteins and dead skin cells

Creams

- less greasy
- less viscous
- soft
- good spreadability
- removed easily
- more cosmetically attractive

- Creams ⟶ long lasting effect on skin (viscosity nature, promotes percutaneous absorption of drugs)

- This study aims to formulate facial skincare cosmetic cream from mature unripe fruit extract of *Carica papaya* L. and which can be used to prevent premature aging of the skin.

OBJECTIVES

1. To formulate facial cosmetic cream containing fruit extract of *Carica papaya* L.
2. To evaluate the pharmaceutical properties of all formulated creams
3. To determine antioxidant activity of formulated creams
4. To evaluate the skin irritation test from the most antioxidant rich formulation

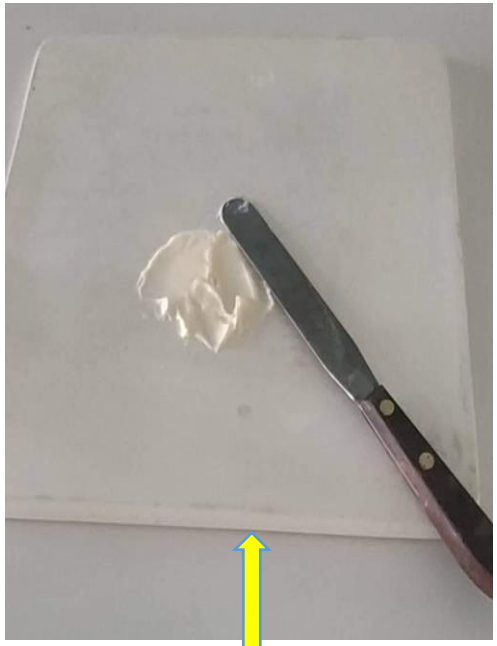
MATERIALS AND METHOD

Formulation of Antioxidant Cream

- The 50% ethanolic fruit extract → Soxhlet extraction
- Oil in water (O/W) emulsion-based cream (semisolid formulation)

Stearic acid
Liquid paraffin
Cetyl alcohol
(heated to 75°C)

Methyl paraben
Glycerol
Propylene glycol
(heated to 75°C)



After heating, the aqueous phase was added to the oil phase with continuous stirring until cooling of emulsifier took place.

Levigation with extract and perfume

Table 1. Formula for F1a, F1b, F2a, F2b, F3a, F3b

Formulation Code	F1a (% w/w)	F1b (% w/w)	F2a (% w/w)	F2b (% w/w)	F3a (%w/w)	F3b (% w/w)
50 % ethanolic extract of *C.papaya* L. fruit	5	10	5	10	5	10
Stearic acid	10	10	8	8	6	6
Tween 80	-	-	-	-	2	2
Cetostearyl alcohol	6	6	6	6	6	6
Liquid paraffin	6.6	6.6	6.6	6.6	3	3
Glycerol	3	3	3	3	2	2
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
Propylene glycol	30	30	30	30	4	4
Water	q.s	q.s	q.s	q.s	q.s	q.s
Perfume	q.s	q.s	q.s	q.s	q.s	q.s

Table2. Physical properties of formulated creams containing 5% and 10% w/w of 50% ethanolic extract of *C.papaya* L. fruit

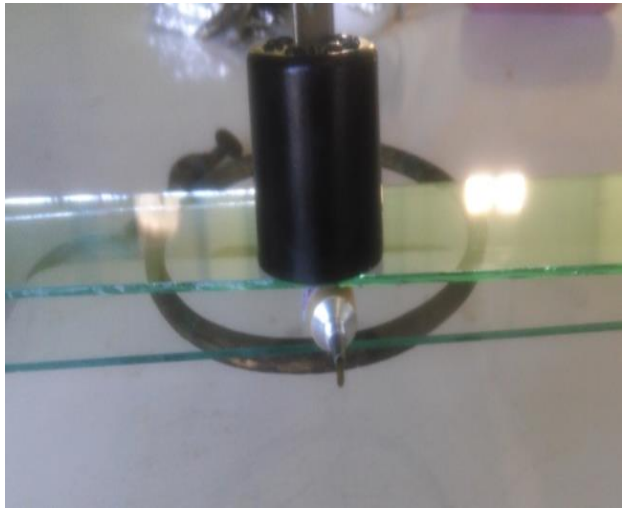
Formulations Code	Color	Odor	Homogeneity
F1a	Pale brown	Characteristic	Good
F1b	Pale brown	Characteristic	Good
F2a	Pale brown	Characteristic	Good
F2b	Pale brown	Characteristic	Good
F3a	Pale brown	Characteristic	Good
F3b	Pale brown	Characteristic	Good

Evaluation parameters for cream

Measured by Brook field Viscometer using spindle TL-5, 6, 7 at varying speed and shear rates. (Ashwini et al., 2014).

determined at 27°C using digital pH meter (Shahlla et al., 2015).

