Phytochemical Profiling, In-Vitro Antioxidant and Anti-Inflammatory Evaluation, and Topical Gel Formulation of Ethanolic Seed Extract of Cleome viscosa Linn

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Abstract

Traditional medical systems continue to play a vital role in healthcare, particularly in regions where plant-based remedies remain culturally and economically significant. Among these systems, Siddha medicine, originating in South India, emphasizes the therapeutic use of medicinal plants for maintaining health and managing disease. Cleome viscosa Linn., a sticky annual herb belonging to the family Capparaceae, is widely distributed across tropical and subtropical regions and is frequently encountered as a wild-growing plant. In traditional practice, various parts of this species have been employed for the management of digestive disturbances, infections, inflammatory conditions, and wound-related disorders. In the present investigation, seeds of Cleome viscosa were selected to examine the phytochemical profile and in-vitro biological performance of their ethanolic extract (NKS ET). Qualitative screening confirmed the presence of multiple classes of secondary metabolites, including flavonoids, phenolic compounds, tannins, steroids, carbohydrates, and proteins. Quantitative assessment revealed a comparatively higher flavonoid concentration at lower extract levels and measurable phenolic content at increased concentrations, indicating the presence of antioxidant-associated constituents. Free radical scavenging ability was evaluated using the DPPH model, where the extract demonstrated substantial radical neutralization, achieving notable inhibition at 100 mg/ml with a defined IC_{50} value when compared with a reference antioxidant. Anti-inflammatory potential was examined through inhibition of protein denaturation, and the extract produced moderate suppression of denaturation at lower concentrations, showing activity comparable to the standard drug used in the assay. The collective findings highlight the biological relevance of Cleome viscosa seed extract and provide experimental support for its traditional usage. Furthermore, the observed in-vitro activity indicates that the extract holds promise for incorporation into topical gel-based systems intended for localized antioxidant and anti-inflammatory applications.

Keywords: Cleome viscosa Seed (NKS ET), antioxidant, DPPH assays, Phytochemical and anti-inflammatory.

