FORMULATION AND *IN-VITRO* EVALUATION OF ANTIOXIDANT GEL OF *CHOMOLAENA ODORATA*

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ABSTRACT

The phenolics present in the herbal extract of Chomolaena odorata was reported to possess admirable antioxidant and anti-aging potentials. The objective of the present work is hence to develop an antiaging gel of Chomolaena odorata and assess the potency of herbal formulation via in-vitro methods. Totally, eight batches of herbal gels were prepared with Carbapol 934 and the optimized formulation was selected on the basis of texture, homogeneity and physical stability. The optimized formulation was further evaluated for its physico-chemical parameters such as P^H, viscosity and spreadability; in-vitro antioxidant potential; skin irritancy and storage stability. In-vitro antioxidant potential was evaluated by by DPPH assay method by comparing with that of ascorbic acid. Skin irritancy was assessed via the Hen's egg test method. Among the eight batches, formulation F6 was found to possess good homogeneity, texture and physical stability and hence selected for further investigations. PH, viscosity and spreadability of the gel was 6.89±0.25, 318±10 cp and 50 ± 3.54 mm respectively. In-vitro assessment of skin irritation demonstrated no signs of toxicity; therefore, the product is safe for topical application. A lower IC50 value of herbal gel (155.6472±11.54 µg/ml) compared with that of vitamin C (843.8595±12.04 µg/ml) indicates that former possess super antioxidant potential. Hence the topical formulation of Chomolaena odorata might be effective for the management of skin aging.Present study demonstrated that Chomolaena odorata has a good potential for cosmetic product development. Researches should upgrade to translational clinical level to confirm these findings.

Keywords: *Chomolaena odorata, Antiaging herb, Antioxidant herb, herbal cosmetics, antiaging gel.*

INTRODUCTION

The oxidative damage induced by "Reactive Oxygen species" (ROS) was found to be the underline cause of skin a [1-4]. Thus, the foremost way for delaying skin aging is the regular use of antioxidants. Phenolics present in herbs are an effective source of antioxidants and possess a superior safety profile in comparison with synthetic antioxidants. *Chomolaena odorata,* commonly considered as a weed plant was recently reported to possess admirable antioxidant and antiaging properties [5-14]. *Chomolaena odorata* belongs to the family Asteraceae is native to the Americas, from Florida and Texas in the United States south through Mexico and the Caribbean to South America. It also has been introduced to tropical Asia, West Africa, and parts of Australia. The major active constituents of the plant are identified to be phenolic compoundssuch as protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic and vanillic acids;flavonoid aglycones such as flavanones, flavonols and flavone; alkaloids such as pyrrolizidine, saponins and tannins; terpenes and terpenoids; essential oils; phenolic acids such as ferulic acid, protocatechuic acid and phytoprostane compound such aschromomoric acid [5,14,15]. The plant has been already in use for wound healing, anti-inflammatory, anthelmintic, antimalarial, analgesic, , antipyretic, antispasmodic, antioxidant, antibacterial, antigonorrheal, antimycobacterial, insecticidal, fungicidal, , diuretic and blood coagulation activities [5].

The plant extract was found to possess excellent antioxidant potential [5-14] comparable with that of vitamin C [7,11]. Also, the Patent submitted by UNILEVER PLC reported that the topical application of *Chomolaena odorata* in cosmetic composition effectively prevent and treat skin aging markers such as appearance of wrinkles, lines, sagging, hyperpigmentation and age spots very few attempts had made to raise a commercial product [13]. The current shreds of evidence demonstrate the potential benefit of *Chomolaena odorata* in antiaging products. However, there no antiaging product of the herb are available though it possesses potent antiaging property. Hence, further researches focusing on formulation development and subsequent translational clinical research are required to develop a commercial antiaging product. The present investigation was undertaken for preparation and subsequent *in-vitro* evaluation of herbal gel of *Chomolaena odorata*. The herbal gel formulated was subjected to physicochemical, pharmacological and stability evaluations to confirm the potency and stability.

