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Evaluation of Antioxidant and Anti-Inflammatory Activity of Formulated Herbal Cream

Soorya. Ps, Agna Boban, Angel Anto, Anusree Udayan, Shaziya T F, Aneesha P P,Nagalekshmi R*, Deena Jose*

Department of Pharmaceutics, ELIMS College of Pharmacy, Ramavarmapuram P.O, Villadam, Thrissur, Kerala, India- 680751.

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ABSTRACT

In this study, we collected and powdered the leave of Chromolaena odorata, extracted the plant using ethanol as a solvent and formulated it into a herbal cream. Ethanolic extracts of Chromolaena odorata were screened for phytochemical constituents. Tests for tannins, alkaloid, flavonoids and saponin were positive in the ethanolic extract of Chromolaena odorata. The formulated cream was evaluated for its physical parameters like organoleptic characteristics, spreadability and viscosity test. The formulated herbal cream have a pH value of 6.5 and viscosity of 53820 cp. The evaluation of the antioxidant and Anti-inflammatory activity of formulated herbal cream was also carried out. These findings indicate that this formulation of C. odorata extract is stable and suitable for further testing as a topically applied product. Pharmacological activities evaluated using DPPH method and protein denaturation method which indicated that the formulated cream has antioxidant and anti-inflammatory activity**.**

Keywords: Chromolaena odorata, phytochemicals, antioxidant activity, medicinal plant

I. INTRODUCTION

Herbal plants used whole over the world in the field of health care. Our ancient ancestors used plants and herbs in the past to preserve and flavor food, reduce pain, treat headache, and even prevent diseases including epidemics.

Chromolaena odorata is a tropical and subtropical species of flowering shrub in the family Asteraceae. Phytochemical studies on the leaf of Chromolaena odorata shows the presence of tannins, alkaloid, flavonoids and saponins. Biological investigations have also shown wound healing, anti-inflammatory, analgesic, antipyretic, diuretic, antimicrobial, anti- mycobacterial, anti-cancer, anti-diabetic, anti-hepatotoxic, and antioxidant properties.[1]

Antioxidants are groups of compounds that neutralize free radicals and reactive oxygen species (ROS) in the cell. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti- inflammatory, anti-aging, anti-atherosclerosis and anticancer.[2] Anti-microbial activity refers to the process of killing or inhibiting the disease causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. [3]

II. MATERIAL AND METHODS

Plant collection, authentication and drying

The plant Chromolaena odorata L. is a commonly using herb and it is easily available. The sample were collected from villadam, Thrissur district, Kerala, India. The plant material was then washed for removing impurities and dried under shade for about 14 days, powdered with mechanical grinder and stored in an air tight container.

Figure 1 Chromolaena odorata

Extraction

The plant material was shade dried and coarsely powdered. Around 100g of dried powder was weighed, moistened with the solvent and then extracted by using 1000 mlethanol for 5 hours (Cold maceration). The extract was then filtered through Whatman No. 1 filter paper and concentrated. The extract obtained was then subjected toqualitative and quantitative phytochemical analysis. Calculate the yield of

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extract.[4]

Preparation of herbal cream

To prepare 40g of herbal cream, take 8g of beeswax and 28.8 ml of liquid paraffin in china dish. Then heat on a water bath for uniform mixing. After few minutes oil phase was formed. 2g of herbal extract, 0.576g of borax, 0.048g of methyl paraben and 7.6 ml of water was taken in a beaker. Mixing all the ingredients by heating on a water bath, the aqueous phase was formed. Oil phase was added into aqueous phase. Add few drops of peppermint oil was added for fragrance.[5]

Table 1: Formula of cream formulation

Sl. no	Ingredients	Quantity(g)
1	Beeswax	8 g
2	Liquid paraffin	28.8 ml
3	Borax	0.576 g
4	Methyl paraben	0.048 g
5	Extract	2 g
6	Water	7.6 ml
7	Peppermint oil	q.s

Figure 2: Herbal cream

Evaluation of herbal cream Irritancy

Mark the area (1 cm2 Washability) on the left-hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and oedema if any for an interval up to 24 h and reported.

Washability

A small amount of cream was applied on the hand and it is then washed with tap water

pH

0.5 g cream was taken and dispersed in 50 ml distilled water and then was measured digital pH Viscosity meter.[6]

Viscosity

Viscosity of cream was done by using Brooke field viscometer at a temperature of 250C using spindle No.6.