INTRODUCTION

Throughout history, plants have served as raw materials for pharmaceutical purposes. In India, a wealth of knowledge regarding the therapeutic value of plants exists among the populace. Approximately 3,500 plant varieties are recognized as sources for raw pharmaceuticals in the country. Out of these, around 2,500 species hold significant medicinal relevance. Certain plants, which may be classified as weeds, are also deemed important for their medicinal properties. Both traditional and contemporary therapeutic practices incorporate plant-based products. In today's landscape, the popularity of herbal remedies is expanding. The discovery of new medications is facilitated by advancements in modern biotechnological and bioinformatics methods (Packialakshmi LN and Oviya K 2014). In today's world, the use of herbal remedies is becoming increasingly popular as people's trust in natural healing methods continues to grow. New therapeutically effective medications are being identified from natural substances extracted from higher plants and microorganisms (Brindha P, et al 2012). *Cleome viscosa* Linn commonly known as wild mustard or dog mustard belongs to the Capparaceae family. This sticky herb is frequently seen as a common weed in the plains of countries such as Pakistan, India, China, Ceylon, and Africa, as well as in various tropical areas around the globe throughout the year. In traditional medicinal practices, the leaves, seeds, and roots of this plant are extensively utilized for a variety of purposes, including acting as an anthelmintic, antiscorbutic, antiseptic, cardiac stimulant, carminative, febrifuge, sudorific, anticonvulsant, and antidiarrhoeal, and they are also employed to address skin ailments (Mohtasheem ul Hasan M, et al 2011). It is known as 'Hurhur' in India. Traditionally, the *Cleome viscosa* Linn plant serves as a treatment for malaria and is beneficial for blood-related ailments and issues with the uterus. The strong-flavored seeds and seed pods act as an alternative to mustard in various curries (Panduraju T, et al 2011). *Cleome viscosa*, part of the Capparaceae family, is a yearly herb commonly referred to as wild mustard or dog mustard. Across various nations, this plant is utilized within traditional medicine to remedy numerous ailments, and it is also recognized for its outstanding nutritional value, antioxidant capabilities, and ability to neutralize free radicals. In India, it serves as a conventional remedy for a variety of conditions, such as epilepsy, irritable bowel syndrome, and infections caused by protozoa and worms. Cleome is more than just an invasive plant; it is a vital medicinal herb that is examined through both its chemical and pharmacological properties, providing substantial evidence regarding its healing benefits (Joshi, T., et al 2015). *Cleome viscosa* is a native herbal plant that exhibits significant seasonal variation in biodiversity across the northwestern regions of India. This prevalent weed is typically located in agricultural fields, neglected areas, and moist, grassy environments throughout the Indian plains. The plant produces yellow blossoms and elongated, thin seed pods that bear seeds resembling mustard seeds, emitting a strong fragrance during the rainy and post-rainy periods. The seeds, which are rich in oil, are utilized for producing cattle cake. The oil extracted is abundant in unsaturated fatty acids and essential nutrients, particularly Vitamins A and C, as well as minerals like calcium, iron, and protein. Additionally, the plant harbors free gallic acid, gallotannins, iridoids, saponins, and polyphenolic compounds of terpenoid nature, all recognized for their antioxidant properties. Its oil shares a fatty acid profile that closely resembles that of non-edible oils derived from rubber, jatropha, soybean, safflower, linseed, and rapeseed. As a result of its oil content, this plant serves as a promising source for green biodiesel (Upadhyay, R.K., 2015). The leaves of *Cleome viscosa* exhibited total phenolic and flavonoid levels of 66.38±0.82mg/g and 0.54±0.04mg/g, along with an antioxidant activity of 77.30%. The significant free radical scavenging capacity of *Cleome viscosa* leaves is demonstrated by the low IC50 values recorded in both DPPH (1,1-diphenyl-2-picryl hydrazyl) (373.18 μg/ml) and hydroxyl radical (573.55 μg/ml) assays. Compared to the stem, *Cleome viscosa* leaves show elevated levels of phenolics and flavonoids as well as notable antioxidant capabilities, including reducing capacity and free radical neutralization (Gupta, P.C., et al 2011). C. viscosa underwent a series of extractions using hexane, chloroform, ethyl acetate, and methanol in order. Among these various extracts, the methanol exhibited a notable concentration of phytochemicals and demonstrated significant antioxidant properties. The phytochemicals identified included alkaloids at 15.42 mg/g, followed by phenols at 15.23 mg/g, fatty acids at 5.06 mg/g, flavonoids at 14.52 mg/g, tannins at 7.38 mg/g, and carbohydrates at 5.37 mg/g. Furthermore, the methanolic extract of C. viscosa displayed remarkable antioxidant effects in tests such as DPPH, ABTS, ferrous ion chelation, and FRAP assays. Given the impressive antioxidant performance of the methanolic extract, C. viscosa may serve as an excellent resource for isolating anticancer compounds (Govindan, L., et al 2018). The flavonoid glycoside's ability to reduce inflammation could be attributed to its ability to hinder the production of prostaglandins. A targeted toxic effect of flavonoid glycoside was observed against the gram-positive bacteria, specifically S. aureus (Senthamilselvi, M.M., et al 2012). The anti-inflammatory properties of the plant extract, *Cleome viscosa*, are similar to those of the standard medications Aspirin and indomethacin. Within the extracts that were evaluated, the methanol extract from C. viscosa showed the highest level of effectiveness. These results offer scientific validation for its traditional medicinal applications and suggest a strong potential for creating anti-inflammatory drugs derived from the plant (Pillai, L.S. and Nair, B.R., 2014). The ethanolic extract of C. viscosa at 95% concentration demonstrated significant anti-inflammatory properties, as indicated by the suppression of NO and TNFα production. This evidence reinforces the traditional Thai practice of utilizing C. viscosa to promote health by alleviating inflammation. Therefore, it is essential to conduct additional research on its ethanolic extract in animal models and to isolate specific active components for their antiinflammatory effects (Namphonsaen, K., et al). The investigations into phytochemicals reveal that the unrefined leaf extracts of *Cleome viscosa L* contained tannins, flavonoids, terpenoids, and alkaloids, which were predominantly present in all five types of solvent extracts from the leaves. Saponins, however, were detected solely in the acetone extracts (Rajani, A., et al 2014. The petroleum ether extract demonstrates remarkable activity in promoting wound recovery (Singh, H., et al 2017). Pharmacognostic research on C. viscosa seeds could provide vital information for recognizing the raw materials and aid in distinguishing the plant from its related species and potential contaminants (Singh, H., et al 2017). This investigation focuses on evaluating the in vitro antioxidant properties using the DPPH method and assessing the anti-inflammatory effects via the protein denaturation technique of the Cleome viscosa Seed (NKS ET), to analyze its phytochemical and pharmacological traits.

