International Journal of Chemical Sciences

Research | Vol 18 Iss 2

Development of Herbal Sunscreen Cream Enriched with Antioxidants from Canna (red) Flowers and Evaluation of in Vitro Sunscreening and Antioxidant Activity

Sujeewa Krishanthi Hettihewa* , **Shakthi Apsara Thejani Opatha**

Department of Pharmacy, University of Ruhuna, Sri Lanka

***Corresponding Author:** Dr.Sujeewa Krishanthi Hettihewa, Department of Pharmacy, University of Ruhuna, Sri Lanka, Email: krishanthi2001@yahoo.com

Received: March 21, 2020; **Accepted:** March 28, 2020; **Published:** April 20, 2020

Abstract

The aim of the present study was to develop herbal sunscreen cream enriched with antioxidants extracted from flowers of *Canna* (red) grown in Sri Lanka and, evaluate *in vitro* sunscreening, radical scavenging and antioxidant activity. The crude extract prepared was subjected to quantitative analysis of total phenolic, flavonoid contents, in vitro radical scavenging activity, antioxidant activity and sunscreening activity. Different formulations were prepared by incorporating freeze dried powder of flower extract and were tested for the physical stability parameters to select the most stable formulation, followed by evaluating antioxidant and sunscreening activities. The results of the total phenolic and total flavonoid contents of the extract were 5389.067±681.343 mg Gallic acid equivalent (GAE)/100 g and 6017.442±158.343 mg Catechin equivalents (CAE)/100 g dry weight (DW) of flowers. The extract revealed promising sunscreening activity (SPF=32.75) at the concentration of 1 mg/ml. The results of the SPF of formulated cream was determined as 37.73 at the concentration of 7 mg/ml of cream. The formulated herbal cream was found to be homogenous, semi-solid, washable and pink caramel colour with pleasant odor and the pH 6 to 7. Physical stability parameters of the herbal cream showed no remarkable variation during 45 days. It is concluded that the formulated cream has promising sunscreening and antioxidant activity and can be commercialized as novel herbal sunscreen cream.

Keywords: ***Formulations; Radical scavenging activity and sunscreening activity***

Introduction

Over exposure to solar Ultra Violet (UV) radiation by outdoor activities, causes harmful effects such as premature aging, low immunity against infections, sunburns and wrinkles etc. Ultraviolet radiation also induces the immunomodulatory effects that may lead to skin cancer and studies have shown that both UVA and UVB radiations are having an immunosuppressive effect. It is reported that epidermal melanin accumulated in keratinocytes, blocks the penetration of UV photons into the skin and acts as a "natural sunscreen" [1]. In addition, the use of personal protection items such as sunglasses, hats and the clothing are recommended to use to protect the skin against UVR. The skin parts which are not protected by clothes must be protected by sunscreen containing both UVB and UVA filters. The application of sunscreen is recommended in many countries as sunscreens rank among the best photo protective measures [2]. Sunscreens have become the most popular way of protection against UV radiation in Western countries more than 40 years.

Sunscreens are the main components that used in lotions and creams to prevent UV-induced skin damage or to overcome harmful effects of sun burns. There are three types of sunscreens namely; stimulators of repairing mechanisms, antioxidants, and physical photon blockers [3]. A sunscreen composition includes a combination of both UVB and UVA filters of full range (280 nm to 400 nm) to prevent damaging human skin as well as to obtain protection from the reactive oxygen species (ROS).

The sun protective factor (SPF) is an index which indicates that when compared to the personal sunburn time how much longer the person is able to stay exposed to sunlight without having sunburn. Manufacturers make sunscreen products with SPF of 15 to 20, and 50 or higher. Use of sunscreen products with SPF $\geq$ 15 is associated with significantly decreased chances for malignant melanoma risk and prevent sunburn compared with SPF < 15 use [4]. It is recommended that an extra protection can be obtained only by applying a sunscreen product with a high-SPF sunscreens (SPF 70 and above) [5].