MATERIAL AND METHODS

Chemicals and reagents

Carbopol 934, Methyl paraben, Propyl paraben (SD fine-chem ltd) Propylene glycol, tri ethanolamine, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Isopropyl alcohol, chloroform and Glacial acetic acid, Million’s reagent, ferric chloride, (Spectrum reagents and chemicals pvt.ltd). DPPH Antioxidant Assay Kit (abcam).

Preparation of fresh extract of *Chromoleana odorata*

The fresh leaves were collected from college campus, prime college of pharmacy, kodumbu village, Palakkad district, kerala, India. The plant material was authenticated by Botanist, Dept of Botany, Victoria College Palakkad kerala, India (PEN NO: 656830 on 06.01.2022). The leaves were cleaned and fresh extract was collected by crushing the leaves.

Pre-formulation Studies:

Tests for flavonoids

The potent antioxidant activity of the plant extract is attributed to its flavonoid compounds. Thence the following tests were performed to confirm the presence of flavonoids in the fresh extract [16].

Table 1: Tests for flavonoids.

TEST
a) Alkaline test Test solution treated with sodium hydroxide solution. b) Ferric chloride test To the alcoholic solution of extract add few drops of neutral ferric chloride solution. b) Tyrosine test Heat 3 ml extract and 3 drops of Million's reagent.

Drug excipient compatibility studies

Test for physical incompatibility

Polymer: drug in 1:1 ratio has been mixed and stored for one month at room temperature (30-32°C) and analyzed for any visible change in Color / odour / Physical state of the powder.

Test for chemical incompatibility

The chemical compatibility between the herbal extract and excipients were analysed using Fourier Transform Infrared Spectroscopy (FTIR, Schimadzu IRSpirit) spectroscopy. FTIR spectra of pure extract, polymer and 1:1 mixture of extract and polymer were recorded and compared [17].

Formulations of herbal gel

Carbapol 934 was kept aside in distilled water for half an hour to swell. Added 5ml propylene glycol to a mixture of methylparaben and propylparaben and added the content to the swelled polymer. Stirred the content under a mechanical stirrer. Added fresh extract of chomolaena *odorata* and stirred firmly with glass-rod to uniformly distribute the herbal extract. Make up the volume up to 150 ml using distilled water and added triethanolamine (drop wise) to adjust the PH of the gel to 6.8-7. Multiple batches were prepared by varying the concentration of Carbapol, herbal extract, stirring time, and stirring speed [18].

Table 2: Formulation of herbal gel

Formulation	Concentration of Carbapol (g)	Stirring speed (RPM)	Stirring time (min)	weight of fresh extract (g)
F1	1	1000	15	10 g
F2	2	1000	15	10 g
F3	3	1000	15	10 g
F4	2	1200	15	10 g
F5	2	1400	15	10 g
F6	2	1200	30	10 g
F7	2	1200	45	10 g
F8	2	1200	30	20 g

Evaluation of herbal gel

Physico-chemical evaluation of gel

Organoleptic properties of gel

Organoleptic properties such as colour, odour, clarity and homogeneity of the herbal gel were observed by visual inspection.

Viscosity

The viscosity of the gel was measured using Brookfield viscometer,spindle no. 62 at 20 RPM.

PH

PH of the herbal gel was determined using digital PH meter without any dilution .

Spreadability

The spreadability of the gel formulation was analyzed by measuring the spreading diameter of 1g of gel between two horizontal plates (20 cm x 20 cm) after one minute. The standard weight applied on the upper plate was 125 g 19-31].

