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FORMULATION AND EVALUATION OF A CLEOME VISCOSA SEEDS EXTRACT-BASED TOPICAL GEL WITH COMBINED ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES

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Article Info

Abstract

Cleome viscosa is a medicinal plant widely recognized in traditional medicine for managing inflammation, fever and skin-related conditions. Despite its extensive ethnopharmacological usage, scientific validation of its therapeutic claims remains limited. This study aimed to investigate the phytochemical composition, antioxidant activity and topical anti-inflammatory potential of C. viscosa seed extract, with an additional focus on the formulation and evaluation of its herbal gel. Seeds of C. viscosa were collected, authenticated, and subjected to methanol extraction. The extract underwent preliminary phytochemical screening to identify key bioactive constituents. Antioxidant potential was evaluated using the DPPH free radical scavenging assay. To assess topical anti-inflammatory activity, herbal gel containing 6%, 7%, and 8% extract concentrations were formulated and tested for stability and physicochemical properties. In vivo anti-inflammatory efficacy was tested using a carrageenan-induced paw edema model in Wistar rats, with diclofenac sodium gel used as a standard reference. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, glycosides, steroids, saponins and fixed oils. The extract exhibited minimal antioxidant activity i.e. 16.72% scavenging at the highest tested concentration. However, notable topical anti-inflammatory effects were observed. Among the formulations, the 7% C. viscosa gel showed the highest inhibition of paw edema (11.24%), closely approximating the standard diclofenac gel (13.02%), suggesting a dose-dependent anti-inflammatory response. Despite weak antioxidant activity, C. viscosa demonstrated significant topical anti-inflammatory potential, supporting its traditional medicinal use. The 7% gel formulation proved both effective and stable, indicating its promise as a plant-based alternative for topical inflammation management and warranting further pharmacological investigation.

Keywords: *Cleome viscosa, methanol seed extract, topical gel, anti-oxidant and anti-inflammatory.*

1. INTRODUCTION

1.1 Background

Herbal medicine continues to be a vital resource for drug discovery, particularly in the search for novel therapeutic agents derived from plants. Among such species, Cleome viscosa (family Cleomaceae) (Singh et al., 2017) commonly known as "wild mustard" or "dog mustard," has presented the significant attention due to its wide range of pharmacological activities and traditional medicinal applications (Donkor et al., 2022). Studies on the medicinal properties of Cleome viscosa seeds highlighted several unique features. This sticky, annual herb is commonly found in tropical and subtropical areas and has long been used in traditional medicine to treat infections, inflammatory disorders and gastrointestinal disturbances. The seeds are small, kidney-shaped and range in color from brown to black. When soaked in water, they form a gel-like coating (Upadhyay et al., 2015). Microscopic analysis shows a seed coat composed of thick-walled polygonal epidermal cells, abundance of aleurone grains, oil globules, and calcium oxalate crystals (Onoja., 2016). Powder microscopy further displays seed coat fragments, mucilage cells and lipid inclusions, which serve as diagnostic features. Phytochemical investigations confirm the presence of bioactive constituents including flavonoids, terpenes, tannins, alkaloids, saponins, essential fatty acids, notably linoleic and oleic acids (Chand et al., 2022) & (Ding et al., 2016).