Organic and inorganic filters are widely used in commercial sunscreens as UVA and UVB filters. Organic UV filters (salicylates, cinnamates, *p*-methoxycinnamic acid esters anthranilates, benzophenones, *p*-aminobenzoates and dibenzoylmethanes derivatives) are usually aromatic compounds act by three different ways namely release incident energy as heat or undergo conformational molecular changes or emit radiation at higher wavelength, when they receive the energy of UV photons [6]. Some inorganic pigments such as titanium dioxide and zinc oxide are commonly used as active ingredients for sunscreens as they absorb, scatter or reflect UV radiation [7]. Further inorganic and organic filters are divided as physical (UVA, UVB and visible radiation is reflected and scattered) and chemical agents sunscreens (UV radiation is absorbed and re-emitted as heat or light) [8].

Several synthetic filter molecules are known to have limited use because of the adverse effects like contact dermatitis, photoirritation and photosensitization on human skin [9]. Also research studies have shown that active ingredients such as titanium dioxide and zinc oxide in sunscreens, themselves become possibly

carcinogenic and toxic by generating reactive oxygen species (ROS) like H_2O_2 and singlet oxygen, $^1O_2$ and highly oxidizing radicals ($^{\bullet}OH$ and O_2-) when exposed to UV radiation [10,11] . Since there is a need of protection of the skin by harmful effects caused by UV radiation and prevention from these side effects occur by synthetic filters [9], scientists started seeking naturally occurring photo protective agents with antioxidant activities known for lesser side effects [8].

Herbs and herbal preparations have been used throughout the history in medicines, pharmaceutical and cosmetic preparations for ages in Sri Lanka and other countries [12-14]. Their potential to provide protection from ultraviolet (UV) radiation has been lead to find ways to satisfy the need for protection from solar radiation and prevent side effects of UV-radiation [14].

Antioxidants such as high molecular weight polyphenols, phenolic acids, flavonoids, vitamin C, vitamin E and carotenoids like naturally occurring phytochemicals have shown to be beneficial as photo protective agents to protect human skin against number of harmful effects [15]. Large number of natural phenol derivatives (polyphenols) have shown both antioxidant and photo protective activity, therefore, they could be used as components for pharmaceutical photo protection formulations [15] . Even though isolated plant compounds have shown a high potential in protection of the skin, a better potential has been shown by the whole herbs extracts, due to their complex composition [9].

Therefore, the aim of this research study was to formulate herbal sunscreen cream using freeze dried extract of *Canna* (red) flowers grown in Sri Lanka, and evaluate the effectiveness (*in vitro* sunscreening activity and antioxidant activity) and the physical stability of the formulated cream**.**

Materials and methods

Sampling plant materials and authentication

The fresh flowers of *Canna* (red) hybrid and Aloe vera leaves were collected from Galle district in Sri Lanka (geographical coordinates; latitude: 6.053519; Longitude: 80.220978) and authenticated at National Herbarium in Peradeniya botanical garden, Sri Lanka.

Chemicals

Folin-Ciocalteu phenol reagent, Hydrochloric acid, Catechin, Sodium carbonate, Sodium hydroxide, Sodium nitrite, Aluminium chloride, Gallic acid, 2-2-diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tripyridyl-s-triazine), Trolox, Methanol, Ethanol, Acetone, $FeCl_3.6H_2O$ Stearic acid, Triehanolamine, Beeswax, Cetyl alcohol, Polyethylene glycol, Methylparaben, Rose water, Ethylene diaminetetraacetic acid, Araliya oil, Olive oil and all other chemicals were purchased from local agents in Sri Lanka.