Spreadability

The spreadability of the cream is determined using the parallel plate method. Two glass slides are used for this purpose. One slide is fixed on a wooden block, and 1.0g of the cream sample is placed on it. The second slide is placed on top, and a weight of1 kg is applied for 5 minutes to remove air bubbles and create a uniform film of the cream. Excess cream is scraped off from the edges. Then, a string is attached to the top slide, and a weight of 20 g is used to pull the top slide, separating it from the bottom slide. The distance covered (7.5 cm) is noted.

S=ML/T

Where, S=Spreadability

M=Weight tide to upper slide L=Length moved on the glass slide

T=Time taken to separate the slide completely from each other.[7]

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Dye test

The scarlet red dye is mixed with the cream. Place a drop of cream on a microscopic slide then cover it with a cover slip, and examine it under a microscope. If the disperse globules appear red the ground colourless. The cream is O/W type. The reverse condition occurs in W/O type cream i.e. the disperse globules appear colourless.[8]

Biological activity studies Anti-oxidant study
DPPH scavenging assay

To different concentration, extract (100,200,300,400 µl) 0.5 ml of methanolic solution of DPPH was added and made up to 2ml using methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518nm in a spectrophotometer. The procedure was repeated for the standard ascorbic acid and the assay was calculated as:[9]

$$\text{Radical scavenging activity} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Anti- inflammatory activity Inhibition of protein denaturation

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of plant extracts of 50, 100, 150, 200 µg/ml concentration and pH were adjusted to 6.3 using 1 N HCL. The samples were incubated at 37^0c for 20 min and then heated at 57^0c for 3 min. Ibuprofen was used as a standard drug (50, 100, 150, and 200 µg/ml), after cooling the samples, 2.5 ml phosphate buffer saline pH 6.3) was added to each tube. Absorbance was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.[10]

$$\text{Percentage Inhibition} = \frac{Abs_{Control} - Abs_{Sample/Standard}}{Abs_{Control}} \times 100$$

Antimicrobial activity
Clinical microbial cultures and culture media

The antimicrobial property of the Herbal cream examined against clinical K. pneumoniae, E. coli, E. faecalis. and S. aureus. Clinical Microbial cultures were procured from microbiology lab Coimbatore, Tamil Nadu. Muller- Hintonagar media of Himedia Pvt. Bombay, India used for the media for the microbial test. The antibacterial activity evaluated by using the Himedia zone reader.

Inoculum preparation

100µl clinical K. pneumoniae, E. coli, E. faecalis. and S. aureus microorganisms were inoculated individually in 5.0 ml of sterile nutrient broth (NB) media, and incubated at 37°C for 24h. 200µl from the organisms' 24 h fresh culture was dispensed into 30ml sterile nutrient broth and set 2-4 h to standardize the bacterial culture to 108 CFU/ml (colony forming units).

Kirby-Bauer method - well diffusion method

Herbal cream standard drug (antibacterial-Ciprofloxacin 200mg/100ml) antimicrobial activity conducted initially using agar well plate method. K. pneumoniae, E. coli, E. faecalis. and S. aureus inoculums prepared using sterile nutrients broth media. Mueller Hinton agar double strength media were made by autoclaving 0.760 g in 100 ml. Fresh inoculum inoculate on the Mueller Hinton agar plates by using sterile cotton swabs. Agar wells prepared using sterile cork-borer, 101 and 104 (100µg) and Ciprofloxacin 50µl (50µg) were placed agar well using micropipette under aseptic conditions. Agar plates incubated for 30 min at the refrigerator to diffuse the formulation into the agar, and finally, plates incubated at 37˚C for 24h. Antibacterial activity evaluated by using the Himedia zone reader.[11]

III. RESULT AND DISCUSSION

Evaluation of herbal cream Irritancy:

The prepared formulation is applied to the skin of the hand. The formulation does not show any

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irritation, redness, oedema, inflammation during the studies when applied on the skin and safe to use.

Washability:
Formulation was applied on the skin can be washable with water.

pH :
The pH of cream was measured by using digital pH meter. According to the result, pH of the cream was found to be 6.5 which is suitable as the skin pH so it can be safely used on the skin.

Viscosity:
The prepared cream at least rpm of 10 exhibited a viscosity of 53820 cp. That indicates the formulation has the desired viscosity required for semi solid formulation for proper packaging. It was found that the viscosity decreases as the rotational speed of viscometer increased suggesting that greater the shearing the lower viscosity favours easy spreadability further confirmed by spreadability and rheological testing.

Table 2: Result for viscosity measurement

RPM (Shear Rate)	CP (Viscosity)	%Torque (Shear Stress)
10	53820	89.7%
12	42800	85.6%
20	25590	85.3%
30	16560	84.8%
50	10080	83.9%
60	8270	82.7%

Spreadability :
If spreadability decreases, the topical cream is good because applying it to the skin is easy. cream should spread easily without too much drag and should not produce greater friction in the rubbing process. Here parallel plate method is used and average spreadability obtained is 7.1g.cm/s. That indicate the formulation show desired spreadability.

