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# *IN-VITRO* ANTI-INFLAMMATORY STUDIES IN *CLEOME VISCOSA L.* AND *CLEOME BURMANNI* W. & A. (CLEOMACEAE)

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**Keywords:**

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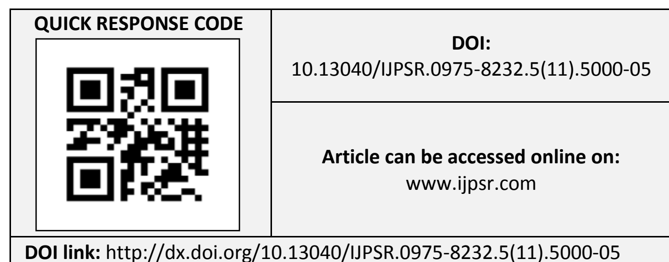
**ABSTRACT:** The present study evaluates the anti-inflammatory potential of the methanol and chloroform extracts of *Cleome viscosa* and *C. burmanni* by *in-vitro* methods. The anti-inflammatory property of the extracts was assessed using albumin denaturation assay, proteinase inhibitory activity and hyaluronidase inhibition assay and the results compared against reference drugs such as Aspirin and Indomethacin. The present findings exhibited a concentration dependent inhibition of albumin denaturation and hyaluronidase enzyme by the extracts of both plants. Proteinase activity was also significantly inhibited by both plant extracts. The methanol extract was more effective than chloroform extract and was comparable to the standards. From the present study, it can be concluded that the extracts of *C. viscosa* and *C. burmanni* possessed marked *in- vitro* anti-inflammatory activity and the effect could be due to the presence of various phytochemicals present in the plants.

**INTRODUCTION:** There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicines. Traditional medicines are sources of easily available, effective healing agents to the people. It is in this context, that the people consume several plants or plant derived preparations to cure different diseases. Therefore, traditional medicines are being re-evaluated and extensive research involving different plant species and their active therapeutic principles is being carried out. Screening of plants for their biological activity is done on the basis of either their ethnobotanical knowledge or the results of chemotaxonomic investigation.



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Purified natural compounds from plants can serve as template for the synthesis of new generation drugs with low toxicity and higher therapeutic value [1]. Thus it is expected that natural products would play a significant role in human health in relation to the prevention and treatment of disease conditions [2].

An uncontrolled and persistent inflammation may act as an etiologic factor for many of the chronic illnesses [3]. Inflammation is a primary physiologic defense mechanism that helps the animal body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli in order to eliminate or limit the spread of injurious agents. It is a local response of living mammalian tissues to foreign substances such as bacteria, trauma chemicals and heat. It is characterized by pain, swelling and redness which are brought about by various inflammogens like histamines, bradykinins, prostaglandins, leukotrienes. By inhibiting these mediators, the inflammatory response could be suppressed [4]. Although it is a

defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [5].

It is not a single event but a series of events occurring in an orderly sequence and is closely interwoven with the process of repair. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury as it is a very complex, dynamic and multifactored phenomenon. Such components of inflammation include oedema formation as a result of extravasation of fluid and proteins, leukocyte infiltration and granuloma formation at the inflammatory site [6, 7]. No known anti-inflammatory drugs are equally effective in suppressing all the facets of inflammatory reactions.

The treatment of inflammation depends on non-steroidal or steroidal anti-inflammatory agents [8]. Non-steroidal anti-inflammatory drugs (NSAIDS) reduce the pain and inflammation by blocking the cyclooxygenase enzyme (COX) and thus production of prostaglandins, but long term administration of NSAIDs may induce gastrointestinal ulcers and renal disorders due to their non-selective inhibition of both isoforms of COX enzyme, the constitutive [COX-1] and the inducible [COX-2] isoforms [9, 10, 11]. COX-2 inhibitors have been associated with cardiovascular side-effects [12]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates. All these drugs possess well known side and toxic effects [13]. Moreover, synthetic drugs are very expensive to develop and their cost of development ranges from 2 to 8 million dollars. It is likely that purified compounds from plants can act as precursors for the synthesis of anti-inflammatory drugs with low toxicity and higher therapeutic value

The genus *Cleome* (Cleomaceae) is used in traditional systems of medicine [14, 15, 16, 17, 18]. Two species of *Cleome, viz, C. viscosa* L. and *C. burmanni* W. & A both annual herbs, are reported to possess many biological properties [19, 20, 21, 22, 23, 24, 25, 26].

The present study is aimed at evaluating the anti-inflammatory potential of the methanol and chloroform extracts of *C. viscosa* and *C. burmanni* since such reports are lacking.

## MATERIALS AND METHODS:

The plant samples, *Cleome viscosa* and *C. burmanni* were collected from Kariavattom, Thiruvananthapuram.

