IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY CREAM CONTAINING *CYNODON DACTYLON*

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Abstract: Inflammatory conditions like redness, swelling, dermatitis and burns are commonly treated with synthetic topical anti-inflammatory formulations which may cause side effects when use for long time period. *Cynodon dactylon* is a medicinal plant traditionally known for its anti-inflammatory properties. The study focuses on the formulation and evaluation of anti-inflammatory cream prepared using *Cynodon dactylon*. The formulation was developed to effectively use of water soluble phytoconstituents of the plant. The cream was evaluated for various physicochemical parameters like appearance, pH, viscosity, spreadability and homogeneity. Its anti-inflammatory activity was examined by using suitable in-vitro method and the results showed that the cream exhibited acceptable physicochemical properties. The anti-inflammatory effect is observed due to presence of compounds like flavonoids and phenolic compounds found in aqueous extract. Over all the study shows that the aqueous extract of *Cynodon dactylon* has strong potential for use in herbal anti-inflammatory cream as a safe and natural alternative for synthetic topical creams.

Key words: Anti-inflammatory, *Cynodon dactylone*, herbal cream, aqueous extract.

1. INTRODUCTION

1.1. Inflammation

The term inflammation comes from flame, reflecting the warmth and redness seen in inflamed tissues. These visible changes are part of the four signs of acute inflammation redness, swelling, heat and pain described by Aulus Cornelius Celsus (1. Antonelli M, et al.). Inflammation is a natural protective response of the body to harmful stimuli such as infection, tissue injury or other damaging condition, it plays a vital role in host defence by helping eliminate harmful agents and supporting tissue repair (Aug, et al.). Inflammation is commonly caused by physical, chemical or biological insults to tissues. Mechanical factors such as trauma, foreign bodies, pressure or vibration metabolism. Cold exposure

can also trigger inflammation by causing tissue stress and swelling. Chemical agents including corrosive substances and reducing compounds damage tissues directly or interfere with metabolic processes, while biological agents such as enzymes and microorganisms promote inflammation through tissue breakdown and immune activation (4. Stankov SV. Definition of inflammation, et al.). Acute inflammation is a rapid, short term and protective response that occurs when the body faces infection or tissue injury. It develops quickly and usually resolves once healing begins. This response is triggered when the immune system detects signals from microorganisms or damaged cells. Once activated, inflammatory mediators are released, blood vessels become more permeable and immune cells are recruited to the affected site. Together, these actions help eliminate harmful agents, clear damaged tissue and promote repair (1. Antonelli M, et al.). Chronic inflammation is long lasting and occurs when the causative agent of acute inflammation persists or when the stimulus itself is capable of inducing a prolonged inflammatory response from the outset. It is characterized by the presence of chronic inflammatory cells such as lymphocytes, plasma cell and macrophages which contribute to ongoing tissue damage and repair occurring simultaneously (Publishers, et al.). However, modern research has revealed that inflammation is far more complex at the molecular level than previously understood, involving tightly regulated processes of initiation, control and resolution with the identification of multiple forms of inflammation driven by diverse stimuli and regulatory pathways. It is now evident that inflammation influences nearly every aspect of human physiology and disease (Aug, et al.).

1.2 Anti-inflammation

Anti-inflammation refers to the ability of a substance or treatment to reduce inflammation in the body. Inflammation is commonly associated with redness, swelling, pain and heat, and anti-inflammatory agents act by calming this response. They do it by interfering with the body's inflammatory pathways, often by inhibiting the release of key mediators such as prostaglandins and cytokines that are produced during injury or infection. As a result, anti-inflammatory activity leads to visible and symptomatic improvements, including reduced redness, swelling and pain at the affected site. For example, a decrease in joint swelling in arthritis or reduced redness around an injured areas indicates that the inflammatory response has been effectively controlled (5. Serafini M, et al.). These beneficial effects occur because anti-inflammatory agents like NSAIDs act on body's internal pathways that drive inflammation helping to control the response at its origin. The NSAIDs act primarily by inhibiting the cyclooxygenase (COX) enzyme which converts arachidonic acid into prostaglandins. Prostaglandins are mediators of pain, fewer, inflammation, platelet aggregation and gastric mucosal protection, by blocking COX, they reduce prostaglandin synthesis producing their gastric and platelet side effects. Most of the agents reduce COX at therapeutic concentrations and their anti-inflammatory potency closely correlates with their ability to inhibit COX. Some of these may also exert additional effects, such as scavenging reactive oxygen radicals or inhibiting inflammatory gene transcription which can contribute to their anti-inflammatory action. Paracetamol acts differently from most NSAIDs. It mainly inhibits COX in the brain rather than in peripheral tissues which explains why it is effective as an analgesic and antipyretic but has little anti-inflammatory action and minimal gastric or platelet side effects. This lead to the idea that different forms of the COX enzyme exist (6. Serhan CN, et al.).