The cream was filled in aluminum collapsible tube 5 grams. Tube extrudability was then determined by measuring the amount of cream extruded through the tip when a pressure was applied on tube (Ashwini et al, 2014).



Exactly 1 g of the sample was placed between 20×20 cm two glass plates. The spread diameter was measured under 125 g weight after one minute.

Evaluation of In-Vitro Antioxidant Activity of All Formulated Creams

- One gram of cream were extracted with 95% ethanol in a separating funnel, and then shaken rapidly for 5 min
- The extract was filtered and the filtrate extract were collected

- DPPH stock solution, 60 µM (DPPH 2.4 mg in 100 mL of 95% ethanol) was freshly prepared and stored in brown coloured volumetric flask at +5°C (no longer than 24 hours) before use.
- Different concentrations of ascorbic acid (100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml, 1000 µg/ml) were prepared by serial dilution with 95% ethanol.
- Different concentrations of formulated cream extracts (100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml, 1000 µg/ml) were prepared by serial dilution with 95% ethanol.

- Test sample solutions was prepared by mixing of 3 mL of 60 µM DPPH solution and 1 mL of different concentrations of different cream extracts by a vortex mixer

- Standard solution was prepared by mixing of 3 mL of 60 µM DPPH solution and 1 mL of different concentrations of ascorbic acid

- These solution mixtures were kept for 30 minutes in the black box.

- Measurement of absorbance was done at 517 nm using UV-visible spectrophotometer.

- Absorbance measurements were done in triplicate and calculated to obtain the % inhibition using the formula

- Percent (%) inhibition $= \frac{\text{Abs DPPH alone} - \text{Abs sample}}{\text{Abs DPPH alone}} \times 100$

- The antioxidant activity of each sample was expressed in terms of IC_{50} calculated from the inhibition curve

- The lower IC_{50} value, the higher antioxidant activities (Raghovendra et al., 2013)

Skin irritation test (Dermal Irritation Test)

- To ensure dermal safety condition for formulation F3b, skin irritation study was carried out by the method given by (Draize, Woodward & Clavery, 1944).
- Six adult female albino rabbits weighing 1.5 to 2.5 g were chosen from Department of Medical Research, Yangon.

individually housed in metal cages fitted with perforated floors

- temperature of 25 ± 2° C,
- humidity of 60 - 90 relative humidity (RH)
- 12hours light/ 12hours dark cycle
- animals were provided with commercial rabbit diet and drinking water
- a minimum of 7 days acclimation
- each rabbit cage was attached with a tag marked with the animal number, the test date and the product name

- Hair was removed
  - to ensure good contact between the skin and the test material and
  - to allow the effects on the skin to be clearly observed
- Only animals with healthy intact skin should be used.

- The area of the back portions of each rabbits was shaved 24 hours before the test (dose application)
- This area was approximately 4 cm^2