Topical gel systems represent a practical approach for delivering plant-derived bioactive compounds at the site of action. Extracts exhibiting antioxidant and anti-inflammatory properties are particularly relevant for such systems. In view of the biological responses observed in the present investigation, the Cleome viscosa seed extract was considered for topical formulation feasibility within the scope of pharmaceutical development.

REVIEW OF LITRATURE

1. **Patil and Patil, 2024.** In this study, Cleome viscosa Linn., seeds were reviewed for their significant medicinal importance in traditional and modern medicine. The seeds are rich in bioactive constituents such as flavanoids, alkaloids, phenolic compounds, tannins, saponins, and fatty acids. Pharmacological investigations revealed notable anti-inflammatory, antioxidant, antimicrobial, and analgesic activities. The presence of phenolics and flavanoids was found to play a major role in inhibiting inflammatory mediators and scavenging free radicals. The study concluded that C. viscosa seeds possess promising therapeutic potential, warranting further experimental validation.

2. **Kumar et al., 2023**. In this study, the phytochemical composition and antioxidant activity of Cleome viscosa Linn., seed extracts were evaluated using different solvents. Qualitative screening confirmed the presence of alkaloids, flavanoids, glycosides, phenols, and fixed oils. Antioxidant activity assessed by DPPH and FRAP assays showed significant free radical scavenging potential, particularly in ethanolic seed extracts. The results suggested that C. viscosa seeds could serve as a natural source of antioxidants useful in managing oxidative stress–related disorders.

3. **Sivakumar and Devi, 2022.** In this study, the anti-inflammatory activity of Cleome viscosa Linn., seed extract was investigated using in-vitro protein denaturation and membrane stabilization methods. The seed extract exhibited dose-dependent inhibition of protein denaturation comparable to standard anti-inflammatory drugs. The observed activity was attributed to the presence of flavanoids and phenolic compounds that stabilize cell membranes and suppress inflammatory pathways. This study supports the traditional use of C. viscosa seeds in inflammatory conditions.

AIM AND OBJECTIVE

Aim:

To evaluate the antioxidant and anti-inflammatory activity of Cleome viscosa Linn., seed extract and to formulate and evaluate a herbal gel incorporating the extract for topical therapeutic application.

Objective:

1. To prepare and extract bioactive constituents from Cleome viscosa Linn., seeds using a suitable extraction method.

2. To perform preliminary phytochemical screening of the seed extract.

3. To evaluate the in-vitro antioxidant activity of the extract using standard methods (e.g., DPPH assay).

4. To evaluate the in-vitro anti-inflammatory activity of the extract using suitable models (e.g., protein denaturation method).