1.3 Cynodon dactylon

Among the many medicinal plants found in India, *Cynodon dactylon* commonly known as Durva. It exists in different variants such as neel, shwet and gand and has been traditionally recognizes for its medicinal value in classical Ayurvedic literature (7. Singh V, et al.). In the Plant List, *Cynodon dactylon* (L.) Pers. is recorded under the Sanskrit name "Durva." This identification was made by correlating its morphological characteristics with the descriptions provided in the classical Ayurvedic text *Bhavprakash Nighantu* (Chunekar, 2009) (7. Singh V, et al.). *Cynodon dactylon* belongs to the family Poaceae and is commonly known by various names across different regions, including Durva grass, Bermuda grass, Dog's tooth grass, Bahama grass, Devil's grass, Couch grass, Indian Doab, Scutch

grass, Dhub, Doob and Durba (10. Nagori BP, et al.). *Cynodon dactylon* thrives in a wide range of soil types, including heavy clay, medium loam, and light sandy soils. It is also capable of growing in saline, alkaline, and highly acidic conditions. However, the plant does not develop well in heavily shaded areas and requires adequate soil moisture for proper growth. According to various researchers, this species is widely distributed across warm-temperate and subtropical regions, primarily cultivated as lawn grass or used as forage, especially in saline habitats (13. Parihar S, et al.). As described in the Ayurvedic Pharmacopoeia, the plant has a pungent and bitter taste, a distinct aroma, and exhibits a cooling effect. In contrast, the Unani system of medicine characterizes the plant as having a sharp, hot taste along with a pleasant odour (8. Khatun P, et al.). It is a perennial grass that shows considerable variation and spreads rapidly through creeping stolons, which root at the nodes and create a thick mat over the soil surface; these runners may sometimes reach up to 20 m in length. The leaves measure about 2.5–20 cm long and 2–6 mm wide and may be flat, folded, or rolled. The flowering stalks (culms) range from 15 cm to 1 m in height and bear an inflorescence made up of 2–12 spikes arranged in a radiating, star-like pattern at the tip. Each spike, 2.5–10 cm long, carries many spikelets aligned in two rows along one side. The spikelets are flattened, 2–2.5 mm in length, lack awns, and contain a single floret. The glumes are of unequal size, with the upper glume being longer and about one-third to three-fourths the length of the floret (2016, et al.). The cultivated *Cynodon dactylon* L. (Durva) was verified by a qualified botanist, and its macro- and microscopic characteristics were examined prior to preservation. Fresh green juice of the plant was extracted and subsequently freeze-dried. In addition, water, hydroalcoholic, and alcoholic extracts were also prepared from the plant material (7. Singh V, et al.). *Cynodon dactylon* is characterized by high survival ability, fast propagation, and strong tolerance to trampling, which makes it highly suitable for use in lawns, animal fodder, soil stabilization, and rehabilitation of polluted soils (14. Wang M, et al.). Phytochemical screening revealed that *Cynodon dactylon* contains various bioactive constituents such as flavonoids, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, as well as volatile and fixed oils. The plant has been reported to exhibit multiple pharmacological activities, including effects on the central nervous and cardiovascular systems, as well as antidiabetic, gastrointestinal, antioxidant, immunomodulatory, antiallergic, anti-inflammatory, antipyretic, analgesic, anticancer, dermatological, diuretic, protective, antimicrobial, antiparasitic, insecticidal, and repellent properties (2016, et al.). Phytochemical analysis indicated the presence of flavonoids and glycosides in *Cynodon dactylon*, which are likely responsible for the observed anti-inflammatory effect. Among these, flavonoids are especially known for their, ability to reduce inflammation by limiting the formation of chemical mediators involved in the inflammatory process (12. Garg VK). The anti-inflammatory activity of Cynodon dactylon aqueous extract produced a significant reduction in inflammation indicating strong anti-inflammatory potential (11. Ashokkumar K, et al.).

