ORIGINAL ARTICLE

Development and evaluation of anti-inflammatory ointment using aqueous extract of *Coccinia grandis* L.

Saraswathy Nachimuthu, Krithika Suresh, Balaji Sadhasivam, Veerabhuvaneshwari Veerichetty, Ramalingam Ponnusamy

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India

Abstract

Background: According to global burden of disease study that was measured in disability-adjusted life years, skin diseases contribute to 1.79% of the global burden when compared to other serious diseases. *Coccinia grandis* (C.G.) is used in folk medicine to treat various skin disorders and diseases. The present study was carried to evaluate the *in vitro* anti-inflammatory activity of aqueous extract of C.G. and to formulate an ointment to treat the skin inflammation. **Materials and Methods:** The aqueous leaf extract of this plant was assessed for their *in vitro* antioxidant by 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid and ferric reducing antioxidant power assays and anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method. The ointment formulations containing extract of the above-mentioned herb were formulated and their physical parameters such as color, odor, pH, spreadability, consistency, diffusion, solubility, washability, and stability were evaluated. **Results:** The membrane stabilization anti-inflammatory test showed that the aqueous leaf extract exhibited similar membrane stabilizing activity of 90.14 ± 0.942% compared to that of standard indomethacin which exhibited 93.63 ± 0.910% at 10 mg/ml concentration, respectively. **Conclusion:** The physical evaluation of ointment indicated that the formulation C.G IV showed better suitability than other formulations and their effectiveness should be explored further in harnessing the potential of the plant for treating skin inflammatory diseases. **Clinical Impact:** At present, there is no effective cure for skin inflammatory diseases, but it can be controlled by various treatments, namely topical, drugs, and biological. However, most of these therapies are of higher cost, causing a number of side effects that include organ toxicity, carcinogenicity, and immunosuppression and are effective only for short term. The present study shows that the C.G. ointment will be useful for the treatment of skin inflammatory diseases.

Key words: Anti-inflammatory, antioxidant, *Coccinia grandis*, ointment, skin inflammation

INTRODUCTION

When the tissue gets injured due to microbial infections, physical trauma, chemical agents, tissue necrosis, and hypersensitivity reactions, human body activates the inflammatory reactions to counteract the damage and heal them.[1] If the regulatory mechanisms of the inflammatory responses are defective or if there is an inappropriate immune response, an acute inflammatory response may progress to the chronic stage. The most common problem in dermatology is the inflammatory skin diseases, which are associated with mild conditions in case of rashes, itching, redness, and irritation, to chronic conditions such as eczema, rosacea, seborrheic dermatitis, and psoriasis. According to global burden of disease study that was measured in disability-adjusted life years, skin diseases contribute to 1.79% of the global burden when compared to other serious diseases.[2] The commonly available treatment options for inflammation are the usage of steroids. Nevertheless, the steroids are often accompanied with systemic reactions in the body such as hyperglycemia, glaucoma, immunosuppression, adrenal insufficiency, complications in gastrointestinal tract, renal failure, and cardiovascular effects.[3] Recently, biological agents are considered effective for treating inflammation when all other alternatives have failed, but it is much costly and sometimes linked with immunosuppressive effects.[4]

Address for correspondence:
Dr. Saraswathy Nachimuthu, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India. Phone: 0422-2661100.
E-mail: saraswathy.n.bt@kct.ac.in