5. To formulate a herbal gel incorporating Cleome viscosa Linn., seed extract.

6. To evaluate the physicochemical properties of the formulated gel (pH, viscosity, spreadability, homogeneity, and stability).

7. To assess the overall therapeutic potential and suitability of the formulated gel for topical application.

MATERIAL AND METHODS

Collection of samples: Seeds of Cleome viscosa were collected from the Tiruchengode region of Namakkal district, Tamil Nadu, India. The collected seeds were cleaned to remove extraneous matter, shade-dried at room temperature, and coarsely powdered prior to extraction.

Method of preparation of samples: A quantity of 250 g of the powdered Cleome viscosa seeds was subjected to maceration using ethanol as the extraction solvent. The powdered material was soaked in a sufficient volume of ethanol in a closed container and kept at room temperature for an appropriate duration with occasional stirring to facilitate extraction. After completion of the maceration process, the extract was filtered to separate the marc, and the filtrate was concentrated under reduced pressure to obtain the ethanolic extract. The prepared extract was preserved in an airtight container and used for subsequent phytochemical and in-vitro pharmacological evaluations.

PHYTOCHEMICAL ANALYSIS TEST

Phytochemical analysis is the technique employed to detect different substances present in plant extracts. Plants consist of a wide range of chemical components that can trigger various physiological effects and provide health benefits. As a result, it is common to investigate plants for the presence of biologically active and therapeutically important phytochemicals. These compounds contribute to specific biological activities. Examples of phytoconstituents include alkaloids, steroids, carbohydrates, saponins, tannins, and flavonoids among others (Farnsworth NR. et al 1966 & Haseen, A., et al 2024).

ESTIMATION OF TOTAL FLAVONOIDS:

The amount of flavonoids in the plant extract was measured using the Aluminium chloride method. 1 ml of Cleome viscosa Seed (NKS ET) ethanolic extract after hexane (1mg/ml) in methanol and 1ml of the standard quercetin (200,400,600,800, and 1000µg/ml) in methanol are kept in ten millilitre of the standard measuring flask, & then 4ml of distilled water was added and then 0.3 ml of 5% sodium nitrite was added and after 5minutes 0.3ml of 10% Aluminium chloride was added. Two ml of 1Molar NaOH was added after five minutes and made up to 10 ml with distilled water. Using a UV-VIS spectrophotometer. The absorbance of both the crude extract and standard quercetin was checked at 510 nm against the reagent blank. The flavonoid content in each gram of dried crude extracts was expressed as milligrams of quercetin equivalents. The test sample's absorbance was measured three times (MohdNurNasyrig, et al 2014 &A. Muchandi, C. et al 2017).

ESTIMATION OF TOTAL PHENOL:

The overall amount of polyphenols in the crude extract was measured using the Folin-Ciocalteu method and a UV-VIS spectrophotometer. 1ml of the different extract of Cleome viscosa Seed (1mg/ml) in methanol and 1 inl of the standard Galhe acid (200, 400, 600. 800, 1000µg/ml) in methanol were taken in a different 25 al volumetric flask. 9ml of the distilled water was added to each volumetric flask and mixed well. The mixture was combined with: one millilitre of the folin Ciocalteu reagent and forcefully shaken. 10ml 7percentage NaCO3 solution was added to the mixt are after 5 minutes. Using distilled water, the volume was adjusted to 25ml after a ninetyminute incubation at room temperature. The absorbance of both the test and standard was measured at 550nm with a reagent blank using a UV-VIS spectrophotometer. The absorbance of the test sample was taken three times. There was a linearity curve for the Gallic acid (Blainski, A., et al 2013 & Nikolaeva, T.N., et al 2022).