Cleome viscosa seeds has been traditionally used in various cultures for treating a wide range of ailments, including diarrhea, fever, inflammation, liver diseases, bronchitis, skin disorders and malarial fever (Elufioye et al., 2016). In traditional medicine systems like Ayurveda and Unani, the plant is valued for its cooling, stomachic, laxative, diuretic and anthelmintic properties. In Ayurveda it is particularly used to treat malarial fevers, indigestion-related fevers, skin diseases, leprosy, blood disorders and uterine complaints. Additionally, C. viscosa is used in folk remedies as a decoction for digestive stimulation and as an expectorant and its vapor is inhaled to relieve headaches (Joshi et al., 2015) & (Mali 2010). These traditional uses highlight its extensive therapeutic potential and is supported by various studies that validates the medicinal uses and pharmacological potential of C. viscosa. As suggested by different research studies, its fixed oil demonstrates potent antiemetic effects, outperforming chlorpromazine (Singh et al., 2015), while methanol extract show significant antidiarrheal, antipyretic, analgesic and anti-inflammatory properties, comparable to standard drugs like paracetamol and diclofenac (Upadhyay RK., 2015) & (Mali 2010). The plant also displays hepatoprotective and anti-fibrotic effects, protecting against carbon tetrachloride-induced liver damage and has demonstrated substantial antitumor and anticonvulsant activity (Upadhyay 2015) & (Singh et al., 2015). It has also been used for its wound healing properties specially in rats (Singh et al., 2017). Furthermore, C. viscosa exhibits notable antimicrobial, antifungal and insecticidal properties, as well as immunomodulatory and anthelmintic effects (Upadhyay 2015) (Mali 2010) & (Singh et al., 2015). The extract has antimalarial potential, gastroprotective activity even against Helicobacter pylori (Elufioye et al., 2016). It has also been found to be effective in cardiac and respiratory disorders (Upadhyay 2015) (Mali 2010) (Singh et al., 2017) (Singh et al., 2015). These findings suggest that Cleome viscosa holds considerable therapeutic potential across various medical fields, including gastrointestinal, hepatic, neurological and infectious diseases.

Skin is the largest organ of the human body and is very essential in protecting the human body, that's why it is used as an important route of drug administration, which involves three different modes of drug administration that includes regional, topical and transdermal, among which topical dosage form is the most common and widely used route of delivery (Suthar et al., 2024) (Garg et al., 2015). Specially in case of skin injury or a wound in which not only drug delivery is important but a protective layer is of the most significance (Ferraz 2025). Topical gels are semi solid dosage form that not only provides these characteristics but are superior in spread ability and patient compliance compared to ointments, pastes and creams (Sowmya et al., 2013). Their ability of providing drug to site of action while minimizing side effects and providing good soothing effect. In addition to making a protective layer makes it a desirable dosage form for topical application (Goh et al., 2019).

2. EXPERIMENTAL

2.1. Material and Methods

2.2. Collection and Identification of Cleome Visoca Seeds

The collection of seeds of C. viscosa was done from University of Karachi in the month of July and Professor Dr. Mothe Shem ul Hassan confirmed their identification. A voucher specimen (CVS-02- 16/18) was submitted to the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical sciences University of Karachi.

2.3. Extraction of Cleome Viscosa Seed

The seeds of C. viscosa was crushed after being removed from the capsule of the plant to be extracted using methanol as the solvent. The extraction was performed in Soxhlet's apparatus at 70^{o}C until most of the constituents were extracted. Finally, the extract was concentrated using rotary evaporator (Singh et al., 2017) (Ahmed et al., 2011).

2.4. Phytochemical Analysis of Extract

Phytochemical analysis was carried out for the presence of flavonoids, fats, steroids, tannins, saponins, glycosides and alkaloids as described by Iqbal et al., 2015, Auwal et al., 2014, Nath et al., 1946, UC et al., 2015 and Gul et al., 2017.

2.5. DPPH Radical Scavenging Assay

The antioxidant or radical scavenging activity of C. viscosa seeds extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A methanol solution of DPPH was prepared and added to different concentration of extract which were 5, 10, 20, 40, 60, 80, 100µg/ml. Ascorbic acid with same concentrations were used. Percent of inhibition (Pi) was calculated using the following calculation (Jadid et al., 2017).

$$\text{Percentage of Inhibition (Pi)} = \{(A0 - A1)/A0\} \times 100$$

Where A0 is the absorbance of the control reaction

A1 is the absorbance of the standard or extract.