In-vitro Pharmacological evaluation of herbal gel

In-vitro skin irritation test

The Hen's Egg Test method was adopted to evaluate the skin irritancy of herbal gel. By making a minor opening on the shell, the Chorio-allantoic Membrane of fertilized chicken eggs were exposed to test samples. Fertile eggs of average weight 50- 60g were selected and incubation for 8 days with rotating 5 times daily. Incubated another 24 hours without rotation and then removed a small part of egg shell, moisten the white inner membrane with normal saline. Applied the optimized formulation (2mg) to the chorio allantoic membrane (CAM) and observed for 30 minutes for any haemorrhage / Lyesis / Coagulation. Isopropyl alcohol and normal saline were used as positive and negative control respectively [32].

In-vitro antioxidant assay

Optimized formulation was diluted with water to prepare various concentrations in the range of 10 ug/ml to 50 ug/ml. To 1 ml of dilution added 5 mL 0.004% (w/v) solution of DPPH. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and the absorbance was read in a spectrophotometer at 517nm. Ascorbic acid was used as positive reference standard [33, 34].

Storage stability study.

Optimized formulation kept in a tightly closed airtight container and stored at room temperature of 37±50C for 3 months and observed for any possible change in physical stability, appearance, PH and *in-vitro* antioxidant potential [35,36].

RESULTS

Preformulation studies

Tests for flavonoids

Table 3 : results of the tests for flavonoids

Test	Observation	Result
Alkaline test	Increasing in the in the intensity of yellow colour which become colourless on addition of dilute acid	presence of flavanoids.
Ferric chloride test	Appearance of green colour	presence of flavanoids.
Tyrosine test	Solution shows dark red color in presence of amino acids.	presence of flavanoids.

Drug excipient compatibility studies

Test for physical incompatibility

Physical mixture of dry extract and polymer had shown no observable changes in colour, odour and physical state.

Test for chemical incompatibility

The characteristic peaks of phenolics [37] detected in herbal extract are enlisted in table no 4.

Table 4: Functional groups and corresponding wave no of herbal extract

Functional group	Peak wave no (cm-1)
Wagging of OH of phenolic compounds	3433
flavanoids	1640
Due to flavonoids and aromatic ring	1457
C=C and C=O vibrations	1637
aromatic vibrations	1454
C–O of polyols	1383
primary and secondary alcohols	1039

The FTIR spectrum of plant extract, polymer and 1:1 physical mixture of plant extract and polymer is shown in fig.1,2,3 below.