Preparation of extracts

The oven dried flowers were subjected to extraction by performing the method published [15]. Briefly, the powder of the dried flowers of *Canna* (red) was steeped in acidified 70% aqueous acetone (AAD), (200 ml) in a Scott Duran bottle overnight in the dark conditions at room temperature (28 ± 2 °C). The extracts were filtered by using four layers of muslin cloth and concentrated on the rotary evaporator (HAHN HS-2005S-N) below 35 ^{0}C under vacuum. The concentrated filtrate was freeze dried (BIOBASE BK-FD10PT) and stored at -40^{O}C until further used. Total phenolic content (TPC), total flavonoid content (TFC), *in vitro* sunscreening, radical scavenging activity and antioxidant activity of the extract were determined [15].

The Aloe vera leaf extraction was performed by soaking fresh aloe gel (50 g) peeled off from the leaf, in 70% ethanol (200 mL) for 12 hours. The gel extract was filtered through a muslin cloth and it was stored in the refrigerator (-20^{O}C) until being used in the herbal cream formulation.

Determination of total phenol content and total flavonoid content

The Folin-Ciocalteu assay and the aluminium chloride colorimetric method were performed for the 70% acidified aqueous acetone dried flower extract and the total phenol content (TPC) was expressed as mg Gallic acid equivalent (GAE)/100 g dry weight (DW) of the flowers and total flavonoid content (TFC) was expressed as mg Catechin equivalent (CAE)/100 g DW of the flowers respectively [15].

Determination of ferric-reducing antioxidant power by FRAP assay

Ferric reducing antioxidant power (FRAP) assay was used to determine the antioxidant power of the extract and the results were expressed in mmol Fe(II) equivalent/100 g DW of the flowers [15].

Determination of radical scavenging activity by DPPH assay

The radical scavenging activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and expressed as mmol Trolox equivalent (TE)/100 g DW of the flowers [15].

Determination of in-vitro sun protective factor (SPF)

Mansur mathematical equation was used to calculate the SPF value, which substitutes *in vitro* method published utilizing the UV spectrophotometry [16]. SPF values were calculated using the Mansur equation 1**.**

Mansur equation ----------(1)

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times I(\lambda) \times \mathrm{Abs}(\lambda)$$

EE (λ) –Erythemal Effect spectrum, I (λ) – Solar Intensity spectrum, Abs (λ) – Absorbance of sunscreen product, CF – Correction Factor (10), The values of EE x I are constants and predetermined.

Herbal cream formulation

Different semisolid emulsion-base oil in water (O/W) formulations were prepared according to the compositions given in the (Table 1).

TABLE 1. **The composition of different formulations (F1-F6) prepared of herbal cream with *Canna* (red) flower extract (g)**

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Freeze dried powder of flower extract	0.400	0.400	0.500	0.500	0.500	0.500
Stearic acid	0.500	1.000	1.000	0.800	0.800	0.800
Cetyl alcohol	0.100	0.200	0.200	0.200	0.200	0.200
Bees wax	1.500	0.500	0.500	0.500	0.500	0.500
Olive oil	2.000	1.000	1.000	1.000	1.000	1.000
Polyethylene glycol	0.200	0.200	0.200	0.200	0.300	0.300
Triethanolamine	0.135	0.135	0.135	0.130	0.135	0.130
Rose water	2.000	1.000	1.000	1.500	1.500	1.500
Moisturizing conditioner (Aloe)	1.500	1.500	1.500	2.000	2.500	2.200
Methylparaban	0.020	0.020	0.020	0.020	0.020	0.020
Ethylene diaminetetraacetic acid	0.010	0.010	0.010	0.010	0.010	0.010
Araliya oil (perfume)	Qs	Qs	Qs	Qs	Qs	Qs
Water	Qs	Qs	Qs	Qs	Qs	Qs

Qs = Quantity sufficient

Evaluation of physical parameters of the cream

The physical stability test was performed for all formulations (F1-F6) prepared, to determine the properties of pH (using pH meter), appearance (odor, colour and roughness), homogeneity (by visual appearances, the touch appearances of the formulations), wash ability (washing with tap water) for 45 days at room temperature. The most stable formulation was selected and subjected for further analysis.