### Preparation of extract

Methanol and chloroform extracts of *Cleome viscosa* and *C. burmanni* were prepared from shade-dried, powdered plant parts by soxhlet extraction (About 20 g each of the dried powder in 300 ml solvent [methanol and chloroform]).

## METHODS:

### Inhibition of albumin denaturation

The method of Mizushima and Kobayashi [27] was followed. The reaction mixture consisted of test extracts and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using small amounts of 1N HCl. The sample extracts were incubated at 37$^{o}$C for 20 min and then heated at 51$^{o}$C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

% inhibition = [{Abs control – Abs sample} / Abs control] x 100

### Protein inhibitory action

The test was performed according to the modified method of Oyedepo and Femurewas [28]. The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml of 20mM Tris HCl buffer (pH 7.4) and 1 ml test sample at different concentrations. The reaction mixture was incubated at 37$^{o}$C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. About 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated as follows:

% inhibition = [{Abs control – Abs sample} / Abs control] x 100

**Hyaluronidase inhibition assay:** The assay was performed according to the method of Ling *et al*. [29]. The assay medium consisted of 3, 5-U-Hyaluronidase in 100 µl 20 mM sodium phosphate (pH 7) with 77 mM NaCl, 0.01% BSA pre-incubated with different concentrations of test extract (in DMSO) for 15 min at 37°C. The assay was initiated by adding 100µ hyaluronic acid (0.03% in 300 mM $NaPO_4$, pH 5.35) to the incubation mixture and incubated for further 45 min at 37°C. The undigested hyaluronic acid was precipitated with 1 ml acid albumin solution made up of 0.1% BSA in 24mM sodium acetate and 79 mM acetic acid (pH 3.75).

After keeping the reaction mixture at room temperature for 10 min, the absorbance was measured at 600 nm. The absorbance in the absence of the enzyme was used as a reference value for maximum inhibition. The inhibitory activity of the test sample was calculated as percentage ratio of absorbance in presence of test sample versus absorbance in absence of enzyme. The enzyme activity was checked by a control experiment run simultaneously, in which the enzyme was pre-incubated with 5 µl DMSO instead, followed by assay procedures described above. Compounds were tested in the range of 0.2 – 0.8 mg/ml in reaction mixture. Indomethacin was used as reference standard.

## RESULTS:

**Inhibition of albumin denaturation:** Protein degradation is considered to be the cause of inflammation. Anti-inflammatory activity reflects the ability of extracts to block protein denaturation. Both methanol and chloroform extracts of *C. viscosa* and *C. burmanni* were effective in inhibiting heat induced albumin denaturation **(Table 1)**.

**TABLE 1: ALBUMIN DENATURATION ASSAY IN THE EXTRACTS OF *C.VISCOSA* AND *C. BURMANNI***

| Conc. mg/ml | % inhibition (Mean±SD) | | | | |
|---|---|---|---|---|---|
| | Cv (MeOH) | Cb (MeOH) | Cv ($CHCl_3$) | Cb ($CHCl_3$) | Aspirin |
| 0.2 | 38.82±0.2 | 23.75±1.2 | 20.66±0.3 | 18.77±0.7 | 42.69±0.2 |
| 0.4 | 66.43±0.5 | 35.82±0.4 | 32.42±1.2 | 30.64±0.01 | 70.54±1.2 |
| 0.6 | 75.89±0.01 | 55.43±1.6 | 48.29±0.2 | 42.98±0.2 | 89.79±0.2 |
| 0.8 | 88.92±0.2 | 78.92±0.3 | 59.75±0.1 | 50.42±0.5 | 95.82±0.01 |

The maximum inhibition (88.92%) was observed in the *C. viscosa,* methanol extract treated sample and minimum inhibition (50.42%) in *C. burmanni* chloroform extract treated sample at 0.8 mg/ml concentration. Aspirin, the standard anti-inflammatory drug showed the higher value of 95.82% inhibition comparable to *C. viscosa,* methanol extract.

**Protein inhibitory action:** The methanol and chloroform extracts of both plants exhibited significant anti-proteinase activity.

**TABLE 2: PROTEINASE INHIBITION ASSAY IN THE EXTRACTS OF *C.VISCOSA* AND *C.BURMANNI***

| Conc. mg/ml | % inhibition (Mean±SD) | | | | |
|---|---|---|---|---|---|
| | Cv (MeOH) | Cb (MeOH) | Cv ($CHCl_3$) | Cb ($CHCl_3$) | Aspirin |
| 0.2 | 28.88±1.1 | 22.40±0.3 | 20.16±0.2 | 18.21±0.01 | 33.61±0.7 |
| 0.4 | 43.20±0.2 | 38.49±0.01 | 35.20±0.2 | 31.24±0.01 | 47.35±0.05 |
| 0.6 | 59.70±0.3 | 46.74±0.05 | 44.78±1.1 | 40.78±0.9 | 64.44±1.1 |
| 0.8 | 78.26±0.01 | 65.43±0.6 | 60.94±1.1 | 56.49±0.6 | 88.20±0.3 |

The maximum inhibition was again observed for the methanol extract of *C. viscosa* compared to the other test samples at similar concentrations. The inhibition percent value was highest for the standard drug, Aspirin in this assay **(Table 2)**.