PHARMACOLOGICAL EVALUATION

In vitro Antioxidant activity

Cleome viscosa Seed (NKS ET) ethanolic extract investigated for in vitro antioxidant activity by DPPH for the estimation of anti-oxidant potential of Cleome viscosa Seed (NKS ET) ethanolic extract. Cleome viscosa Seed (NKS ET) also tested by DPPH. Antioxidant Activity by DPPH Assay

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay

A popular way to evaluate the effectiveness of antioxidants in neutralising free radicals is through the DPPH assay. This process involves combining a stable free radical called DPPH assay with a plant extract and then observing the change in colour. A more noticeable colour shift suggests a greater antioxidant activity (Baliyan S. et al., 2022).

Principle: The capacity of the extracts to neutralize free radicals was evaluated through the DPPH radical scavenging assay, following the methodology outlined by Blois and Desmarchelier (Blois MS (1958) and (Desmarchelier et al., 1997). The potential of plant extracts to donate hydrogen atoms was examined by observing the decolorization of a methanol solution containing 2,2-diphenyl-1- picrylhydrazyl (DPPH).When antioxidants are present, the DPPH solution, which initially appears violet or purple, transitions to yellow hues (Rahman MM et al., 2015).

Procedure: A arrangement of DPPH at a concentration of 6×10-5M in methanol was made by dissolving 7.89mg in 100ml, which implies for 250ml, you'd utilize (7.89/100) ×250. At that point, 500µl from each test arrangement was set into an Eppendorf tube. Each concentration was tried three times. Following, 500µl of the DPPH solution was included to the test arrangement and blended well. This blend was shaken and cleared out at room temperature for half an hour. The absorbance was at that point measured at 520nm. Ascorbic corrosive served as the positive control, whereas refined water acted as the negative control. The rate restraint compared to the standard was calculated utilizing the condition underneath, and the IC50 values were too decided. Graph Pad Prism 9 software (Blois MS. 1958 and Karan SK et al., 2012).

Analysis: To analyse data statistically, you need to determine the actual absorbance of the plant material. This involves subtracting the blank plant absorbance from the assay absorbance of the plant. Essentially, after preparing your dilution series, you'll measure the colour intensity of the plant material using a spectrophotometer, which will give you the absorbance reading for the blank plant. For instance, after diluting the plant, it will have a certain colour, and when you measure its colour intensity, you might find the absorbance to be A for the blank plant. If the DPPH assay shows an absorbance of B, then the actual absorbance for the assay would be calculated as B minus A. So, the formula for actual absorbance is: Assay absorbance – Blank absorbance. Next, you'll need to calculate the percentage inhibition values using the following equation: % Inhibition = (Blank DPPH solution absorbance – Actual absorbance) x 100% / Blank DPPH solution absorbance. To discover the absorbance of the clear DPPH arrangement, you'll degree it after planning the DPPH arrangement. You'll degree its absorbance spectro photometrically. By rehashing this process for positive and negative controls, you have got to plot a log concentration time chart and the IC50 should be detected employing a nonlinear relapse demonstrate utilizing Graph Pad Crystal computer program. At that point by comparing the test comes about with controls, you'll be able decipher the information discoveries.

In-vitro Anti-Inflammatory Activity

Principle of In vitro Egg Albumin Denaturation Method: The most objective of the egg whites denaturation measure is to discover out in case certain operators or compounds can stop or moderate down the method of egg whites getting to be denatured beneath particular conditions. Denaturation alludes to the alter in a protein's structure that leads to a misfortune of its natural work. In this try, egg whites serves as a demonstrate protein, and denaturation happens when it is uncovered to extraordinary warm, changing pH levels, or other denaturing substances. The test surveys how well a medicate or compound can avoid or diminish the denaturation of egg whites, which makes a difference assess its anti-inflammatory impacts. The fundamental rule of the egg whites denaturation measure is that substances with anti-inflammatory properties might balance out protein structures and anticipate denaturation, a prepare regularly related with irritation and tissue harm. Hence, operators or compounds that altogether diminish egg whites denaturation in this test might possibly display anti-inflammatory impacts. Protein denaturation is accepted to be one of the components contributing to irritation. Non-steroidal anti-inflammatory drugs (NSAIDs) not as it were avoid protein denaturation but too restrain the COX enzyme at the same time. Different concentrations of the test can be blended with egg whites arrangement beneath controlled exploratory conditions, permitting the responses to occur sometime recently measuring the absorbance to calculate the rate of restraint (Dharmadeva S et al., 2016).