Received: 07-08-2018
Revised: 14-09-2018
Accepted: 21-09-2018

Medicinal plant extracts have long been used as an efficient therapeutic in traditional medicine because of its benefits such as patient compliance, minimal or no side effects, easy availability, low cost, and multidirectional mechanism of action. The major drawback for its commercial usage is the lack of availability of pharmacopeias standardization. *Coccinia grandis* (C.G.) (ivy gourd) belonging to the Cucurbitaceae family is found around the tropical regions of India, Africa, and Asia. Various parts of this plant are used in treating skin disease, asthma, bronchitis, joint pains, tuberculosis, tongue sores, and cerebral oxidative stress.[5] Unani system of medicine has revealed the usage of C.G. for treating ringworm, psoriasis, smallpox, scabies, and other itchy skin eruptions.[6] Free radicals are highly unstable molecules, leading to various diseases such as inflammatory diseases, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, cancer, atherosclerosis, diabetes mellitus, hypertension, and aging.[7] Antioxidants are agent that prevents damage to biological molecules such as lipids, proteins, enzymes, carbohydrates, and DNA by neutralizing the free radicals.[8] It has been reported that inflammatory processes are associated with an increase in reactive nitrogen and oxygen species produced by leukocyte activation.[9] Thus, the plant possessing antioxidant property will have good anti-inflammatory activity. During inflammation, lysosomal constituents are released into the cytosol which results in tissue damage, leading to various disorders such as heart attacks, septic shocks, and rheumatoid arthritis.[10] Hence, by inhibiting the discharge of inflammatory mediators, the effect of inflammation can be reduced. Human red blood cell (HRBC) membrane stabilization method is the principle *in vitro* assay to study the anti-inflammatory activity of plant extracts.[11] Herbal extract that offers significant protection to erythrocyte membrane has a potential anti-inflammatory activity.[12] The dermal delivery of drug is highly suitable for treating skin inflammation, because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks, and non-persistent nature of the treatment.[13] Ointments offer a protective barrier as well as controlled delivery system for active ingredients into the skin. Herbal drugs are formulated in the form of ointment and are used topically for several purposes such as antiseptics, emollients, astringents, antipruritic, and keratinolytics.[14] Hence, an effort has been made to establish the scientific validity to investigate the possible antioxidant and anti-inflammatory activity of the aqueous extract of C.G. and also to prepare different formulations of the ointment containing the herbal extract and perform possible evaluation tests for ointments, which may further unfold its potential for future drug development.

MATERIALS AND METHODS

Chemicals

2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), neucoprine, ammonium persulfate, ammonium acetate, N-1-naphthyl ethylenediamine, sulfanilamide, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride ($FeCl_3$), cupric chloride, dextrose, sodium citrate, citric acid and sodium chloride, cetostearyl alcohol, petroleum jelly, and hard paraffin were purchased from HiMedia Ltd., Mumbai, India. All other chemicals and solvents used were of analytical grade.

Plant Material

The fresh leaves of C.G. L. Voigt. Cucurbitaceae were collected in and around Coimbatore district, Tamil Nadu, India, during the month of July 2017 and were authenticated by Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore (Ref No BSI/SRC/5/23/2017/Tech.884), dated July 25, 2017.

Preparation of Plant Extract

The collected plant part (leaves) was separated from undesirable materials and washed with distilled water. The leaves were dried under shade for 3 weeks. The dried leaf samples were ground into a coarse powder using a clean blender and stored in polythene bags at room temperature. About 10 g of plant powder was loaded into Soxhlet extractor and subjected to extraction with water. The extract thus obtained was dried and stored at 4° C until further use.

Estimation of Phenol

A method proposed by Singleton and Rossi[15] was adopted to determine the total phenolic content. To 0.1 ml of the extract, added 3.9 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent. The tube was incubated at 37° C for 3 min. About 2 ml of 7% (w/v) sodium carbonate was added to the reaction mixture and the contents were kept in a boiling water bath for 1 minute. The blue color formed was read against a blank at 650 nm. Quantification was done using the standard curve of gallic acid (20–100 µg/ml). The results were expressed in gallic acid equivalents (GAE) mg GAE/g of the dry weight.

Estimation of Flavonoid

Total flavonoid content was estimated spectrophotometrically by the method of Aiyegroro and Okoh (2010)[16] with minor modifications. About 1 ml of sample extract was mixed with 3 ml of methanol, 0.3 ml of 10% (w/v) aluminum chloride, 0.2 ml of 1 M potassium acetate, and 6 ml of distilled water, and the reaction mixture was kept at 37° C for 30 min. The absorbance was measured at 510 nm. Rutin was used as the standard for constructing a calibration curve (50–250 µg/ml). The results were expressed as mg of rutin equivalent RE/g of the dry weight of extract (DW).

In Vitro Antioxidant Activity

ABTS radical scavenging assay

The ABTS assay was done using the method of Rakholiya *et al*.[17] The ABTS radical cations (ABTS+) were produced by reacting ABTS (solution (7 mM) with an equal volume of ammonium persulfate solution (2.45 mM). The mixture was left at the room temperature in the dark for 16 h before use. The initial absorbance was measured at 745 nm. The extracts of varying concentrations (2–10 mg/ml) were prepared. About 30 µL of the sample added to 1 ml of the ABTS solution was allowed to stand at room temperature. After 6 min, the absorbance was measured at 744 nm immediately in the spectrophotometer.