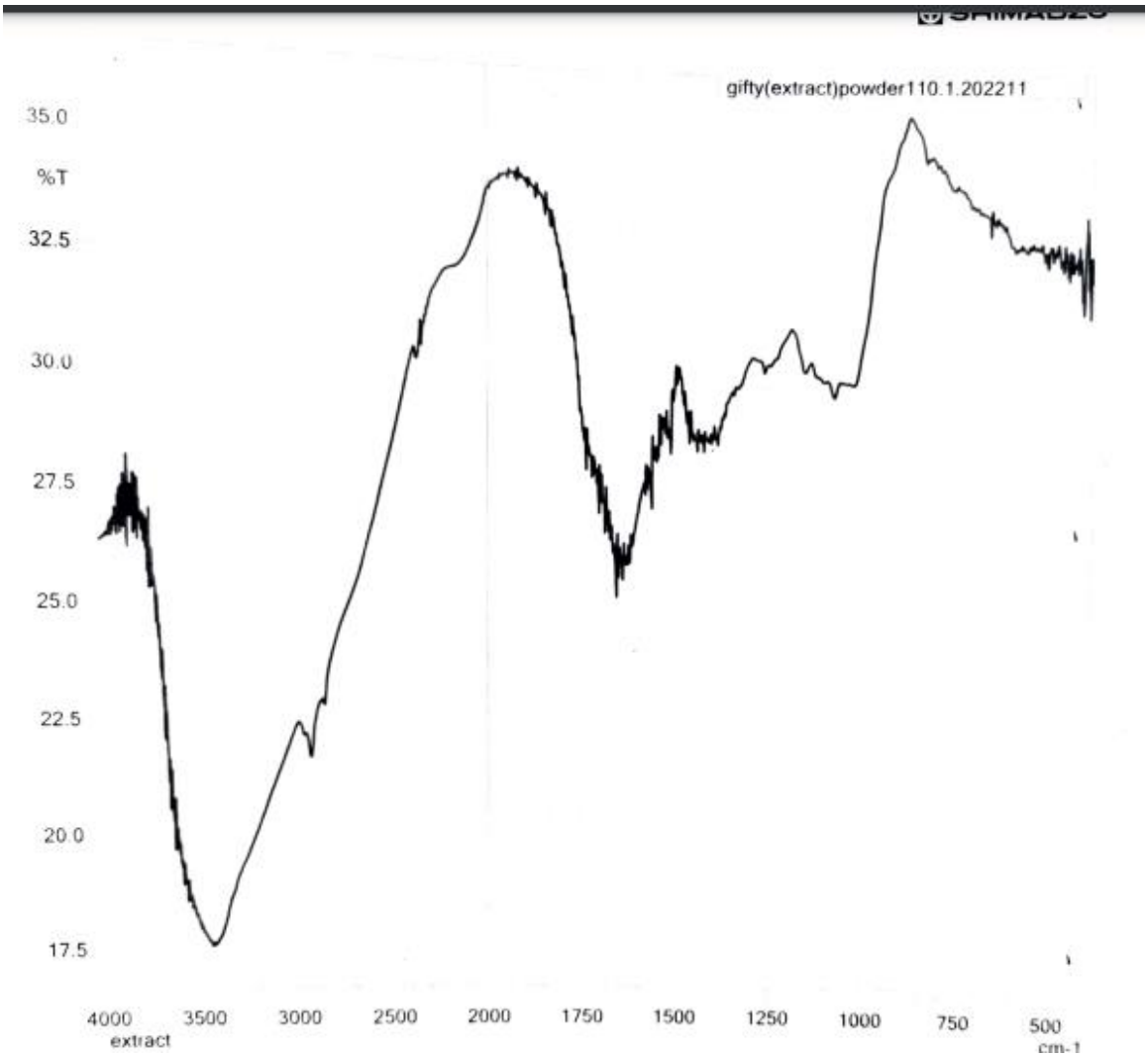

Figure 1 : FTIR spectrum of Herbal extract

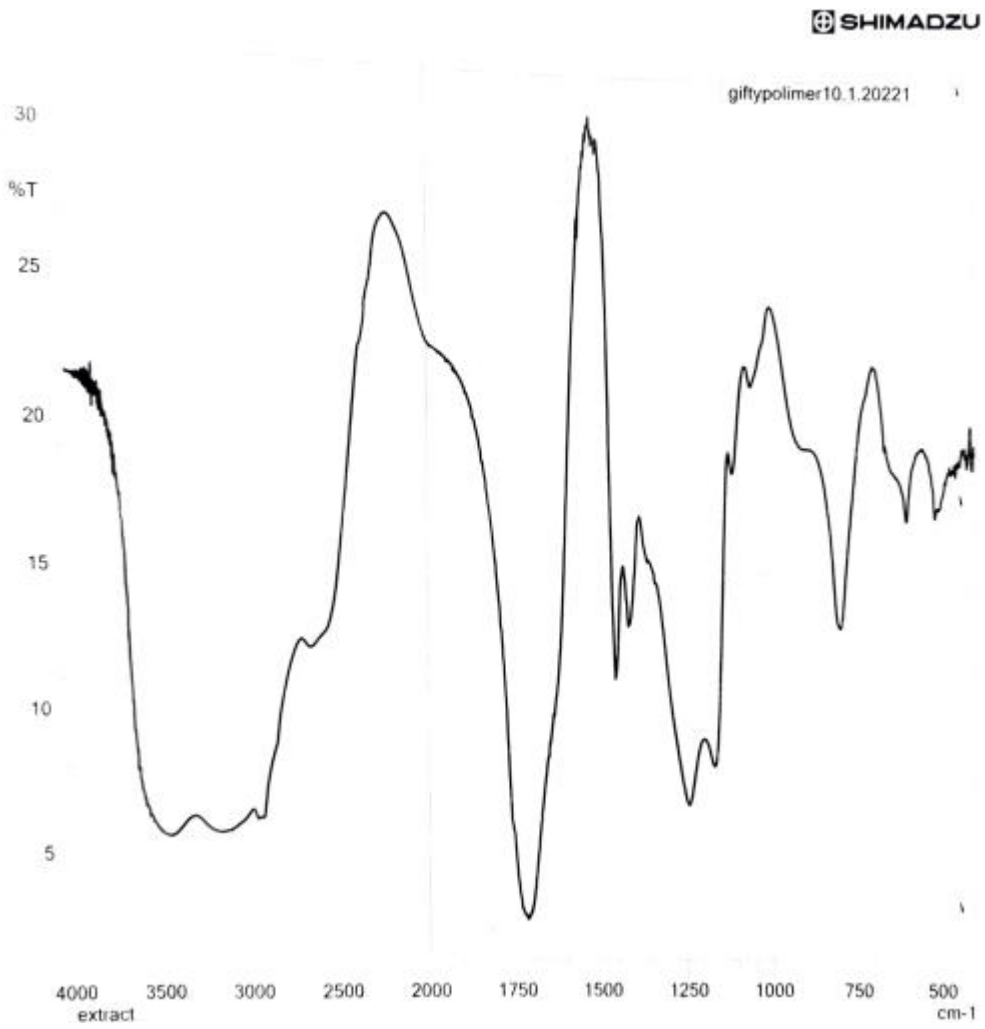

Figure 2: FTIR spectrum of Carbapol 934

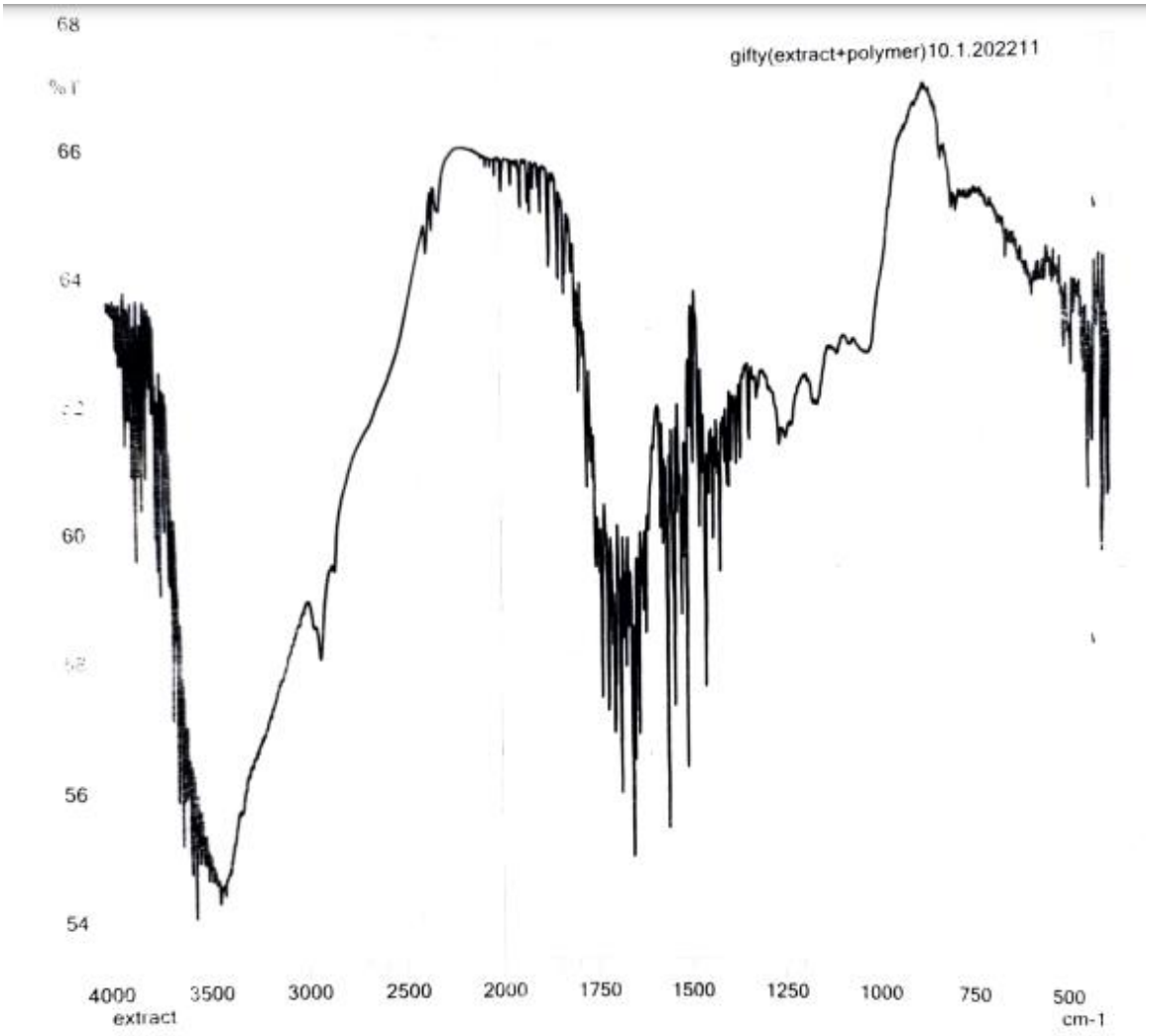

Figure 3: FTIR spectrum of herbal extract + Carbapol 934

Formulation of herbal gel

Among the eight batches, formulation F6 was found to possess good homogeneity, texture and physical stability. Hence formulation F6 was selected for further investigations.

Evaluation of herbal gel

Physico-chemical evaluation of gel

Physicochemical characteristics of the optimized formulation is summarized in table no 4.

Table 5: Physiochemical evaluation of gel

Colour	Green
Odour	Rose fragrance
Homogenity	Homogenious
Ph	6.89±0.25
Viscosity	318±10 CP

Spreadability	50 ± 3.54 mm