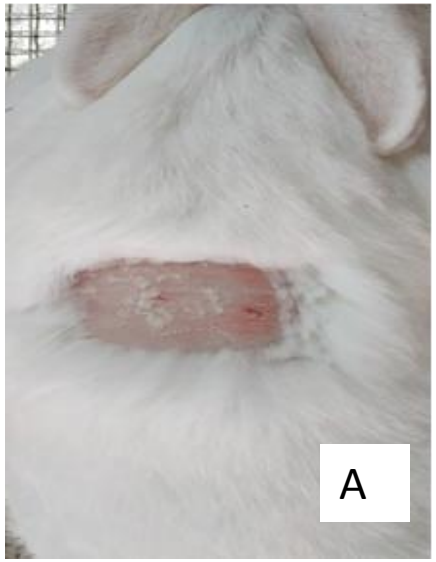


Figure 1. Negative control rabbit

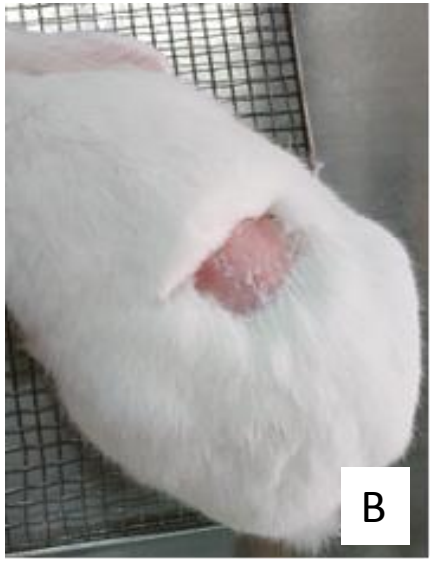


Figure 2. Positive control rabbit

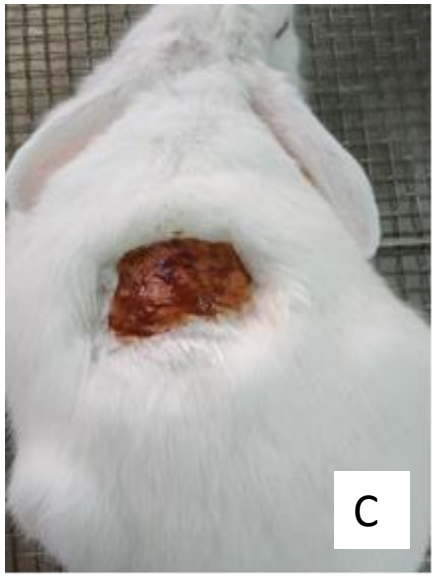
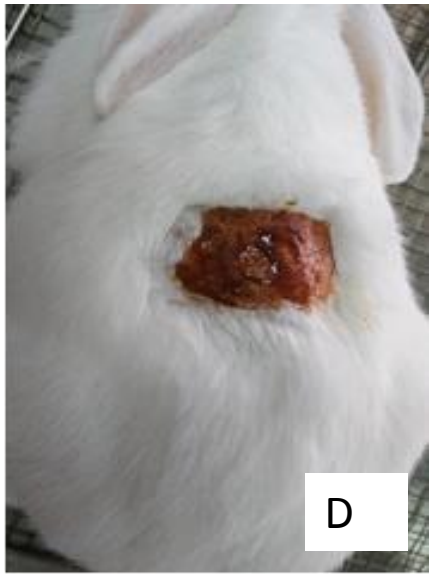
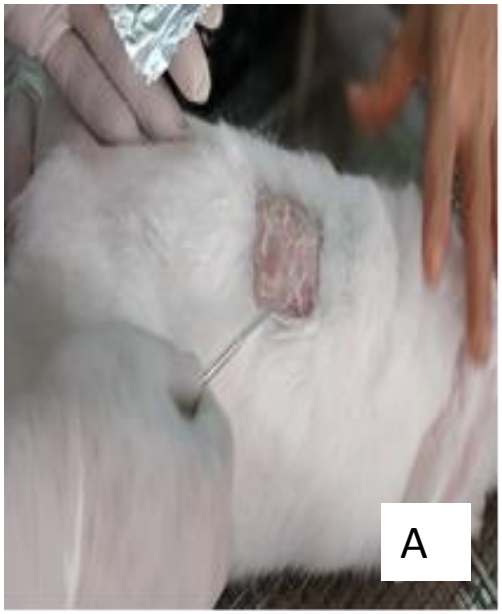


Figure 3. Application of 50% ethanolic extract of *Carica papaya* L. on rabbit No. 3 (A) &4 (B)





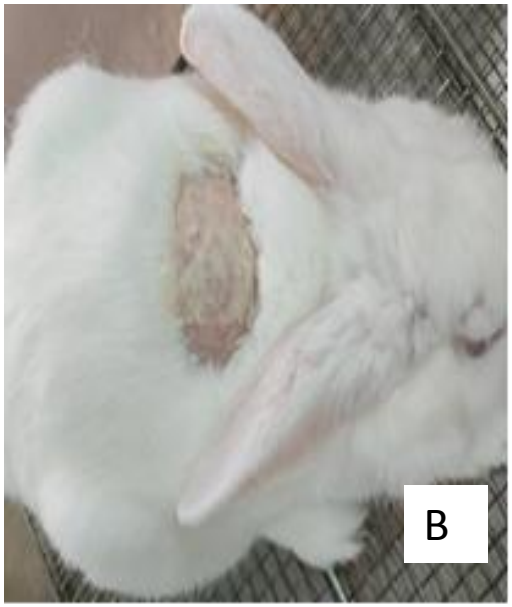


Figure 4. Application of formulation F3b containing 50% ethanolic fruit extract of *Carica papaya* L. on rabbit No. 5 (A) & 6 (B)

- Observed at 2 hours, 24 hours,48 hours and 72 hours

- Observations were continued until 14 days to determine the reversibility of the effect.