2.6. IC_{50} Value

IC_{50} value in DPPH assay is a measure of free radical scavenging activity. The concentration of the sample (x-axis) was plotted against the percent of inhibition (y-axis) from the graph, $IC_{50}$was the concentration of sample at 50% percent of inhibition (Rehman et al., 2015)

2.7. Preparation of Gel

A gel of Cleome viscosa seeds extract was formulated in three different concentrations which were 6%, 7%, and 8%. Aqueous phase was prepared at 65-70°C by dissolving methyl paraben in purified water then carbomer 934p was added as the gel base and hydrated for 3 hours. Separately, oil phase was prepared by melting non-emulsifying wax, arlacel and menthol crystals. C. viscosa extract was dissolved in polysorbate 80 and then added in the oil phase. Finally, the aqueous phase was added into oil phase with continuous stirring after passing through a sieve of 80 mesh size, and then butylated hydroxyanisole and butylated hydroxytoluene dissolved in isopropyl alcohol was added and mixed well. pH was maintained at 6.5±0.2 with triethanolamine and volume was made up with purified water (Table 1) (Aiyalu et al., 2016).

Table 1. All the ingredients and their Quantities used in Formulation of C. Viscosa Gel

S.No.	Chemical Constituents	Quantity (%w/w)		
		Formulation1	Formulation 2	Formulation 3
1	Cleome viscosa methanol seeds extract	6%	7%	8%
2	Arlacel 170-PA-(SG)	3.00	3.00	3.00
3	Non-ionic emulsifying wax	2.00	2.00	2.00
4	Carbomer 934P	0.25	0.25	0.25
5	Menthol crystals	0.25	0.25	0.25
6	Methyl paraben preservative	0.18	0.18	0.18
7	Polysorbate 80 surfactant	9.00	9.00	9.00
8	Butylated hydroxytoluene	0.02	0.02	0.02
9	Butylated hydroxyanisole	0.02	0.02	0.02
10	Isopropyl Alcohol	3.00	3.00	3.00
11	Triethanolamine 99%	Q.S to make pH 6.5	Q.S to make pH 6.5	Q.S to make pH 6.5
12	Water purified vehicle	Q. S	Q.S	Q.S

2.8. Evaluation of Gel

2.8.1. Extrudability

Collapsible tubes were loaded with 20g of gel, tightly clamped to avoid any roll backs. The gel was ejected by taking the cap off and extrudability was calculated by weighing the amount of gel that was ejected (Aiyalu et al., 2016).

2.8.2. pH Measurement

Digital pH meter was used to determine the pH. The glass electrode was submerged in the gel to detect the pH (Aiyalu et al., 2016).

2.8.3. Appearance and Homogeneity

The appearance and homogeneity of the formulation was visually assessed (Aiyalu et al., 2016).

2.8.4. Viscosity

Brookfield viscometer at a temperature of 25^{o}C and a spindle speed of 12 rpm was used to determine the viscosity of the gel (Aiyalu et al., 2016).

2.8.5. Spread ability

Two standard glass slides were used. Gel was applied on to one slide, then covered with the second slide. A 100 g weight ensured even spreading over 7.5 cm. After removing excess gel from sides, the slides were mounted vertically. A 20 g weight was added to the upper slide to initiate movement, and the time it took to slide the 7.5 cm and detach was recorded (Aiyalu et al., 2016).

Spread ability was calculated by using the following formula.

$$S= m \times l/t$$

where, S= spread ability, m= weight tied to upper slides, l= length of the glass slide and t= time taken in seconds.

2.8.6. Topical Anti-Inflammatory Evaluation of Herbal Gel

In order to induce paw edema carrageenan was used for Albino Wistar rats of both sexes male and female weight ranging from 150-300g. Rats were divided in six different groups, each containing 6 animals (n=6). Edema was induced by injecting 0.1ml of 1% v/w of carrageenan (prepared in distilled water) in the sub planter surface of the left hind paw. Group 1 (control inflamed) received carrageenan only (n=6), group 2 (control) was treated with 2g of sodium carbomer 934p gel base (n=6), group 3 (standard) was treated with 2g of commercial diclofenac sodium gel (standard drug) along with carrageenan (n=6), Group 4, 5 and 6 (test group) were treated with 2g of 6%, 7%, and 8% of sodium carbomer 934p gel infused with C. viscosa seeds extract respectively. Inflammation was quantified by measuring the volume (mL) displaced

by the paw using paleothermometer at 30mins and 180mins after the administration of carrageenan (Jain et al., 2019).