2. MATERIAL AND METHODOLOGY

2.1. Material

Table 1 Formula for Cream

INGREDIENTS	QUANTITY
Beeswax	10 gm
Liquid paraffin	25.5 gm
Sodium benzoate	0.5 gm
Durva extract	8 ml
Rose oil	Quantity Sufficient

2.2. Method of preparation

2.2.1. Preparation of Durva Powder Extract

Powdered plant (5g) in 100ml water

↓

Heated at 50°C for 60ml, to reduce the volume to 25ml

↓

Then filter it and Durva extract is obtained

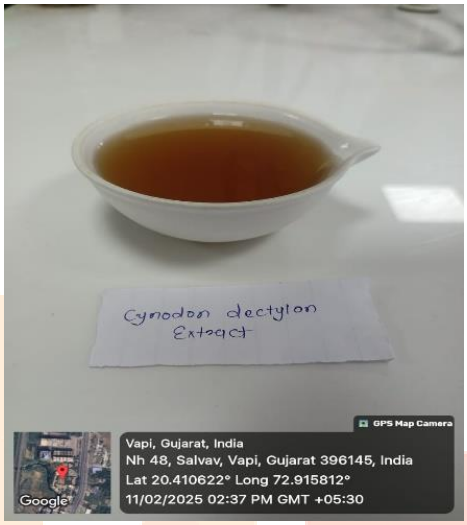

Fig 1 Durva Extract

2.2.2.Preparation of oil & aqueous phase

Oil phase: In china dish take beeswax and liquid paraffin at 75°C temperature. Aqueous phase: Take 3ml extract of Durva are added to beaker, add 0.1ml of borax and heat at 75°C on water bath.

Fig 2 Oil phase

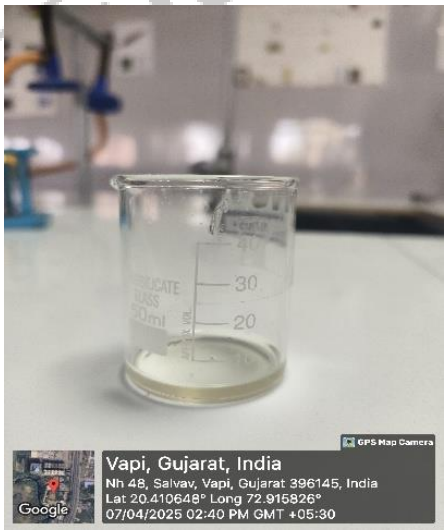

Fig 3 Aqueous phase

2.2.3.Preparation of cream

The gently add aqueous phase in heated oily phase with continue stirring.

↓

When cream is formed, then add rose oil as fragrance.

↓

This method is called as fusion technique or extemporaneous method of preparation of cream.

Fig 4 anti-inflammatory cream

3. EVALUATION PARAMETER

3.1 Physiochemical Properties of Cream

3.1.1. pH Evaluation

The pH of the cream was evaluated by using electronic pH meter which determine the acidity and alkalinity of cream by measuring its pH level, using glass electrode. To measure the pH of cream first the pH meter was calibrated using the freshly prepared buffer solution with the known pH values of 4, 7 and 10. Prepare the cream sample by taking a 0.5gm of crem dispersed in 50ml of distilled water then pH of cream is measured. According to result the pH was found to be nearer to skin pH so it is safely used on the skin.

Fig 5 pH evaluation

3.1.2. Washability

Wash ability test was carried out by applying a small amount of cream on the hard and then washing it with tap water. Formulation was easily washable.

Table 2 Washability test

Sr no.	Standard	Result
1.	Easily washable	Easily washable

3.1.3. Irritability

Make the area (1 to 2cm) on 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 edema if any for an interval up to 24 h and reported. According to the results all the three formulations that showed no sign of irritancy, erythema and edema.

Table 3 Irritability test

Sr no.	Standard	Irritant effect	Erythema	Oedema
1	No	No	No	No

3.1.4. Greasiness

Here the cream was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like. According to the result, we can say that all formulations were non-greasy.

Table 4 Greasiness test

Sr no.	Standard	Greasiness
1	Non-greasy	Non-greasy

3.1.5. Spread ability

The formulation was sand witch between two slides, of dimension 20*5cm, by placing a weight of 100g uniformity on the slide. The weight was removed and excess of cream was scrapped off. the slides were fixed to a stand at 45 angle without a slightest disturbance so that only the lower slides were held firmly by the clamp, allowing the upper slides to slip off freely under a weight of 20g. The time taken for the upper slide to separate from the lower glass plate under the direction of the weight was noted. Experiment was done in triplicate and spread ability was calculated as follows;

$$S= (W*l/T)$$

Where,

S= spread ability

L=Length of glass plate

W= weight tied to the upper plate

T=time(sec)

Table 5 Spread ability test

Sr no.	Standard	Result
1	9.0-31.02g cm/s	21.4g cm/s

3.1.6. Viscosity

The viscosity of the cream was found by using digital viscometer which measure the cream's resistance to flow by rotating spindle. Viscosity of cream was found by taking the sufficient amount of cream in viscometer by rotating at 50rpm. The viscosity was found to be 8775centipoise.