- The reactions defined as erythema and oedema were evaluated according to the scoring system for skin reaction.

Table 3. Scoring system for skin irritation on rabbit skin (Draize Method, 1944)

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation	4
Oedema	
No oedema	0
Very slight oedema	1
Well defined oedema	2
Moderate oedema (raising approximately 1mm)	3
Severs oedema (raising more than 1mm and extended beyond the area of exposure)	4
Total Possible score for primary irritation	8

The Score of Primary Irritation

- Scores for erythema and oedema at 24, 48 and 72 hours will be summed and divided by the number of observations for the treated sites.
- The SPI for the control sites were calculated in the same fashion as test.

$$SPI = \sum \frac{Erythema\ and\ oedema\ grade\ at\ 24,48\ and\ 72\ hrs}{Number\ of\ observation}$$

Response categories of irritation in rabbits (Draize Method, 1944)

- The difference between the summation of SPI scores of five animals from the treated sites and control sites were calculated and were used for Primary Irritation Index (PII) determination.
- PII was calculated as the means of the SPI values of the five rabbits.
- The irritation degree was categorized as negligible, or slight, moderate, severe irritation based on PII.

$$PII = \frac{\sum SPI\ (Test) - \sum SPI\ (Control)}{Number\ of\ aminals}$$

Table 4. Response categories of irritation (Draize Method, 1944)

Primary Irritation Index (PII)	Category
0 - 0.4	Negligible
0.5 - 1.9	Slight irritation
2 - 4.9	Moderate irritation
5 - 8	Severe irritation

RESULTS AND DISCUSSION

- the FTIR spectra of all formulated creams containing extract did not result any additional peaks for the formation of new functional groups

- there was no physical and chemical interactions between the extract and all the excipients used in formulation

Liquid paraffin	Cream base
Propylene glycol	Cream base
Stearic acid	Emulsifier
Tween 80	Emulsifier
Cetostearyl alcohol	Emollient property
Glycerol	Moisturizer
Soft extract	Homogenous appearance

- All formulated creams showed good homogeneity and good organoleptic properties and no foreign particles were detected.
- The pH of all formulated creams → around 6 → suitable for topical application because the pH of the skin is between 4.5 - 6 (Ali & Yosipovitch, 2013).
- The viscosity → acceptable for cream formulation.
- Formulation F3a with viscosity of 4.8 cps and formulation F3b with 3.3 cps showed better viscosity characteristics.