Percent inhibition of edema was calculated by using following formula,

$$\%\text{inhibition} = (1 - Vt / Vc) \times 100$$

Vt = mean volume of paw edema in drug treated group

Vc = mean volume of paw edema in control group.

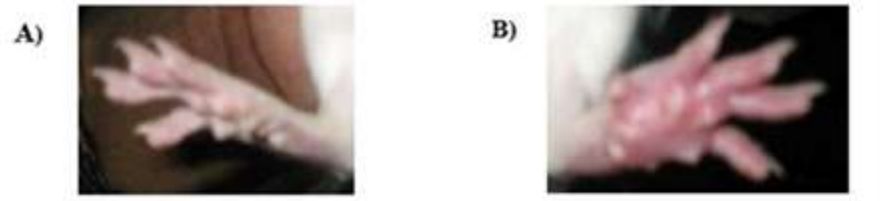

Fig.1. (A) normal paw of a rat, (B) carrageenan induced edematous paw of the same rat.

Table 2. Study Design to Evaluate Topical Anti-Inflammatory Activity of Herbal Gel

Animals Groups	Dose Received
Group 1 Control Inflamed	Received only carrageenan
Group 2 Control	Treated with 2g of sodium carbomer 934p gel base
Group 3 (Standard drug) +inflammation	Treated with 2g of commercial diclofenac sodium gel (Standard drug) along with carrageenan.
Group 4 (6 % gel treated) + inflammation	Treated with 2g of 6% gel of sodium carbomer 934p- Cleome viscosa extract along with carrageenan.
Group 5 (7% gel treated) + inflammation	Treated with 2g of 7% gel of sodium carbomer 934p- Cleome viscosa extract along with carrageenan.
Group 6 (8 % gel treated) + inflammation	Treated with 2g of 8% gel of sodium carbomer 934p- Cleome viscosa extract along with carrageenan.

2.8.7. Statistical Analysis

To visualize and interpret the data, GraphPad Prism v8.0.1 was used to make graphs and carry out statistical tests.

3. RESULTS

3.1. Phytochemical Analysis of C. Viscosa

The phytochemical analysis of C. viscosa seeds extract showed the presence of alkaloids, tannins, saponins, glycosides, steroids, flavonoids and fixed oils (Table 3).

Table 3. Phytochemical Analysis of C. Viscosa Seed Extract.

Phytoconstituents	Tests	Observation	Result
Alkaloid	Wagner Reagent	Reddish brown ppt	+Ve
Tannin	Ferric chloride test	Bluish black color	+Ve
Flavonoid	Lead acetate test	Yellow ppt	+Ve
Steroids	Liebermann test	Blue ring	+Ve
Glycoside	Keller-killiani test	Violet	+Ve
Fixed oil	Copper sulfate test	Blue color	+Ve
Saponin	Foam test	Foams appeared	+Ve

3.2. Antioxidant Activity

DPPH assay was used to determine radical scavenging activity of C. viscosa seeds extract and compared with the standard ascorbic acid. As compared to standard the anti-oxidant activity of C. viscosa was negligible as shown in Table 4. The highest concentration of C. viscosa which was 100µg/ml showed 16.72% of radical scavenging activity.

Table 4. Percentage of Inhibition of free Radicals at different Concentrations of Ascorbic Acid and C. Viscosa Extract

S.No.	Concentration µg/ml	DPPH Scavenging Effect (%) of Standard drug (Ascorbic Acid) at 517 nm	DPPH Scavenging Effect (%) of Tested drug (C. viscosa) at 517 nm
1.	5	17.77%	3.77%
2.	10	23.55%	6.00%
3.	20	94.00%	6.55%
4.	40	96.77%	11.22%
5.	60	97.11%	13.72%
6.	80	97.16%	14.77%
7.	100	99.77%	16.72%

3.3. IC_{50} Value

The IC_{50} value for standard was 13.513 while no IC_{50} value was determined for the tested drug since it didn't cause 50% inhibition and only 16.72% of scavenging activity was observed at the highest concentration (Fig.2).