Fig 6 Viscosity test

3.1.7. Anti-inflammatory Activity

The anti-inflammatory activity of cream was found by using albumin denaturation process. The 5ml of reaction mixture was prepared by 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac

sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below.

Percentage of inhibition= 10 x (Abs control- Abs test /Abs control)

Table 6 Diclofenac sodium test

Sr. no	Conc. µg/ml	% of inhibition
1	100 µg/ml	34.65 %
2	200 µg/ml	39.26 %
3	300 µg/ml	42.72 %
4	Mean	38.87 %

Table 7 Cream formulation test

Sr. no	Conc. µg/ml	% of inhibition
1	100 µg/ml	41.09 %
2	200 µg/ml	45.64 %
3	300 µg/ml	49.56 %
4	Mean	45.43%

3.2 Preliminary Test

3.2.1. Test for carbohydrates:

Table 8 Test for carbohydrates

Sr. no.	**Test**	**Observation**	**Interference**
1.	Molisch's test (General test): To 2-3 ml sample extract then add few drops of alpha-naphthol solution in alcohol, shake and add concentrated H_2So_4, from sides of the test tube.	Violet ring is formed at the junction of two liquids	Carbohydrates present.
2.	Fehling's test: Mix 1 ml Fehling's A and 1 ml Fehling's B solutions with sample extract then Heat in boiling water bath for 5-10 min.	First yellow, then brick red precipitates is observed in the test tube	Reducing sugars present.
3.	Benedict's test: Mix equal volume of Benedict's reagent and sample extract in test tube. Heat in boiling water bath for 5 min.	Solution appears green, yellow or red in test tube depending on amount of reducing sugar present in sample extract	Reducing sugars present.
4.	Barfoed's test: Mix equal volume of Barfoed's reagent and sample extract. Heat for 1-2 min. in boiling water bath and cool.	In the test tube red precipitates is observed	Monosaccharides present.

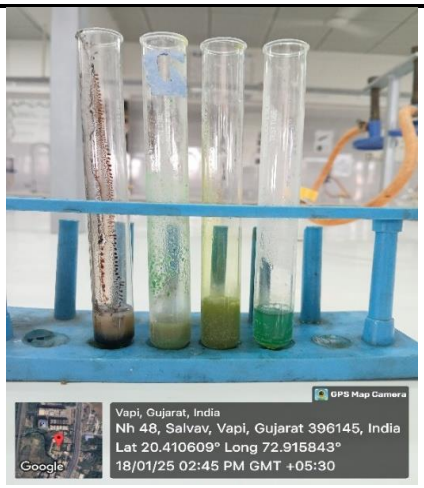

Fig 7 Test for carbohydrates

3.2.2. Test for alkaloids:

Table 9 Test for alkaloids

Sr. no.	Test	Observation	Interference
1.	Dragendorff's test: To 2-3 ml sample extract, add few drops Dragendorff's reagent.	Orange brown precipitates is formed .	Alkaloids present.
2.	Mayer's test: 2-3 ml sample extract with few drops Mayer's reagent gives precipitates.	Precipitates form	Alkaloids present.
3.	Hager's test: 2-3 ml sample extract with Hager's reagent.	Give yellow precipitates	Alkaloids present.
4.	Wagner's test: 2-3 ml sample extract with few drops Wagner's reagent.	Give reddish brown precipitates	Alkaloids present.

Fig 8 Test for Alkaloids

3.2.3. Test for flavonoids:

Table 10 Test for flavonoids

Sr. no.	Test	Observation	Interference
1.	To small quantity of sample extract, add lead acetate solution.	Yellow colored precipitates formed	Flavonoids present.
2.	Addition of increasing amount of sodium hydroxide to the sample extract shows coloration.	Which decolourises after addition of acid	Flavonoids present.

Fig 9 Test for flavonoids

3.2.4. Test for tannins:

Table 11 Test for tannins

Sr. no.	Test	Observation	Interference
1.	Aqueous extract with 5% $FeCl_3$ solution.	Deep blue-black color.	Tannins present.
2.	Aqueous extract with Lead acetate solution.	White precipitates	Tannins present.
3.	Gelatine solution with Aqueous extract.	White precipitates	Tannins present.
4.	Dilute iodine with solution Aqueous extract.	Transient red color	Tannins present.