Determination of radical scavenging activity of the cream by DPPH assay

The radical scavenging activity of the most stable cream formulated was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and expressed as mmol Trolox equivalent (TE)/100 g of the cream. The results were expressed as mmolTrolox equivalents to 100g of the cream [15].

Determination of ferric-reducing antioxidant power of the cream by FRAP assay

Ferric reducing antioxidant power (FRAP) assay was used to determine the antioxidant power of the most stable cream formulated and the results were expressed in mmol Fe(II) equivalent/100 g of the cream [15].

Evaluation of in-vitro sun protective factor (SPF) of the cream

Mansur mathematical equation was used to calculate the SPF value, utilizing the UV spectrophotometry [16]. The cream was dissolved in methanol and a concentration series of solutions 0.5 mg/ml, 1.0 mg/ml and 1.5 mg/ml was prepared. A series of positive control was prepared by dissolving Dermatone in methanol (0.1 mg/ml, 0.5 mg/ml, 1.0 mg/ml and 1.5 mg/ml). SPF values were calculated using the Mansur equation and normalized product function given**.**

Statistical analysis

All experimental measurements were triplicated, and the results were expressed as mean ± SD.

Results and Discussions

Total phenolic and total flavonoid contents of flower extract

The high values of total phenolic content (5389.067 ± 681.343 mg Gallic acid equivalent (GAE)/100 g dry weight (DW) of flowers) and total flavonoid content (6017.442 ± 158.343mg Catechin equivalents (CAE)/100 g DW of flowers) were exhibited by the extract tested.

Ferric-reducing antioxidant power and radical scavenging activity of the *Canna* flower extract

In vitro antioxidant activity of 70% aq. acidified acetone extract was evaluated for 5 mg/ml concentration of samples and the results were expressed as 43.742 ± 2.047 mmol Fe(II) equivalents/100 g DW of the flowers. Radical scavenging activity (17.430 ± 2.673 mmolTrolox equivalents/100 g DW of flowers) was exhibited by the acidified 70% aqueous acetone extract. The results of this study revealed that the acidified 70% aqueous acetone extract of dried *Canna* (red) flowers has promising antioxidant activity by both FRAP and DPPH assays. These results are in the agreement with the data published by Srivastava & Vankar in 2010 for the antioxidant activity of Indian *Canna indica* flowers [17].

Sunscreening activity of the *Canna* flower extract

It was noticed that promising sunscreening activity (SPF=32.75) for acidified 70% aqueous acetone extract whereas Dermatone showed SPF=35.48 at the concentration of 1 mg/ml. The findings obtained from this study for sun screening activity, are with the agreement of the results shown by Patel, Naikawade, Magdum, & Sathe,

in 2016 on the study of UV absorption activity of flower extracts obtained from *Canna indica Linn* (Scitaminaceae) and *Clitoriaternata Linn* (Fabaceae) at the range of 200 to 400 nm, due to the presence of flavonoids. .

Herbal Cream formulation and testing for the physical stability of the cream

F1-F6 formulations prepared were tested for their physical stability and **F4** was selected as the most stable formulation, and subjected for further analysis. The results of the physical parameters obtained for formulation **F4** were shown in Table 2 . The herbal cream formulation was found to be semi solid, homogeneous, emollient, not greasy with good spread ability, washable with water and pink caramel in colour with pleasant odor. The pH of the cream was found to be in the range of 6 to 7, which is good for skin pH.

TABLE 2. **Results of physical parameters of the formulated herbal cream**

Parameters	**Cream with AAD extract**
Appearance (Colour)	Pink caramel
Odor	Pleasant
pH	6-7
Homogeneity	Good
After feel (Emolliency)	Emollient
Spread ability	Good
Type of smear	Not greasy
Removal	Easy

Results of physical parameters of the stability study during 45 days tested

The results of physical parameters observed on 1st, 5th, 30th and 45th day are shown in the Table 3 and no remarkable change of the physical parameters observed at room temperature during 45 days.