Results are given in mean±SD
n=3

Pharmacological evaluation of herbal gel

In-vitro Skin irritation test

Isopropyl alcohol treated membrane exhibited characteristic signs of irritations such as haemorrhage, lysis and coagulation. Whereas, treatment with negative control and formulation induced no such toxicity signs.

In-vitro antioxidant assay

IC50 values of herbal gel and ascorbic acid were found to be 155.6472±11.54 µg/ml and 843.8595±12.04 µg/ml respectively.

Storage stability study

No significant in changes physical state, appearance, viscosity, PH and *in-vitro* antioxidant potential was observed upon 3 months of storage at room temperature The results are shown in table no 5.

Table 6: Storage stability studies

Days	**Apparance**	**Viscosity**	**PH**	***in-vitro* antioxidant potential (IC 50 value)**
0 days	Green in colour	318±10 CP	6.89±0.25	155.6472±11.54
30 days	Green in colour	320±8 CP	6.69±0.20	155.560±10.54
60 days	Green in colour	316±10 CP	6.50±0.30	157.589 ±10.25
90 days	Green in colour	318±8 CP	6.41±0.40	155.589±14.54
120 days	Green in colour	320±7 CP	6.48±0.45	153.256±11.54

Results are given in mean±SD
n=3

DISCUSSION

Phenolics are the principle antiaging molecules present in *Chromolaena odorata* extract. The present investigation used alkaline test, ferric chloride test and tyrosine test to perceive the presence of principle phenolics. Agreeing to the previous reports [14,15], the current investigation also revealed the presence of flavonoids in the fresh extract of *Chromolaena odorata*.