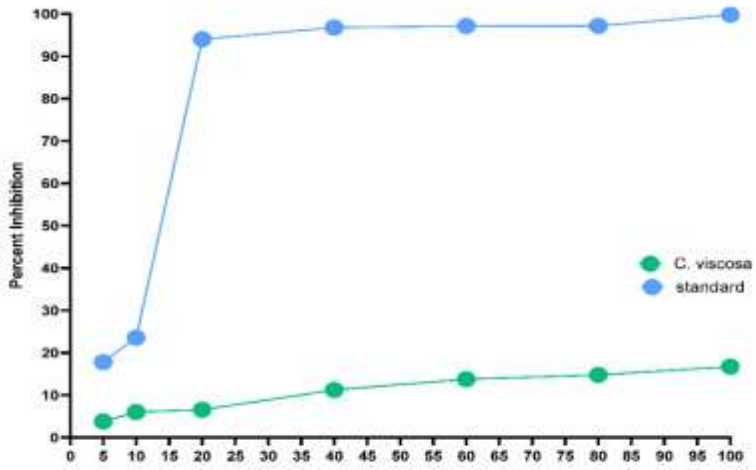

Fig.2. The graph shows percent inhibition of radicals which shows the antioxidant activity and IC50 value was determined using this where standard IC50 value is 13.513 while no IC50 value was obtained for tested drug.

3.4. Characteristics of Formulated Gel

The formulated gel of C. viscosa seed extract was of white color, and homogenous and smooth for all percentages indicating that a physically stable dosage form was prepared. The pH of all formulations varied slightly but was within the expected range which was 6.5 ± 0.2, pH of 6%, 7% and 8% gel was measured as 6.3, 6.7 and 6.5 respectively. The viscosity was within the range of 16000-21000cP and all the gels were found to be in this range, as 6% gel had the viscosity of 16800cP, 7% gel had the viscosity of 17250cP and 8% gel had the viscosity f 19500cP. Since the viscosity was within the specified range the spread ability was also desirable and as the viscosity increased, the spread ability was decreased as shown in Table 5. Extrudability was found to be excellent for all three formulations.

Table 5. Physical and Chemical Characteristics of the Formulated Gel.

Test	Specification	Formulation 1	Formulation 2	Formulation 3
Physical Characteristics	White smooth soft	White smooth soft	White smooth soft	White smooth soft
pH	6.5 ± 0.2	6.3	6.7	6.5
Viscosity	16000 - 21000	16800	17250	19500
Readability	7.95	8.95	7.95	7.64
Extrudability	Excellent	Excellent	Excellent	Excellent

3.5. Anti-inflammatory Activity of Gel

Anti-inflammatory activity of formulated gels was tested using Inflamed paw edema test (Fig.2). The anti-inflammatory activity of the highest concentration of C. viscosa gel 8% was found to be highest and greater than standard drug which was diclofenac sodium. A gradual dose dependent increase in percent inhibition was observed in all 3 concentrations of C. viscosa gel which was proportional with the increase in concentrations (Table 6), (Fig. 3) and (Fig. 4).

Table 6. Evaluation of Anti-Inflammatory Activity of Formulated Gel using Inflamed Paw Edema Test.

Drugs	Doses (g)	Initial	30mins	180mins
Control (gel base)	2	0.420 ± 0.001	0.548 ± 0.001	0.598 ± 0.001
Standard Drug (Diclofenac sodium) 3%)	2	0.350±0.001	0.550 ± 0.002	0.462 ± 0.005
Cleome viscosa gel 6%	2	0.386 ± 0.021	0.598 ± 0.001	0.480 ± 0.001
Cleome viscosa gel 7%	2	0.370 ±0.003	0.568 ± 0.002	0.452 ± 0.003
Cleome viscosa gel 8%	2	0.403 ±0.001	0.564 ± 0.001	0.438 ± 0.003