TABLE 3. **Results of physical parameters of stability studies of the formulated cream**

Day	**C**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
1	RT	6-7	NCC	P	G	E	G	NG	ES
5	RT	6-7	NCC	P	G	E	G	NG	ES
30	RT	6-7	NCC	P	G	E	G	NG	ES
45	RT	6-7	NCC	P	G	E	G	NG	ES

C: Condition; **A1**: pH; **A2**: Appearance; **A3**: Odor **A4**: Homogeneity; **A5**: After feel; **A6**: Spread ability; **A7**: Type of smear; **A8**: Removal; **NCC**: No change in colour; **P**: Pleasant; **G**: Good; **E**: Emollient; **NG**: Not greasy; **ES**: Easy

In vitro radical scavenging activity, ferric reducing power activity and sunscreening activity of the formulated cream during 45 days tested

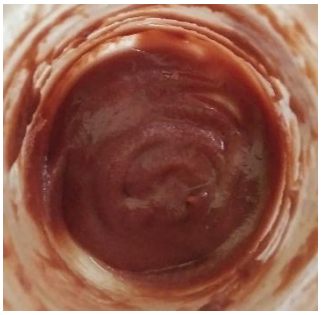
The results of radical scavenging activity of formulated cream which contains 70% acidified aqueous acetone dried flower extract of *Canna* (red) indicated promising values (3.881 mmolTrolox equivalents/100 g weight of the cream by DPPH assay) and antioxidant activity (10.422 mmol Fe(II) equivalents/100 g weight of the cream by FRAP assay). The SPF values of the formulated herbal cream at different concentrations were evaluated and the results revealed that cream formulated has promising sunscreening activity (SPF=37.73) at the concentration of 7 mg/ml of cream. The results showed a concentration dependent response as well. Results of *in vitro* sunscreening activity and antioxidant activity of the formulated cream after 1st, 5th, 30th and 45th day at room temperature were recorded (Table 4 , Figure 4).

TABLE 4. **Results of *in vitro* sunscreening activity and antioxidant activity of stability studies of the formulated herbal cream**

Parameters	**1day**	**5 day**	**30 day**	**45 day**
Sunscreening activity (SPF)	37.73	37.70	37.43	37.30
By DPPH assay (mM Trolox/100 g cream)	3.881	3.879	3.860	3.755
by FRAP assay mmol Fe(II)/100 g cream	10.422	10.420	10.412	10.405

RT: Room temperature

The findings of a research conducted by More, Sakharwade, Tembhurne, & Sakarkarin in 2013, has shown that the formulated creams with *Butea monosperma* leaf extract were having potency to protect against UV-rays and good SPF against UVB. Formulations depending on various concentrations have shown satisfied protection against both UVA and UVB rays [18].