The assay mixture without extract was set as control. Trolox solution was used to prepare the standard curve. The percentage inhibition of the ABTS radical by the antioxidants in the extract was determined by:

$$\%\,\text{inhibition} = \frac{\text{O.D. of Control} - \text{O.D. of Test}}{\text{O.D. of Control}}$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing activity of the plant material extract was estimated based on the method of Benzie and Strain.[18] The FRAP reagent was prepared by mixing 90 ml of acetate buffer (0.2 M, pH 3.6), 9 ml of $FeCl_3$ solution (20 mM), and 9 mL of TPTZ solution (10 mM) prepared in HCl. About 2.7 mL of the freshly prepared FRAP reagent was added to the 90 µL of extract, along with 270 µL of water. Then, the absorbance was measured at 595 nm against the blank after 30 min incubation at 37° C. Trolox was used to prepare the standard curve.

In Vitro Anti-inflammatory Activity

HRBCs membrane stabilization assay

HRBC stabilization evaluation of the leaf extracts was carried out according to the Bouhlali *et al*.[19] method with some modifications. About 10 mL of fresh whole human blood was collected from volunteers (who had not taken any NSAIDS for 2 weeks before the experiment) and mixed with equal volume of sterilized Alsever's solution which was prepared by dissolving 2% (w/v) dextrose, 0.80% (w/v) sodium citrate, 0.05% (w/v) citric acid, and 0.42% (w/v) sodium chloride in distilled water. The reaction mixture was then centrifuged at 2500 rpm for 10 min and the supernatant was removed. The cell suspension was washed with isosaline (0.85% sodium chloride, pH 7.2) and centrifuged at 2500 rpm for 5 min. This step was repeated until the supernatant was clear and colorless. The cellular component was reconstituted to 10% (w/v) with isosaline. Plant extract at various concentrations (1000, 2000, 3000, 4000, and 5000 µg/ml) was prepared using distilled water. About 1 ml phosphate buffer (0.15 M, pH7.4), 2 ml hyposaline (0.36% sodium chloride), and 1 ml HRBC suspension were added to each ml of plant extract. Indomethacin at different concentrations (1000, 2000, 3000, 4000, and 5000 µg/ml) was used as the reference drug and the control was prepared without the extracts. The assay mixtures were incubated at 37° C for 30 min, and the contents were centrifuged at 3000 rpm for 20 min. The hemoglobin content in the supernatant solution was measured at 560 nm using spectrophotometer.

The percentage of membrane stabilization activity was calculated using the formula:[20]

$$\%\,\text{Inhibition} = \frac{\text{O.D. of Control} - \text{O.D. of Test}}{\text{O.D. of Control}}$$

Half Maximal Inhibitory Concentration (IC_{50}) Calculation

A standard calibration graph was plotted with concentration of plant extract or standard drug against percentage inhibition. Linear regression analysis was used to determine the IC_{50} value. IC_{50} is the concentration of extract at which 50% of the target is inhibited.

Statistical Analysis

All tests and analyses were done in triplicate and the results were the average of triplicate determinants. Data were represented as mean ± standard error of mean. Statistical analyses were performed by Student's *t*-test using Microsoft Excel 2010 version and $P < 0.05$ versus control was considered to be statistically significant.

Formulation of Ointment

Four different topical ointments were formulated based on varying degrees of plant extract and type of scent material. The constituents of the base, namely cetostearyl alcohol, petroleum jelly, and liquid paraffin were weighed and melted at 70° C. After melting, the ingredients were stirred gently for 5–10 min and then other agents including aqueous extract in different concentrations and scent material were added until the mixture congeals to become a homogenous mass. The blank ointment base without plant extract served as the control. The prepared herbal ointments were kept in ointment jars, labeled, and stored at 25° C. The compositions of different herbal ointment are listed in Table 1.

Physical Evaluation of Ointment

Preliminary evaluation of formulations was carried out according to Panigrahi *et al*.[21]

Color and odor

Organoleptic parameters such as color and odor were carried out by visual examination.

Table 1: Composition of ointment formulations of the C.G. extract

Composition	C.G. - I	C.G. - II	C.G. - III	C.G. - IV	Control
Cetostearyl alcohol (g)	1.25	2.5	1.265	2.5	2.5
Petroleum jelly (g)	12	11	12	11	11
Liquid paraffin (ml)	11	10	11	10	10
Extract (g)	0.75	1.5	0.75	1.5	1.5
Scent (ml/mg) (rosewater/camphor)	5	5	160	160	160
Total (g)	30	30	25	25	25

Coccinia grandis ointment formulations - C.G. - I, C.G. - II, C.G. - III, and C.G. - IV

Consistency

Smooth and greasiness of the ointment were observed by visual examination.

pH

The pH of various formulations was determined using digital pH meter. About 0.5 g of the ointment was dispersed in 50 ml distilled water, and the contents were dissolved by stirring and then were kept for 2 h.