**Hyaluronidase inhibition assay:** The anti-inflammatory activity was also assessed by hyaluronidase inhibition.

**TABLE 3: HYALURONIDASE INHIBITION ASSAY IN THE EXTRACTS OF *C.VISCOSA* AND *C.BURMANNI***

| Conc.mg/ml | % inhibition (Mean±SD) | | | | |
|---|---|---|---|---|---|
| | Cv (MeOH) | Cb (MeOH) | Cv ($CHCl_3$) | Cb ($CHCl_3$) | Indomethacin |
| 0.2 | 30.44±0.9 | 26.22±1.3 | 23.42±0.7 | 20.61±0.02 | 45.25±0.7 |
| 0.4 | 45.60±0.7 | 41.77±0.2 | 38.22±0.9 | 34.90±0.06 | 59.21±0.12 |
| 0.6 | 66.98±0.02 | 53.22±0.2 | 49.67±1.5 | 43.77±1.2 | 70.90±0.02 |
| 0.8 | 82.11±0.01 | 70.89±0.3 | 68.24±0.02 | 63.88±0.2 | 91.33±0.2 |

In this assay also, the methanol extract of *C. viscosa* showed maximum inhibition percentage (82.11%). The standard drug in this experiment was Indomethacin, which however, exhibited a comparatively higher inhibition value (91.33%) at the same concentration **(Table 3)**.

**DISCUSSION:** Medicinal plants have shown great promise in the management of various inflammatory disorders and have continued to serve as alternative and complementary therapies. Functional foods and beverages are typically developed with specific health goals such as reducing inflammation.

The ethical issues associated with the use of animals in experimental pharmacological research, leaves out the only possibility of using suitable *in vitro* methods for the initial screening of plant extracts for bioactivity studies. Hence, in the present study the *in-vitro* assessment of anti-inflammatory activity was done.

Denaturation of proteins is a well documented cause of inflammation and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* [30]. Therefore, development of anti-inflammatory drugs that could prevent protein denaturation could be of interest. The inflammatory drugs (Salicylic acid, phenylbutazone) have shown dose dependent ability to thermally induced protein denaturation [27]. Similar results were observed for many plant extracts also [31]. The most commonly used anti-inflammatory assay is the albumin denaturation and proteinase inhibition assays. The results of the present study showed that, the test extracts were effective in inhibiting heat induced albumin denaturation at different concentrations **(Table 1)**.

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors [32]. The methanol extract of *C. viscosa* and *C. burmanni* exhibited significant antiproteinase activity at different concentrations almost comparable to the standard aspirin **(Table 2)**.

Another enzyme that is involved in tissue remodeling during inflammation is hyaluronidase, which degrades glycosaminoglycans, including hyaluranan, in human and animal tissues. Hyaluranan polymers are important constituents of the extracellular matrix of connective tissues, including cartilages, the synovial membrane and synovial fluid joints [33]. The enzyme is known to be involved in allergic reactions, cancer metastasis, inflammation and increasing permeability of vascular membrane [34]. Many plant derived polyphenols exert effects on hyaluronidase and other enzymes regulating extracellular matrix metabolism. In this work, the anti-hyaluronidase activity was tested in comparison to the widely used drug, indomethacin. The data obtained from the present study indicated the potent inhibition power of the plant extracts **(Table 3)**.

A total of 37 phytochemicals were qualitatively analysed, and the presence of major groups of compounds such as phenols, flavonoids, terpenoids, glycosides, alkaloids, quinones, saponins and coumarins was detected. Majority of the compounds were present in the methanol extract of *Cleome viscosa* [35]. Quantitative estimation of some of the phytochemicals has also been conducted.

The amount of phenols was the highest, followed by flavonoids and glycosides. It could also be noted that *Cleome viscosa* contained more amount of phytoconstituents than *C. burmanni* [26]. Recent studies have shown that many glycosides, flavonoids and related polyphenols contribute significantly to the anti-inflammatory activity of many plants [36]. Based on the results, the plant extract can be considered as effective and safer alternative to non-steroidal anti-inflammatory drugs.

**CONCLUSION:** In the present study, the anti-inflammatory activity of the both plant extracts, *Cleome viscosa* and *C. burmanni* are comparable with standards Aspirin and indomethacin. Among the tested extracts, the methanol extract of *C. viscosa* appeared to be the most effective. The findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of anti-inflammatory agents from these plants.

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