Table 3: Spreadability test

Formulation	Weight tied to upper slide(M)	Length of glass slide(L)	Time taken(T)	Average spreadability(S)
Herbal cream	20g	7.5cm	21s	7.1g.cm/s

Dye solubility test :
The scarlet red dye is mixed with cream. Place a drop of the cream on a microscopic slide cover it with a cover slip, and examine it under microscope. The dispersed globule appears colourless in the red ground i.e. W/O type cream.

Figure 3: Microscopic examination of globule

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Antioxidant Activity-DPPH Assay

In-vitro scavenging activity of the herbal cream was determined using 2,2,- diphenyl- 1-picrylhydrazyl(DPPH)assay according to a published method. Compared to standard ascorbic acid, herbal cream show antioxidant activity. The percentage radical scavenging of standard and herbal cream are tabulated in the table:4,5 and Comparison of percentage radical scavenging activity of standard, and cream shown in figure 4.

Table 4: Percentage scavenging of standard Ascorbic acid

Concentration (μl)	OD at 518 nm	Percentage of scavenging(%)
Control	1.414	
100	0.663	53.11%
200	0.412	70.86%
300	0.188	86.70%
400	0.105	92.57%

Table 5: Percentage scavenging of herbal cream

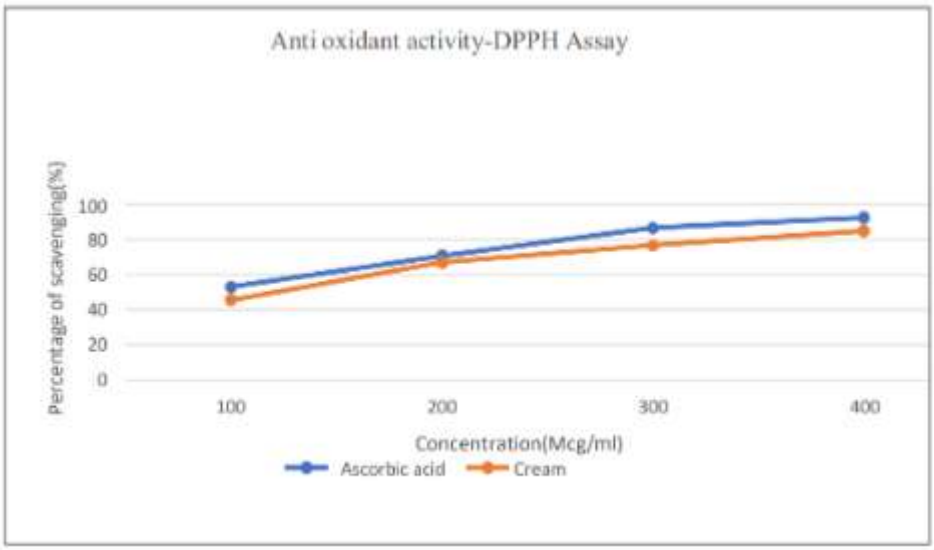

Figure 4: Comparison of percentage radical scavenging activity of standard and cream

Anti-inflammatory Activity by protein denaturation method

The anti-inflammatory activity was performed by protein denaturation method. A dose dependent denaturation was showed by herbal cream. When compared with standard ibuprofen herbal cream show anti-inflammatory activity. The percentage inhibition of standard and cream are tabulated in the table 6 :. The anti-inflammatory activity was demonstrated in the figure 5.

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Table 6 : Anti-inflammatory activity by protein denaturation method

Sl.NO	Sample		Concentration	Absorbance at 660 nm	% inhibition
1	control		-	1.29	-
2	Standard (Ibuprofen)		50	0.69	46.65
			100	0.52	55.03
			150	0.44	65.89
			200	0.29	77.51
3	Cream		50	0.85	36.92
			100	0.72	44.62
			150	0.52	59.69
			200	0.34	73.37