Inhibition of Albumin Denaturation: The response blend was made by combining 0.5 ml of Boswellic acid corrosive and a 0.45 ml fluid arrangement of 5% bovine egg whites. The pH of the blend, which was 6.3, was balanced with a bit of 0.1N HCl whereas keeping it at 37 ˚C for 20 minutes. After that, it was warmed to 57 ˚C for 30 minutes. Once cooled, the arrangement was exchanged to 96-well plates, and the absorbance was measured at 660 nm. Standard was utilized as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml refined water.

Preparation of gel

A topical gel base was prepared by gradually dispersing the required quantity of Carbopol in purified water with continuous stirring to avoid the formation of aggregates. The dispersion was allowed to stand until complete hydration of the polymer was achieved. Separately, the ethanolic extract of Cleome viscosa seeds was dissolved in a measured amount of propylene glycol to obtain a uniform extract solution. This solution was slowly incorporated into the hydrated polymer base under constant mixing to ensure even distribution of the extract throughout the formulation. Methyl paraben, previously dissolved in a small volume of warm purified water, was added as a preservative. The pH of the formulation was carefully adjusted using triethanolamine until a clear, smooth gel of suitable consistency was obtained. The final volume was made up with purified water, and the prepared gel was stored in a closed container for further study.

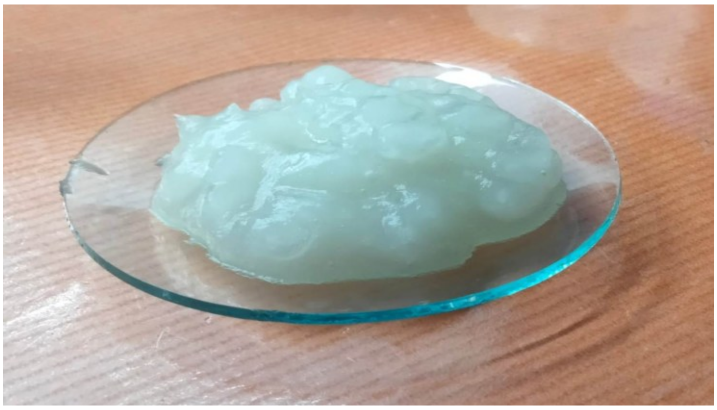

Fig No: 01 FORMULATION OF GEL

RESULT

The ethanolic extract of Cleome viscosa Seed (NKS ET) has demonstrated both anti-inflammatory and antioxidant effects. The ethanolic extract of Cleome viscosa Seed (NKS ET) of yield percentage is 0.8%. Preliminary phytochemical analysis of the ethanolic extract of NKS ET revealed the presence of flavonoids, phenol, protein, tannins, steroids and carbohydrates (see Table 1).The current study reveals that the ethanolic extract of NKS ET, at a concentration of 100, contains a total flavonoid content of 214 mg, which is favorable compared to other concentrations (Table 2) and the ethanolic extract of Cleome viscosa Seed (NKS ET), at a concentration of 1000, contains a total phenol content of 54 mg, which is favorable compared to other concentrations (Table 3). The quantitative analysis of total flavonoids indicates that the ethanolic extract of NKS ET has the highest levels of flavonoids demonstrates that NKS ET exhibits strong antioxidant activity. The ethanolic extract of Cleome viscosa Seed (NKS ET) has demonstrated both anti-inflammatory and antioxidant effects. In the current study, NKS ET exhibited in vitro antioxidant activity as assessed by the DPPH. At a concentration of 100 mg/ml, the DPPH assay revealed an inhibition percentage of 77% (see Table 3 and Figure 2), with an IC50 value of 161.755 mg/ml, in contrast to the standard ascorbic acid, which had an IC50 value of 33.7334 mg/ml (refer to Table 4). This indicates that NKS ET's antioxidant activity via the DPPH assay is significant when compared to Vitamin C. To assess anti-inflammatory activity in vitro, the albumin denaturation method was utilized. At 50 mg/ml, Cleome viscosa Seed (NKS ET) demonstrated a 48% inhibition (see Table 7) and an IC50 value of 106.0796 mg/ml, compared to the standard diclofenac sodium, which had an IC50 value of 121.29 mg/ml (see Table 8). Throughout our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammatory properties of Cleome viscosa Seed (NKS ET). When compared to diclofenac sodium, Cleome viscosa Seed (NKS ET) showed moderate anti-inflammatory effects, indicating it's significant for anti-oxidant activity. The collective findings indicate that the ethanolic seed extract possesses characteristics supportive of its consideration for topical gel-based delivery.