Loss on drying (LOD)

LOD was determined by placing ointment in Petri dish on water bath and dried for 105° C.

Homogeneity

All the developed ointments were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Solubility

Solubility of the ointment in various solvents, namely water, boiling water, methanol, ethanol, petroleum ether, and chloroform was established.

Stability study

The stability studies were carried out in all formulations at different temperature conditions (4° C and 25° C) for 4 weeks.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the ointments readily spread on application to skin or affected part. It was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Spreadability was calculated using the formula: [22]

$$S=(M*L/T)$$

Where, S=Spreadability,
M=Weight tied to upper slide,
L=Length of glass slides and
T=Time taken to separate the slides.

In this present experiment, M = 192 g, L = 10 cm, and T = variable depending on the sample were recorded.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. A hole was bored at the center of the medium and ointment was placed in it. The time taken for the ointment to get diffused after 60 min was noted.[23]

RESULTS

Total Phenolic and Flavonoid Content

Phenolics and flavonoid compounds in plant extracts are associated with antioxidant and anti-inflammatory activity.[24] The standard graph of gallic acid and rutin obtained for the estimation of total phenolic and flavonoid contents from C.G. is depicted in Figure 1a and b. The presence of phenolic and flavonoid content in C.G. was 0.659 ± 0.018 mg GAE/g DW and 0.575 ± 0.02 mg RE/g DW, respectively. The present plant leaf extract was found to show reported by Sathishkumar and Baskar[25] and Gomathy *et al*.[26]

In Vitro Antioxidant Activity

The reduction of the blue-green complex of ABTS radical cation by hydrogen-donating antioxidant is measured at a wavelength from the visible region. Free radical scavenging potential (ABTS) of different concentrations of extract and standard ABTS is shown in Figure 2a and b, respectively. It was observed from Figure 2b that an increase in free radical scavenging activity of the extract was concentration dependent. The inhibition of the extract was 71.53 ± 0.805% at 8 mg/ml and that of Trolox standard was 75.21 ± 1.285% at 1 mg/ml. The IC_{50} of standard was 0.645 mg/ml while the C.G. extract revealed 5.049 mg/ml.

The reducing ability of a compound depends on the presence of antioxidants, which exert their action by breaking the free radical chain by donating a hydrogen atom.[27] FRAP assay measures the reduction of ferric tripyridyltriazine [Fe^{3+}-TPTZ] to the colored product ferrous tripyridyltriazine [Fe^{2+}-TPTZ]. The ferric reducing antioxidant potential of different concentrations of extract and standard were represented in Figure 3, respectively. The absorbance of C.G. clearly increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration that represents increase in antioxidant activity.

The values observed were generally low compared with those reported in other studies. Umamaheswari and Chatterjee[28] showed that the chloroform fraction of the C.G. has strong radical scavenging activity with the IC_{50} value of about 0.145 mg/ml when compared to that of standard ascorbic acid with 0.03 mg/ml. Another study reported that powder form and methanolic extract of C.G. leaves showed good antioxidant property, whereas aqueous extract did not show any significant activity.[29] There was no significant difference between the antioxidant activity of the standard (Trolox) and the plant extract. Thus, the low antioxidant activity of the extract in comparison with the standard Trolox can be attributed to the usage of water as solvent for the present study. The major reason for performing aqueous extraction is that the organic solvents that are generally used for plant extraction are highly toxic due to petrochemical residues and cause irritation when a product is developed.