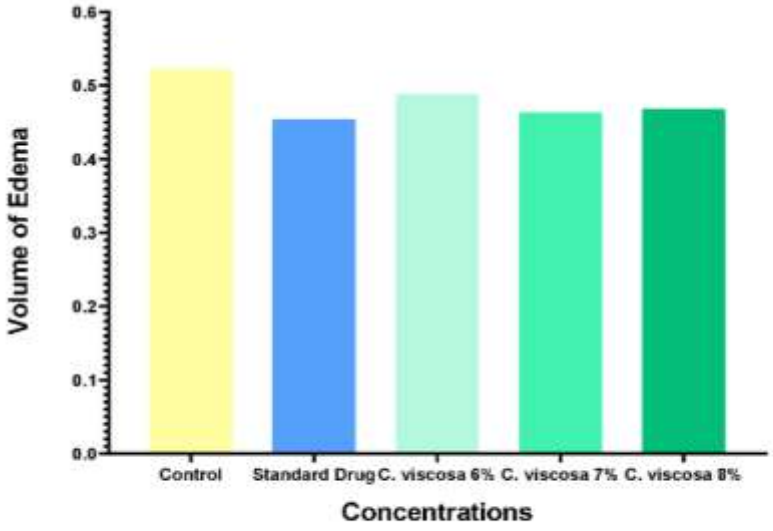

Fig. 3. Mean volume of edema in treated rats was observed and compared with control and standard drug using inflamed paw edema test. The mean volume edema for control, standard drug, *C. viscosa* 6%, 7% and 8% gel was found to be 0.522, 0.454, 0.488, 0.463 and 0.468 respectively.

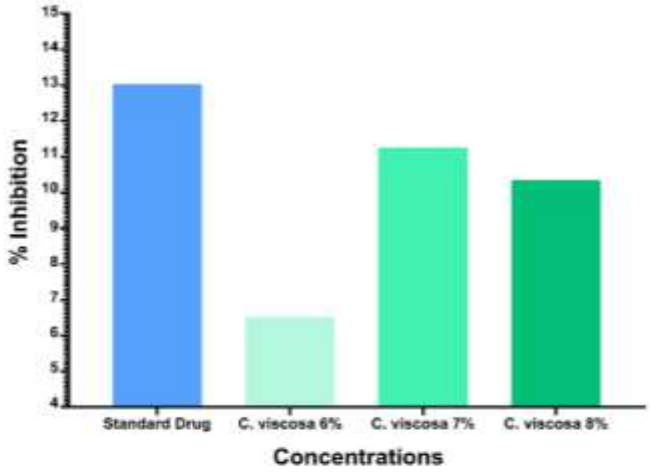

Fig. 4. The mean percentage of inhibition of edema was calculated. % inhibition of standard drug = 13.02%, *C. viscosa* gel 6% = 6.51%, *C. viscosa* gel 7% = 11.24% and *C. viscosa gel* 8% = 10.34%.

4. Discussion

Medicinal plants serve as a critical reservoir and fundamental source of bioactive compounds, playing a vital role due to its significant therapeutic potential. They are widely utilized either independently or in conjunction with synthetic pharmaceuticals due to their cost-effectiveness, widespread availability and cultural compatibility (Ngeranwa et al., 2020). Notably, these plants are extensively employed in the management of various conditions, including inflammatory diseases (Cooper & MA., 2017). In recent years, a lot of research has been focused on plant extracts and their applications in inflammatory diseases (Paun et al., 2020). Key compounds such as flavonoids, polyphenols, terpenoids, saponins, alkaloids and tannins have been identified as major contributors to anti-inflammatory effects (Azab et al., 2016) (Kaymaz et al., 2019) (N guessan et al., 2021). For instance, hyoscine and berberine are commercially available alkaloids are known for their anti-inflammatory properties (Heinrich et al., 2021). Hence why, plant sources are increasingly being used as an alternative therapeutic intervention for many inflammatory conditions (Tasneem et al., 2019).

Despite limited data on phytochemicals of medicinal plants, extensive literature highlights their use in developing countries for treating inflammatory ailments (Hosseinzadeh et al., 2015) (Mbendana et al., 2019). Bioactive compounds extracted from various plant components have demonstrated efficacy against a broad range of inflammatory diseases (Gonfa et al., 2021). Additionally, natural products derived from dietary sources, herbs and medicinal plants are the subject of extensive research, with ongoing pre-clinical investigations exploring their anti-inflammatory potential and bioactive constituents.