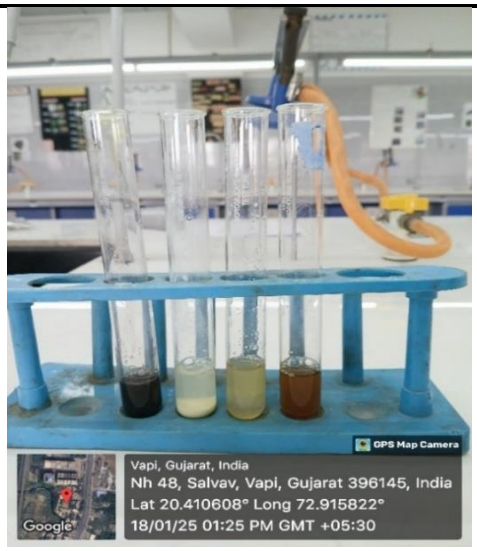

Fig 10 Test for Tannins

3.2.5. Test for glycosides:

Table 12 Test for glycosides

Sr. no.	Test	Observation	Interference
1.	Borntrager's test for anthraquinone glycosides: To 3 ml sample extract, add dil. H_2So_4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia.	Ammoniacal layer turns pink or red	Anthraquinones glycosides present.
2.	Test for deoxysugars (Keller-Killiani test): To 2 ml sample extract, add glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2So_4.	Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green.	Cardiac glycosides present.
3.	Foam test: Shake the sample extract or dry powder vigorously with water.	Persistent stable foam observed.	Saponin glycoside present.

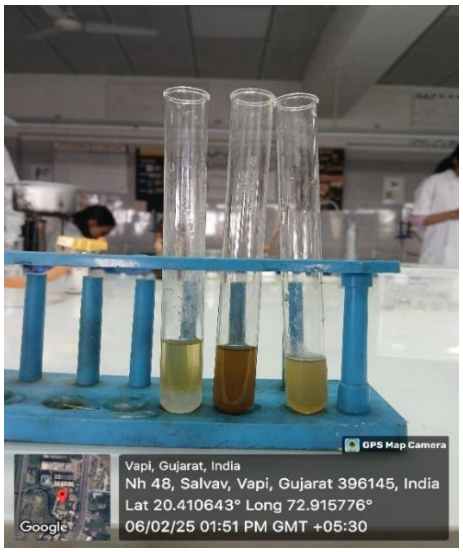

Fig 11 Test for Glycosides

3.2.6. Test for Proteins:

Table 13 Test for proteins

Sr. no.	Test	Observation	Interference
1.	Biuret test (General test): To 3ml of sample extract add 4% NaOH and few drops of 1% $CuSo_4$ solution.	Violet or Pink colour appears	Protein Present.
2.	Millon's test: Mix 3ml of sample extract with 5ml Millon's reagent. White precipitate Warm. Precipitate turns brick red or the precipitate.	Dissolves giving red coloured solution	Protein Present.

Fig 12 Test for Protein

4. RESULT

The tested sample showed a pH value 6.61, along with good washability and left no residue behind During the irritability test no redness, irritation or oedema was observed even after 24 hours, indicating good skin compatibility. The formulation was found to be non-greasy and spread smoothly over skin and the spreadability was recorded as 21 g cm/sec. The viscosity was measured at 8775 centipoise at 50rpm, giving the cream an appropriate semi solid consistency for convenient application and stability. Overall, the results suggest that the formulation is stable, user friendly and safe for topical use.

5. CONCLUSION

The present study demonstrates that the cream formulated using *Cynodon dactylon* possesses favorable physicochemical and dermatological properties. The formulation exhibited a pH of 6.61, which is within the acceptable range for skin application, indicating good compatibility with skin physiology. It showed excellent washability and left no residue, enhancing user convenience.

The irritability studies confirmed that the formulation is safe, as no signs of redness, irritation, or oedema were observed even after 24 hours. The cream was found to be non-greasy, spread easily over the skin, and showed good spread ability, ensuring uniform application.

Additionally, the viscosity indicated an appropriate semi-solid consistency, contributing to formulation stability and ease of use. These characteristics confirm that the cream is stable, user-friendly, and suitable for topical application.

Overall, the formulation developed with *Cynodon dactylon* demonstrates promising anti-inflammatory potential along with enhanced safety due to reduced use of synthetic chemicals. It can be considered an effective natural alternative for managing skin inflammation, providing soothing effects, reducing redness, and supporting skin protection and healing.

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