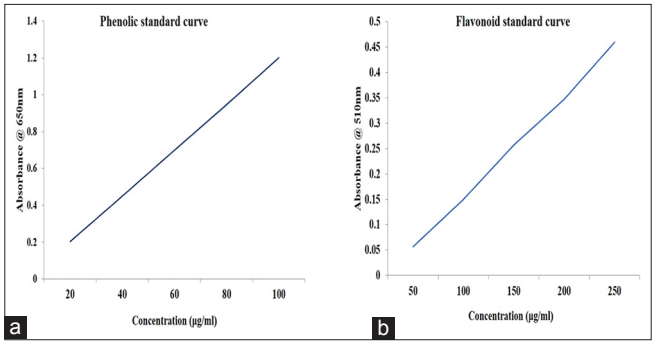

Figure 1: (a) Phenolic standard curve, (b) flavonoid standard curve. Each value is mean ± standard error of mean, $n = 3$

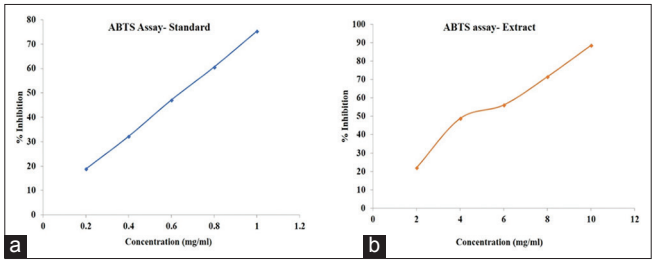

Figure 2: 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity of (a) standard, (b) extract. Each value is mean ± standard error of mean, $n = 3$. *$P < 0.05$ represents the statistical significance of data

In Vitro Anti-inflammatory Activity

The erythrocyte membrane is considered as an analogous to the lysosomal membrane, and hence, the stabilizing effect of drugs on erythrocyte membrane denotes the stabilization of lysosomal membrane. When the red blood cell membrane is exposed to injurious substances such as hypotonic medium, there is an excessive accumulation of fluid from the medium into the membrane that ultimately results in hemolysis and oxidation of hemoglobin. The destruction of erythrocytes will further render the cell susceptible to secondary damage through free radical-induced lipid peroxidation.[30]

The percent inhibition of hemolysis by different concentrations of plant extract and standard was represented in Figure 4. It was observed from Figure 4 that aqueous extract of C.G. leaves exhibited highest membrane stabilizing activity of 90.14 ± 0.942% compared to that of standard indomethacin which exhibited 93.63 ± 0.910% at 10 mg/ml concentration.

The HRBC stabilizing activities of the extracts were concentration dependent, the membrane stabilizing activity increased with increasing concentration. It has been reported that an infusion of indomethacin alters the cerebral, mesenteric, and renal blood flow.[31] Since the percentage hemolysis of both the extract and standard was not statistically significant,

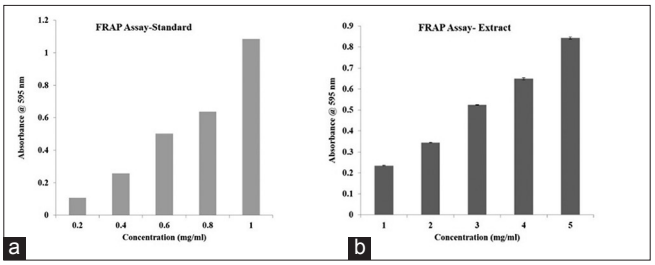

Figure 3: Ferric reducing antioxidant power assay of (a) standard, (b) extract. Each value is mean ± standard error of mean, $n = 3$. *$P < 0.05$ represents the statistical significance of data. $P = 0.99$

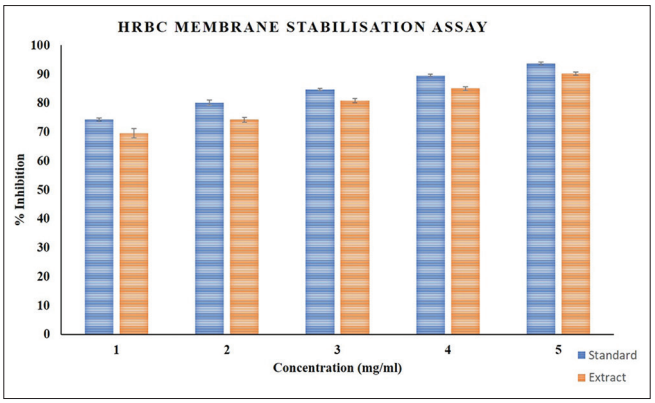

Figure 4: Percentage inhibition of hemolysis by standard and extract. Each value is mean ± standard error of mean, $n = 3$. *$P < 0.05$ represents the statistical significance of data. $P = 0.39$

it is better to use the extract for the anti-inflammatory activity which is considered to have fewer side effects.