Physical mixture of dry extract and Carbapol 934 had shown no observable changes in colour, odour and physical state. Physical compatibility of herbal extract and excipient is hence confirmed. Chemical compatibility was established through FTIR spectroscopy. The FTIR spectrum of physical mixture showed no addition or deletion of major peaks of phenolics. The retention of the characteristic peaks of phenolics (table no 4 in the physical mixture shows the compatibility of herbal extract with excipient.

The formulation development stage included preparation and evaluation of eight batches of herbal gel. The impact of various the process as well as formulation variables on product quality and stability were investigated. Formulations F1, F2 and F3 were prepared by varying the concentration of gelling agent. On comparison with F1, formulation F2 was found to be homogenous, smooth in texture and physically stable. The gel became dense, compact and hard in texture on further addition of gelling agent. Present study also investigated the influence of various volumes of herbal extract on product appearance and physical stability. Gel loses the consistency upon addition of extract than 10 g. Stirring time and stirring speed found to had substantial impact on product homogeneity, texture and physical stability in the present study. The soft, homogenous and physically stable gel was formed at 1200 rpm and stirring time of 30 min. Based on above observations, Formulation F6 was selected and used for further investigations.

The optimized formulation was evaluated for various physico-chemical and pharmacological parameters. Physico-chemical properties of the herbal gel is shown in table no 5. The skin irritation potential of herbal gel was analysed in “Hen’s Egg model". The basic principle of this model is that the inflammatory processes in irritation induce vascular changes, which can be monitored by observing chorioallantoic membrane [36]. The herbal gel treated membrane showed no signs of haemorrage, lysis and coagulation; which indicated safety and acceptability of the formulation for topical administration.

The Total antioxidant capacity (TAC) of herbal gel of *Chromolaena odorata* was evaluated by DPPH assay methods. The assay used IC50 value to measure the antioxidant capacity. IC50 value is the concentration of antioxidant needed to decrease the initial DPPH concentration by 50 %. Thus, the lower IC50 value the higher the antioxidant activity [37]. IC50 values of herbal gel and ascorbic acid were found to be 155.6472±11.54 µg/ml and 843.8595±12.04 µg/ml respectively. A p- value of <0.05 revealed that the herbal gel possesses significant antioxidant capacity compared with that of Vitamin C.

The stability study showed no significant changes in major parameters upon 3 months of storage; which indicate the stability of product at room temperature. The topical gel of *Chromolaena odorata* thus found to possess appropriate physicochemical parameters, storage stability, skin compatibility and excellent antioxidant capacity.

CONCLUSION

Present work involved the formulation and *in-vitro* evaluation of herbal gel of *Chromolaena odorata*. FTIR study evaluated the compatibility of herbal extract with gelling agent (Carbapol 934) showed good compatibility between ingredients. Among the various formulations prepared, formulation F6 was found to be homogeneous, smooth in texture and physically stabile; and hence selected for further investigations. The gel was found to be safe for topical application via Hen's Egg model. The PH, viscosity and spreadability of the gel was found to be 6.89±0.25, 318±10 cp and 50 ± 3.54 mm respectively. Antioxidant capacity of gel evaluated via DPPH assay method has shown significant activity in comparison with vitamin C gel. Hence, the present data suggest that topical formulation of *Chromolaena odorata* could be used for treating the age markers of skin. Researches should upgrade to translational clinical level to confirm these findings.

CONFLICT OF INTEREST

The authors state no conflict of interest

CONTRIBUTION AUTHORS

The study was an institutional research work carried out by Jaseera MP, MK Riyas Ibrahim, Sisbana, Solamy K Geever[1], Swaliha Shirin under the guidance of Dr Gifty M Jojo and D Deborah Evangeline.

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