the lower the viscosity of a cream

the lower the surface tension

the more the cream is easily spread and absorbed into the skin

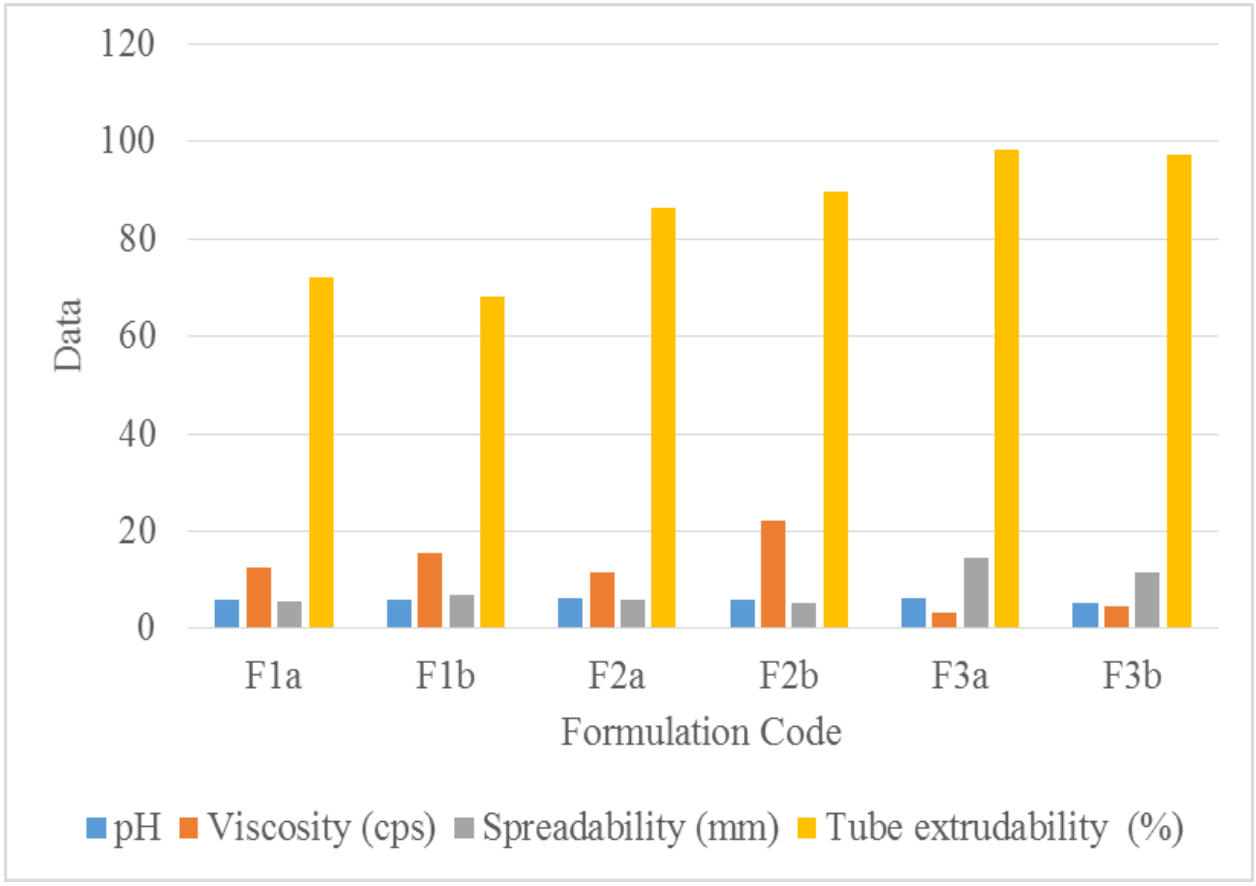


Figure 1. Comparison of pharmaceutical qualities of all formulated creams

- Lower viscosity in formulation F3a and F3b ⟹ more suitable to use as facial antioxidant cream
- The spreadability of all formulated creams showed acceptable results
- Formulation F3a with spreadability value of 14.67 mm and formulation F3b with 11.67 mm ⟹ more appropriate spreadability characteristics
- The values refer to the extent to which the formulations were readily spread on the application surface by applying a small amount of shear.

- The extrudability values of all formulated creams showed no significant difference.
- But formulation F3a and F3b with extrudability value of 98.30% and 97.12% showed better extrudability values than the other formulated creams.
- May be due to decreased in cream base composition and the use of combination of emulsifiers

The IC_{50} values

F1a	1.68 mg/mL
F1b	1.35 mg/mL
F2a	1.22 mg/mL
F2b	1.06 mg/mL
F3a	0.731mg/mL
F3b	0.53 mg/mL
Ascorbic acid	0.0133 mg/mL