Recently, Dependra and Singh[32] reported the membrane stabilization activity of two plant extracts *Acorus calamus* and *Vexillum articulatum* and they also compared them. It was found that *A. calamus* provided slightly more inhibition of denaturation of 76.8% than that of standard drug indomethacin (72.8%), whereas *V. articulatum* provided slightly lesser inhibition (68%) in comparison to the drug. The methanolic extract of C.G. fruits exhibited an anti-inflammatory activity of 44.46% and 84.63% for the concentration of 100 mg/ml and 500 mg/ml, respectively, estimated by HRBC membrane stabilization method.[33] In the line of these studies, Niazi *et al*. [34] also reported the anti-inflammatory activity of *Coccinia indica*, which is in good agreement with our study. However, the present study was undertaken under *in vitro* condition, whereas they have used *in vivo* method using carrageenan-induced paw edema method, which is the major difference between the two studies. A dose-dependent anti-inflammatory effect was observed in the dose range of 25–300 mg/kg, which was equivalent to the standard diclofenac. Further, it was suggested that the effectiveness shown by the extract in the early phase of inflammation may be due to the inhibition of histamine and serotonin release.

There have been several studies in the literature that explains about the mechanism of action of these extract on membrane stabilization. According to Shinde *et al*.,[35] the inhibition of hemolysis is brought about by the increase in surface area/volume ratio of the cells, due to the expansion of membrane and interaction of membrane proteins with the RBC membrane. A possible reason for the stabilizing activity of the extracts may be due to their ability to adjust the intracellular concentration of calcium into the membrane.[36] It can also be due to the binding of phytochemical compounds in the extract to the erythrocyte, thereby changing the charges in their surfaces.[37]

Formulation of Ointment

The present study was done to prepare an herbal ointment of the aqueous extract of C.G. by fusion method using different percipients and different concentrations of extract (3% and 6%) that was compared along with the control without extract.[38] The mechanical evaluation parameters such as pH, spreadability, solubility, diffusion, stability study, as well as irritant effect were evaluated. The pH of all the formulations lies in the range of 4.3–6. The result of spreadability denotes the extent of area to which the prepared formulations readily spreads on application to skin and homogeneity confirms no lump. The solubility study done in different solvents disclosed that the ointments were miscible with chloroform and petroleum ether. The diffusion study revealed the diffusibility of ointment into the agar medium and the stability study observed for 4 weeks indicated that there was no change in the prepared ointments during the treatment period.

An ointment formulation with an herbal gel containing the ethanolic and aqueous fruit extract of *C. indica* that was found effective for wound repair.[39] Another study revealed an ointment formulation with the herbal plant *Cajanus scarabaeoides* containing 1 g of the extract in 10 g of ointment base showed the highest rate of wound closure.[40] The different formulation of ointments and physical parameters are shown in Tables 2-4 and Figure 5, respectively. Results indicated that the formulation C.G. IV showed better suitability than the other formulations for the production of ointment.

Table 2: Physical tests of ointment formulations of the C.G. extract

Formulation	Color	Odor	Consistency	pH	LOD (%)	Homogeneity
C.G. - I	Brown	Characteristic	Smooth and no greasiness	4.70	99.35	Passed
C.G. - II	Brown	Characteristic	Smooth and no greasiness	5.33	98.22	Passed
C.G. - III	Chocolate brown	Good	Smooth and no greasiness	5.28	99.75	Passed
C.G. - IV	Dark chocolate brown	Good	Smooth and no greasiness	6.03	99.19	Passed
Control	White	Good	Smooth and no greasiness	4.80	98.63	Passed

Coccinia grandis ointment formulations - C.G. - I, C.G. - II, C.G. – III, and C.G. - IV. LOD: Loss on drying

Table 3: Physical evaluation of different ointment formulations of the C.G. extract

Formulation	Spreadability (sec)	Diffusion (mm)	Stability	Washability
C.G. - I	7	-	Stable	Good
C.G. - II	6	12	Stable	Good
C.G. - III	6	4	Stable	Good
C.G. - IV	5	13	Stable	Good
Control	12	-	Stable	Good

Coccinia grandis ointment formulations - C.G. - I, C.G. - II, C.G. - III, and C.G. - IV

Table 4: Solubility study on ointment formulations of the C.G. extract

Solvents	C.G. - I	C.G. - II	C.G. - III	C.G. - IV	Control
Water	Immiscible	Immiscible	Immiscible	Immiscible	Immiscible
Boiling water	Miscible	Miscible	Miscible	Miscible	Miscible
Methanol	Immiscible	Immiscible	Immiscible	Immiscible	Immiscible
Ethanol	Immiscible	Immiscible	Immiscible	Immiscible	Immiscible
Chloroform	Miscible	Miscible	Miscible	Miscible	Miscible
Petroleum ether	Miscible	Miscible	Miscible	Miscible	Miscible