Preliminary phytochemical analysis of C. viscosa seed extract showed the presence of several bioactive compounds, including alkaloids, tannins, saponins, glycosides, steroids, flavonoids, and fixed oils. These constituents are known for various pharmacological properties, especially anti-inflammatory and antioxidant activities. The positive identification of flavonoids and tannins, in particular, supports the rationale for further investigation into the therapeutic potential of C. viscosa, as these compounds are associated with membrane stabilization and free radical scavenging effects (Cui & Jia., 2021) (Choy et al., 2019) (Al Masud et al., 2019).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay serves as an efficient method for assessing antioxidant activity through free radical scavenging capacity. This spectrophotometric technique quantifies the ability of test compounds to donate H atoms, thereby reducing the stable nitrogen-centered DPPH radical. In its oxidized state, DPPH exhibits a characteristic deep violet coloration ($\lambda max \approx 517$ nm), which undergoes hypsochromic shift to pale yellow upon reduction to the non-radical form (DPPH-H) through hydrogen atom transfer. The degree of decolorization provides a quantitative measure of antioxidant efficacy, making this method particularly valuable for preliminary screening of radical quenching potential (Baliyan et al., 2022). Despite having these properties, the C. viscosa extract showed negligible antioxidant activity compared to ascorbic acid, and no IC_{50} was determined for C. viscosa extract by the concentrations that we used, which could be because a very low concentration was used for a crude drug extract, as IC_{50} value for C. viscosa ethanol extract was achieved at a much higher concentration in previous literature (Singh et al., 2021).

The formulated gel of 6%, 7% and 8% of C. viscosa exhibited desirable physicochemical properties as they were stable and homogenous, their viscosity, pH, spread ability and extrudability were also in desirable ranges. The anti-inflammatory activity assessed by paw edema test in rats showed promising results as all three gels of C. viscosa 6%, 7% and 8% showed comparable results with the standard diclofenac, specially 7% gel showing highest percent inhibition of 11.24% while diclofenac showing percent inhibition of 13.02%. The anti-inflammatory effect compared to radical scavenging activity was much greater when compared to their standards also indicating that the plant possessed anti-inflammatory activity was mediated through mechanism other than just free radical scavenging, like membrane stabilizing, inhibition of pro inflammatory markers, modulation of Nuclear Factor-Kappa B (NFκB) and suppression of cytokines. These anti-inflammatory mechanisms are due to the presence of steroids, flavonoids and alkaloids (Cui & Jia., 2021) (Choy et al., 2019) (Al Masud et al., 2019). Flavonoids are known to reduce the production of arachidonic acid (AA), prostaglandins and leukotrienes. They are also known to reduce the levels of intracellular Ca2+. While tannins are shown to inhibit cyclooxygenase (CO), and phenolic compounds have also demonstrated anti-inflammatory by regulating the levels of different inflammatory markers such as COX-2 (Karrat et al., 2022).

5. Conclusion

In conclusion, the Cleome viscosa seeds methanol extract represents a significant source of bioactive phytochemicals with demonstrable therapeutic potential. Research has confirmed two key properties: significant antioxidant activity and potent topical anti-inflammatory effects. The efficacy extract, comparable to standard agents' ascorbic acid and diclofenac sodium, showed natural alternative. The anti-inflammatory action is likely mediated through the inhibition of cyclo-oxygenase enzymes by compounds such as flavonoids and polyphenols present in the extract. These findings validate the traditional uses of the plant and open promising opportunities in modern therapeutics, particularly as a topical anti-inflammatory treatment. However, to completely realize this potential, further investigation is crucial. Future studies must focus on elucidating the precise molecular mechanisms of action, conducting clinical trials to validate efficacy and safety in humans, and standardizing optimal concentrations for reliable therapeutic outcomes. Ultimately, Cleome viscosa stands as a compelling candidate for further development, exemplifying the value of plant-derived compounds in advancing health and disease management.

Ethical Approval

Standard guidelines were followed for this research study and it was approved from the Institutional of Bioethical Committee Reference no. IBCKU-269/2022A.

Conflict of Interest

There is no conflict of interest.

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