DISCUSSION

Antioxidant resistances comprise of different compounds and proteins that work together to neutralize and dispense with receptive oxygen species (ROS). (Hawk, M.A., et al 2018). Besides, prior thinks about highlighted that the egg whites denaturation test assesses a medicate or compound's capacity to avoid or decrease egg whites denaturation, serving as an pointer of its anti-inflammatory properties (Dharmadeva S et al., 2016). Cleome viscosa Linn has been studied in detail for its chemical and medical properties. It is clear from the previous discussion that Cleome viscosa Linn serves as a significant healing solution for a range of health issues. The oil extracted from Cleome viscosa Linn seeds is also utilized in the production of biodiesel. A variety of substances have been extracted from the plant that contributes to its medicinal effects (Singh, H., et al 2015). C. viscosa underwent a series of extractions using hexane, chloroform, ethyl acetate, and methanol in order. Among these various extracts, the methanol exhibited a notable concentration of phytochemicals and demonstrated significant antioxidant properties. The phytochemicals identified included alkaloids at 15.42 mg/g, followed by phenols at 15.23 mg/g, fatty acids at 5.06 mg/g, flavonoids at 14.52 mg/g, tannins at 7.38 mg/g, and carbohydrates at 5.37 mg/g. Furthermore, the methanolic extract of C. viscosa displayed remarkable antioxidant effects in tests such as DPPH, ABTS, ferrous ion chelation, and FRAP assays. Given the impressive antioxidant performance of the methanolic extract, C. viscosa may serve as an excellent resource for isolating anticancer compounds (Govindan, L., et al 2018). A triterpenoid substance known as Lupeol was extracted from the petroleum ether extract of Cleome viscosa L. seeds, which likely contributes to the high effectiveness of the ointment in facilitating wound healing. The petroleum ether extract demonstrates remarkable activity in promoting wound recovery (Singh, H., et al 2017). Pharmacognostic research on C. viscosa seeds could provide vital information for recognizing the raw materials and aid in distinguishing the plant from its related species and potential contaminants (Singh, H., et al 2017). Moreover, inquire about on Cleome viscose seed (NKS ET) has approved their capacity to neutralize free radicals, displaying their antioxidant properties. Water extracts of herbs and spices contain significant levels of phenols and flavonoids, which enable their ability to neutralize free radicals to be comparable to that of gallic acid (Shirazi, O.U., et al 2014). The beneficial antioxidant and anti-inflammatory characteristics of medicinal botanicals, as discussed by Krishnaiah, D., et al in 2011, can be credited to the presence of phenolic compounds as noted by Dangles, O. in 2012 and Agati, G., et al in 2012. Our study further emphasizes a multi-herb blend that is rich in phenolics, which may enhance its antioxidant capabilities. Additionally, investigations into the Cleome viscosa Seed (NKS ET) have verified its effectiveness in counteracting free radicals, demonstrating its antioxidant functions and proficiency in alleviating acute inflammation and discomfort. The biological activity demonstrated by the ethanolic extract can be correlated with its phytochemical composition, particularly compounds known to influence oxidative and inflammatory processes. Such activity profiles are often considered advantageous for topical applications, thereby supporting the relevance of the extract for future formulation into gel-based delivery systems.