Coccinia grandis ointment formulations - C.G. - I, C.G. - II, C.G. – III, and C.G. - IV

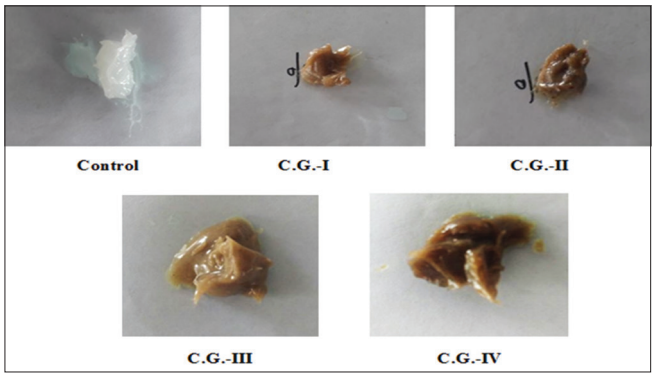

Figure 5: Photographic representation of ointment formulations

CONCLUSION

It was found from the present investigation that the aqueous extract of C.G. serves as a free radical scavengers or inhibitors acting as an antioxidant that has a possible effect on controlling inflammation. C.G. extract exhibited effective membrane stabilization in hypotonicity-induced lysis of erythrocyte membrane and was compared to that of standard drugs. This activity could be due to the strong presence of polyphenolic compounds such as alkaloids, flavonoids, glycosides, terpenoids, tannins, steroids, saponins, and phenols. The prepared ointment of different formulations containing the aqueous C.G. extract was evaluated for its physical parameters. The results of the physical evaluation of ointment preparation indicated that the formulation C.G. IV showed better suitability than the other formulations, and it can be suggested that the application of this ointment will be useful for the treatment of skin inflammatory diseases. Further, phytochemical studies are needed to isolate the purified active compound(s) that are responsible for the anti-inflammatory activity and also to understand the mechanism of anti-inflammation of C.G.

REFERENCES

1. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. Inflamm Res 1995;44:1-0.
2. Karimkhani C, Dellavalle RP, Coffeng LE, Flohr C, Hay RJ, Langan SM, *et al*. Global skin disease morbidity and mortality: An update from the global burden of disease study 2013. JAMA Dermatol 2017;153:406-12.
3. Coondoo A, Phiske M, Verma S, Lahiri K. Side-effects of topical steroids: A long overdue revisit. Indian Dermatol Online J 2014;5:416-25.
4. Ryan C, Korman NJ, Gelfand JM, Lim HW, Elmets CA, Feldman SR, *et al*. Research gaps in psoriasis: Opportunities for future studies. J Am Acad Dermatol 2014;70:146-67.
5. Alagarraja M, Rasika T, Monika G, Rajesh R, Rajavel E, Arunachalam G, *et al*. Updated review on pharmacognosy, phytochemistry and pharmacological studies of *Coccinia indica*. Int J Pharm Sci Res 2017;8:54-8.
6. Perry LM, Metzger J. Medicinal Plants of East and South-East Asia: Attributed Properties and Uses. Cambridge, London: MIT press; 1980.
7. Awah FM. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. J Med Plants Res 2010;4:2479-87.
8. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872-9.
9. Akula US, Odhav B. *In vitro* 5-lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. J Med Plants Res 2013;2:207-12.
10. Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) *Moore ssplaurina*. Bangladesh J Pharm 2008;3:121-4.
11. Lakshmi MA, Srinivas K, Rani SU, Praneetha V. Evaluation of *in vitro* anti-inflammatory activity of *Elaeocarpus ganitrus* of bark extract by HRBC membrane stabilization. Int J Pharm Biol Sci 2016;6:395-9.
12. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs 1995;49:345-61.
13. Daniels R, Knie U. Galenics of dermal products – vehicles, properties and drug release. J Dtsch Dermatol Ges 2007;5:367-83.
14. Mhatre J, Nagaral S, Kulkarni S. Formulation and evaluation of antibacterial activity of a herbal ointment prepared from crude extracts of *Aegle marmelos*, (Bael). Int J Pharm Pharm Sci 2014;6:575-9.
15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.

16. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med 2010;10:21.
17. Rakholiya K, Kaneria M, Chanda S. Vegetable and fruit peels as a novel source of antioxidants. J Med Plants Res 2011;5:63-71.
18. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
19. Bouhlali E, Sellam K, Bammou M, Alem C, Filalizehzouti Y. *In vitro* antioxidant and anti-inflammatory properties of selected Moroccan medicinal plants. J Appl Pharm Sci 2016;6:156-62.
20. Kumari CS, Yasmin N, Hussain MR, Babuselvam M. *In vitro* anti-inflammatory and anti-artheritic property of *Rhizopora mucronata* leave. Int J Pharm Sci 2015;6:482-5.
21. Panigrahi L, John T, Shariff A, Rani S, Hiremath R. Formulation and evaluation of lincomycin HCL gels. Indian J Pharm Sci 1997;59:330.
22. Multimer MN, Riffskin C, Hill JA, Marry E, Cyr NG, Glickman GJ. Synthesis of methyl silyl derivatives of procaine and their diffusion. J Pharm Sci 1956;45:212-20.
23. Sawant SE, Tajane MD. Formulation and evaluation of herbal ointment containing neem and turmeric extract. J Sci Innov Res 2016;5:149-51.
24. Chippada SC, Volluri SS, Bammidi SR. Vangalapati MI. *In vitro* anti-inflammatory activity of methanolic extract of *Centella asiatica* by HRBC membrane stabilisation. Rasayan J Chem 2011;4:457-60.
25. Sathishkumar T, Baskar R. Evaluation of antioxidant properties of *Tabernaemontana heyneana* wall. Leaves. Indian J Nat Prod Resour 2012;3:197-207.
26. Gomathy K, Baskar R, Kumaresan K. Comparison of antioxidant potential in pulp and peel extracts of *Manilkara zapota* (L.) P. Royen. Afr J Biotechnol 2013;12:4936-43.
27. Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. J Med Chem 2014;4:517-22.
28. Umamaheswari M, Chatterjee TK. *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. Leaf extract. Afr J Trad Complement Altern Med 2008;5:61-73.
29. Sargunam JH. Ivy gourd–medicinal and nutritional values. Int J Curr Res 2017;9:47604-7.
30. Ferrali M, Signorini C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. Biochem J 1992;285(Pt 1):295-301.
31. Gal P. Patent ductus arteriosus: Indomethacin, ibuprofen, surgery, or no treatment at all? J Pediatr Pharmacol Ther 2009;14:4-9.
32. Chamlagai D, Singh B. Study of *in vitro* anti-inflammatory activity of ethnomedicinal plants of Sikkim *Viscum articulatum* and *Acorus calamus*. Asian J Pharm Clin Res 2016;3:230.
33. Selvam V, Sumathy R, Kumuthakalavalli R. Phytochemical constituents and *in vitro* anti-inflammatory activity of an ivy gourd, *Coccinia grandis*. World J Pharm Pharm Sci 2014;3:1173-9.
34. Niazi J, Singh P, Bansal Y, Goel RK. Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia indica*. Inflammopharmacology 2009;17:239-44.
35. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 1999;70:251-7.
36. Chopade AR, Somade PM, Sayyad FJ. Membrane stabilizing activity and protein denaturation: A possible mechanism of action for the anti-inflammatory activity of *Phyllanthus amarus*. J Krishna Inst Med Sci Univ 2012;1:67-72.
37. Oyedapo OO. Biological activity of *Plyllanthus amarus* extracts on pragrow-dawley rats. Niger J Biochem Mol Biol 2001;26:202-26.
38. Kumar M, Alok S, Jain VA, Alok M, Sabharwal M. Morphology, pharmacological activity, pharmaceutical preparation, doses and side effect of *Coccinia indica* (Wight and Arn): An overview. J Coast Life Med 2013;1:330-6.
39. Bambal VC, Wyawahare NS, Turaskar AO, Deshmukh TA. Evaluation of wound healing activity of herbal gel containing the fruit extract of *Coccinia indica* wight and Arn. (*Cucurbitaceae*). Int J Pharm Pharm Sci 2011;3:319-22.
40. Pattanayak S, Nayak S, Dinda C, Panda D. Evaluation of herbal ointments formulated with methanolic extract of *Cajanus scarabaeides*. J Pharm All Health Sci 2011;1:49-57.

Source of Support: Nil. **Conflict of Interest:** None declared.