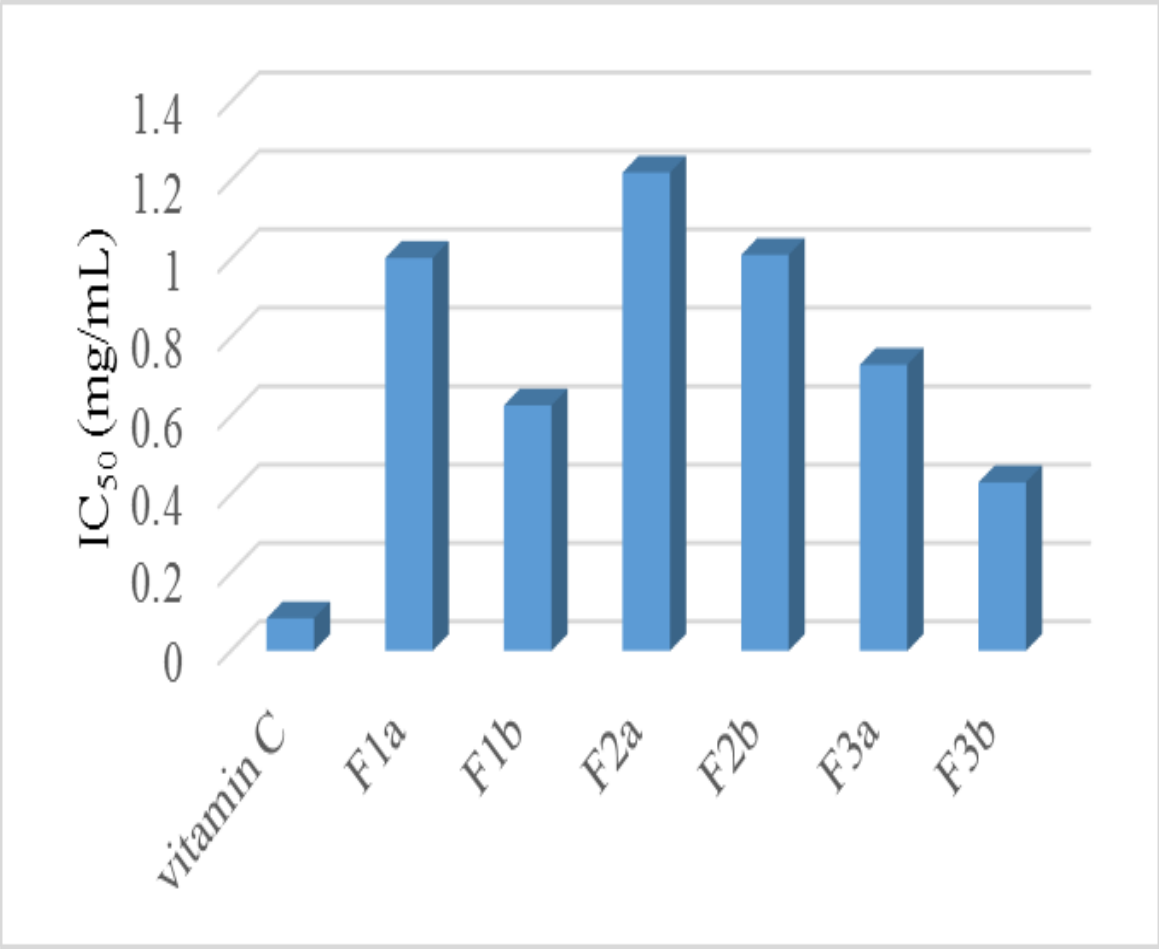


Figure 2. Comparison of IC_{50} values of standard ascorbic acid and formulation F1a, F1b, F2a, F2b, F3a, F3b containing 50% ethanolic extract of C.papaya L. fruit

- The formulation F3a and F3b containing 5% and 10% extract possess more antioxidant activity than the other four formulations.
- Decreasing the base percentage and the use of combination of emulsifier in formulation F3a and F3b increases the antioxidant activity of the formulated creams.

- Increasing extract concentration will increase the antioxidant activity of cream.
- There is no signs of erythema and oedema in No. 3 and 4 rabbits treated with 50% ethanolic fruit extract of *C. papaya* L..
- There is no sign of oedema in No.5 and 6 rabbits but signs of slight erythema was found after 24 hours of sample application.

- It may be due to one of the ingredients used in formulation, possibly, methyl paraben which is preservative used in concentration of 0.02%.
- Preservatives are the second most common cause of skin irritation (Nigam, 2009).
- the score of Primary Irritation (SPI) and Primary Irritaiton Index (PII) for this rabbits were negligible (it was less than 0.4)
- 50% ethanolic fruit extract of *C. papaya* L. and formulation F3b are safe for use as cosmetic product.

CONCLUSION

- all formulated creams showed good homogeneity and organoleptic properties and no foreign particles was detected.

- The formulation F3a and F3b exhibited the best rheological characteristics such as viscosity, spreadability and extrudability.

- the pH value of F3b was more acceptable to use in human skin than formulation F3a.

- IC_{50} values were significantly higher in formulation F3b than other formulations.

- Formulation F3b with the most suitable pH value, acceptable rheological characteristics and highest antioxidant activity was reasonable to be considered as an optimized formulation

- The results of skin irritation revealed that the Score of Primary Irritation (SPI) and Primary Irritation Index (PII) were negligible

- Formulation F3b containing 10% w/w of 50% ethanolic extract of *C.papaya* L. fruit were suitable for formulation of antioxidant facial cosmetic cream

SUGGESTIONS

1. To study both the real time and accelerated stability testing of *C.papaya* L. fruit extract cream
2. To find out the antioxidant activity of the different types of *C.papaya* L. fruit extracts such as soft, dry and lyophilized extracts
3. To determine the antioxidant activity of the different parts of *C.papaya* L. extract
4. To isolate antioxidant compounds from *C.papaya* L. fruit extract

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