Table 1: ***Cleome viscose*** **Seed (NKSET)** ***Invitro*** **antioxidants activity by DPPH assay**

S.NO	**Concentration(mg)**	**COD**	**SOD**	**%inhibition**	**Average (%)**	**IC_{50} Value**
1	50 mg	0.29	0.07	75%	73%	161.755 mg/ml
2		0.29	0.08	72%		
3		0.29	0.08	72%		
1	100 mg	0.29	0.07	75%	77%	168.345 mg/ml
2		0.29	0.07	75%		
3		0.29	0.05	82%		
1	150 mg	0.29	0.09	68%	63%	156.870mg/ml
2		0.29	0.10	65%		
3		0.29	0.12	58%		
1	VitaminC	0.29	0.04	86%	**88%**	
2		0.29	0.03	89%		
3		0.29	0.03	89%		

Table 2: ***Cleome viscosa*** **Seed (NKS ET) Antioxidant activity IC_{50} value (mg/ml) compared to standard Vit C IC_{50}value (mg/ml)**

S.NO	Concentration(mg)	Average(%)	IC50(mg/ml)
1	50 mg	73%	**161.755mg/ml**
2	100 mg	77%	
3	150 mg	63%	
Standard Ascorbic acid vitamin			
1	50 mg	91%	**33.7334 mg/ml**
2	100 mg	87%	
3	150 mg	86%	
4	200 mg	92%	
5	250 mg	84%	

Table 3: ***Cleome viscosa Seed*** **(NKSET)** ***Invitro*** **anti- inflammatory activity**

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)	IC50 (mg/ml)
1	50 mg	0.30	0.14	53%	**48%**	106.0796 mg/ml
2		0.30	0.17	43%		
3		0.30	0.15	50%		
1	100 mg	0.30	0.15	50%	**47%**	
2		0.30	0.16	46%		
3		0.30	0.16	46%		
1	150 mg	0.30	0.16	46%	**45%**	
2		0.30	0.16	46%		
3		0.30	0.17	43%		
1	Diclofenac sodium	0.30	0.06	80%	**79%**	
2		0.30	0.07	77%		
3		0.30	0.06	80%		

Table 4: *Cleome viscosa* Seed (NKSET) Anti-Inflammatory Activity Ic_{50}Value (mg/ml) Compared to Standard Diclofenac Sodium Ic_{50}Value (mg/ml)

S.NO	Concentration(mg)	Average (%)	IC50(mg/ml)
1	50 mg	48%	**106.0796mg/ml**
2	100 mg	47%	
3	150 mg	45%	
Standard Diclofenac sodium			
1	50 mg	91%	**121.29mg/ml**
2	100 mg	93%	
3	150 mg	85%	
4	200 mg	89%	
5	250 mg	88%	

CONCLUSION

The ethanolic extract of Cleome viscosa seeds (NKS ET) demonstrated notable antioxidant and moderate anti-inflammatory activity, supported by the presence of flavonoids, phenolic compounds, tannins, steroids, proteins, and carbohydrates. The extract exhibited strong free radical scavenging activity in the DPPH assay, with 77% inhibition at 100 mg/ml, which may be associated with its high flavonoid content. In vitro anti-inflammatory evaluation using the protein denaturation method showed moderate inhibition, indicating potential anti-inflammatory efficacy. These findings scientifically support the traditional use of Cleome viscosa in Siddha medicine and suggest that the extract may serve as a promising candidate for future topical gel-based pharmaceutical development.

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