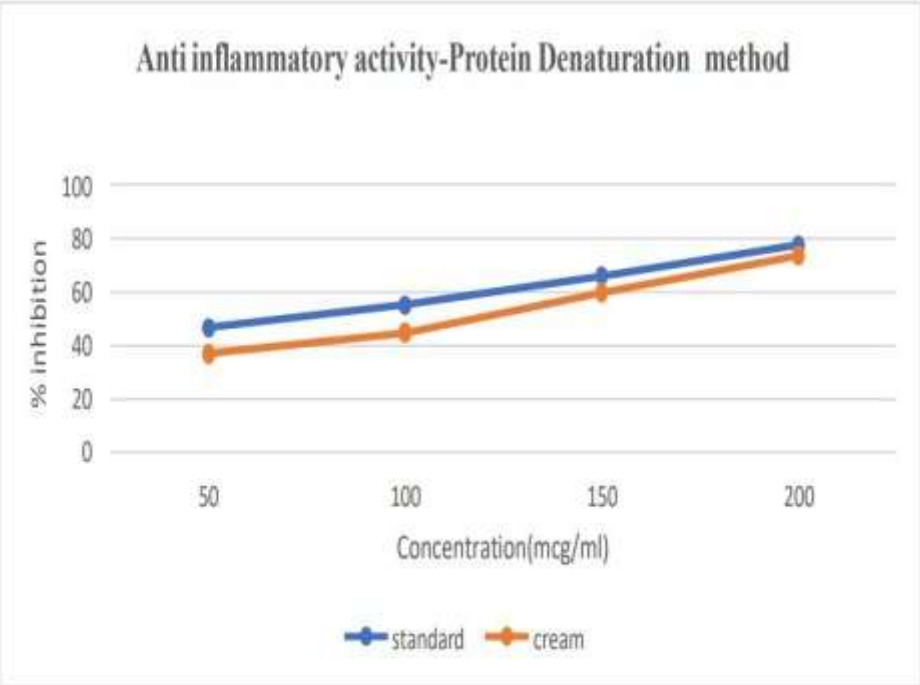

Figure 5: Anti-inflammatory activity by protein denaturation method

Antimicrobial activity

Anti-microbial activity performed by **Kirby-Bauer method - well diffusion method**. The formulated herbal cream examined for antimicrobial property against clinical K. pneumoniae, E. coli, E. faecalis. and S. aureus and the result indicates that the microbial strains is resistant to the herbal cream.

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Table 7: Antimicrobial activity of herbal cream against to clinical micro microorganism

Sl. No	Test organism	Zone of inhibition (mm) n=2			
		K.pneumoniae	*E.coli*	*E. faecalis*	*S. aureus*
1	Herbal cream	R	R	R	R
2	CIP	32	32	10	28

R:Resistance

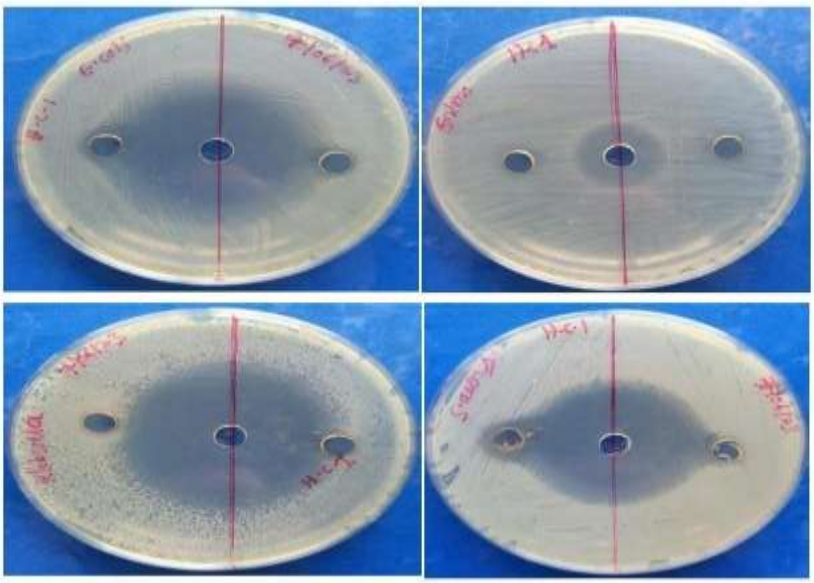

Figure 6: Antimicrobial activity of A and B against clinical microorganism (K. pneumoniae, E. coli, E. faecalis. and S. aureus)

IV. CONCLUSION

1. By using Brookfield viscometer viscosity of herbal cream measured and the prepared cream atleast rpm of 10 exhibited a viscosity of 53820. So it indicates the formulation has desired viscosity.
2. Biological activity of the drug determined by conducting anti- oxidant study, anti microbial study and anti inflammatory study.
3. From the DPHH radical scavenging activity, the drug extract show antioxidant activity.
4. The invitro anti-inflammatory activity was performed by protein denaturation method. A dose dependent denaturation was showed by the herbal cream. From this we can understood that the drug formulation show anti inflammatory activity.
5. The antimicrobial property of the herbal cream examined against clinical k. Pneumonia, E. coli, E faecalis and S. aureus using Muller-Hinton agar media and found that the herbal cream is resistant to the above strains.

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