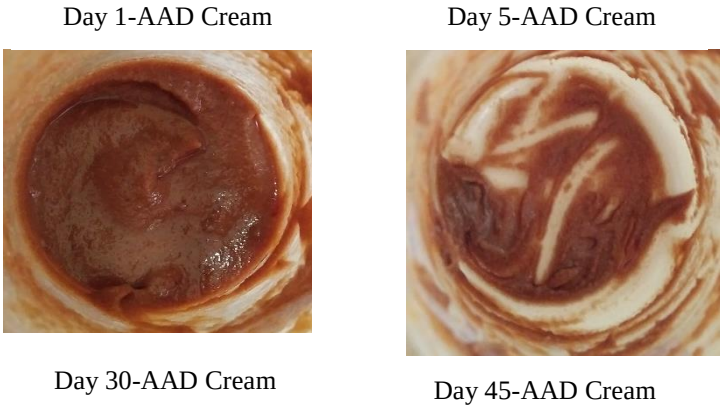


FIGURE 4. **Results of stability study of the herbal cream**

Conclusion

In the present research study, an attempt has been made to formulate a novel herbal sunscreen cream with promising antioxidant activity enriched with *Canna* (red) flowers grown in Sri Lanka. Hence, herbal sunscreen cream with acidified 70% aqueous acetone dried flower extract of *Canna* (red) was formulated and subjected for further analysis. The results revealed SPF as 37.73 at the concentration of 7 mg/ml of cream while antioxidant activity 3.881 ± 0.033 mmol TE/100 g weight of the cream by DPPH assay and 10.422 ± 0.055 mmol Fe(II) equivalents/100 g weight of the cream by FRAP assay at the concentration of 50 mg/ml of the cream enriched with Canna (red) flowers. The sunscreening activity and antioxidant activity of formulated herbal cream showed concentration dependent responses. The formulated herbal sunscreen cream was found to be semi-solid, homogenous, emollient, non-greasy with good spread ability, washable with water and pink caramel colour with pleasant odor. The pH of the cream was found to be in the range of 6 to 7 which is good for skin pH. The stability parameters of the formulated herbal cream showed that there was no remarkable variation during the period of the study (45 days at room temperature), therefore it is concluded that the *Canna* (red) flowers and the formulated herbal sunscreen cream is having promising sunscreening and antioxidant activity thereby the formulated cream can be commercialized as a novel herbal sunscreen cream with antioxidant activity.

REFERENCES

1. D'Orazio J, Jarrett S, Amaro-Ortiz A, et al. (2013) UV radiation and the skin. Internationa l Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI).
2. Bens G (2014) Sunscreens. Advances in Experimental Medicine and Biology, 810, 429 –63.
3. González S, Fernández-Lorente M, Gilaberte-Calzada Y (2008) The latest on skin photoprotection. Clinics in Dermatology, 26(6), 614–626.
4. Ghiasvand R, Weiderpass E, Green AC, et al. Sunscreen Use and Subsequent Melanoma Risk: A Population-Based Cohort Study. Journal of Clinical Oncology, 34(33), 3976–3983.
5. Vanicek K., Frei T, Litynska Z, et al. UV- Index for the Public. Publication of the European Communities, Brussels, Belgium, (28), 1.

6. Manaia EB, Kaminski R C K, Corrêa M. A., et al. (2013) Inorganic UV filters. Brazilian Journal of Pharmaceutical Sciences, 49(2), 201–209.
7. Picatonotto T, Vione D, Carlotti M, et al. Photocatalytic Activity of Inorganic Sunscreens. Journal of Dispersion Science and Technology, 22(4), 381–386.
8. Saewan , Jimtaisong A (2013) Photoprotection of natural flavonoids.
9. Korać R R, Khambholja KM (2011) Poten tial of herbs in skin protection.
10. Serpone N, Dondi D, Albini A (2007) Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare products. Inorganica Chimica Acta, 360(3), 794–802.
11. Dunford R, Salinaro A, Cai L, et al. Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Letters, 418(1–2), 87–90.
12. Al-Snafi, A E (2015) Bioactive Components and Pharmacological Effects Of Canna Indica-An Overview. International Journal of Pharmacology & Toxicology, 5(2), 71–75.
13. Behr S, Duret P, Gendron N, Guay J, et al. Plant Extracts and Dermatological Uses Thereof.
14. Saija A, Tomaino A, Trombetta D, et al. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. International Journal of Pharmaceutics, 199(1), 39–47.
15. Hettihewa SK. Extraction, separation, Characterization of and in vitro biological actitivites of Actinidia macrosperma (A Wild Kiwifruit), Thesis, University of Auckland, New Zealand. 2014.
16. Hettihewa SK, Opatha SAT. (2020) A comp arative study on sunscreening.
17. Srivastava J, Vankar PS (2010) Canna indica flower: New source of anthocyanins. Plant Physiology and Biochemistry, 48(12), 1015–1019.
18. More BH, Sakharwade SN, Tembhurne S V, et al. Evaluation of Sunscreen activity of Cream containing Leaves Extract of Butea monosperma for Topical application. International Journal of Research in Cosmetic Science, 3(1), 1–6.