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# ANTI-INFLAMMATORY ACTIVITY OF *COSMOS CAUDATUS*

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**KEYWORDS:**

*Cosmos caudatus* leaves, Carrageenan, Paw edema, Anti-inflammatory

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## ABSTRACT

*Cosmos caudatus* (Asteraceae) an edible plant is commonly used in folk medicine to treat various diseases and has been reported for the presence of flavonoids and as antioxidant. The present study was aimed to investigate the anti-inflammatory activity of *C. caudatus* leaves against Carrageenan induced paw edema in mice. Petroleum ether, chloroform, methanol and aqueous extracts of *C. caudatus* leaves were prepared by cold maceration technique. Oral administration of the various extracts of *C. caudatus* leaves at 200mg/kg significantly reduced the elevated paw edema volume. Methanol and aqueous extracts showed high significant anti-inflammatory activity. All the results were well comparable with standard drug, diclofenac sodium.

## 1. INTRODUCTION :

Inflammation is a local response which causes injury and forms oedema and granuloma and leukocyte infiltration [1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increases vascular permeability and blood flow [2]. Inflammation that is untreated properly leads to chronic inflammatory disorders. Some of the anti-inflammatory drugs inhibit the lipoxygenase pathway and some inhibit cyclooxygenase pathway. These two pathways can be used for potential interventions against inflammation [3]. Most of the anti-inflammatory drugs, particularly steroids and cyclooxygenase inhibitors are often associated with adverse side effects including GI irritation, ulcers, hypertension and cardiac abnormalities.

Drugs from plant sources have been used for the treatment of various disorders and diseases since ancient times. Nowadays, the use of herbal drugs to cure inflammation and pain is gaining popularity due to their effectiveness, fewer side effects, low cost and availability [4]. Malaysia is one of the world‘s mega bio-diversity rich countries in terms of the number of plant species and compliment the herbal needs of treatment in Malay traditional medical systems. Many of these plant species are used by natives as folk medicine for the treatment of various human ailments [5]. *Cosmos caudatus* (Kunth) is an edible plant which belongs to the family, Asteraceae. This vegetable plant is generally consumed freshly as salad or cooked by boiling with other spices. In Malaysia, it is commonly known as Ulam raja. *C. caudatus* is a very good source of natural antioxidants and rich in minerals such as calcium, phosphorous, iron, magnesium and potassium. It is traditionally used for cleansing the blood [6] and also to strengthen the muscles and bones because of its high calcium content. In East Java, it is traditionally used to reduce the blood pressure and to improve blood circulation in tropical America [7].

Previous studies on *C. caudatus* showed that it had high antioxidant [8] due to the presence of flavanoids [9] and antimicrobial properties [10]. Also it was found to have good inhibitory profile against carbohydrate modulating enzymes such as α-glucosidase and moderate effect against ACE [11]. There is no scientific report for the anti-inflammatory effect of *C. caudatus* leaves. It was hypothesized that the presence of flavanoids in the plant, the chance of having anti-inflammatory action is high. Hence, the present study was undertaken to explore and scientifically prove the anti-inflammatory property of *C. caudatus* leaves in experimental animals.

## 2 MATERIALS AND METHODS

### *2.1 Plant material*

The leaves of *C. caudatus* were obtained from the plant nursery, Sungai Buloh, Selangor, Malaysia in the month of October 2011. The plant material was identified and authenticated by Mrs Nurnida binti Mohd Kamal, Research Officer, Forest Health and Conservation Programme, Forest Biodiversity Division, Forest Research Institute Malaysia (FRIM), Malaysia. A voucher specimen (SBID011/12) was deposited at herbarium, FRIM.

### *2.2 Preparation of extracts*

The leaves of *C. caudatus* were washed thoroughly, shade dried, pulverized into coarse powder. Then it was equally divided into four portions and extracted with petroleum ether, chloroform, methanol and water separately by cold maceration technique using for 6 days. The extracts were collected separately and concentrated at $40^0$C - $50^0$C using rotary vacuum evaporator under reduced pressure [12]. The colour, consistency and the percentage yield were noted and tabulated in table 1. All the extracts of *C. caudatus* leaves were stored in dessicator until further use.

### 2.3 *Animals*

Swiss albino mice (25-30g) were procured from Laboratory Animal Research Unit, Asia Metropolitan University College of Health Sciences. Malaysia and housed in plastic cages. The animals were maintained under room temperature (25 ± 4°C), 12 h light/12 h dark cycle and 35 – 60% relative humidity at the animal house. The animals had free access to the food which is a commercial pellet and drinking water. The study was approved by University Animal Ethics Committee (UAEC), Asia Metropolitan University College of Health Sciences, Cheras, Selangor, Malaysia. All the experiments were performed in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes.

### 2.4 *Screening of Anti-inflammatory activity*

Swiss albino mice (25-30g) were used for this study. The animals were divided into six groups (n=3) and were fasted for 24 h prior to the experiment [13, 14]. The anti-inflammatory activity of various extracts of *C. caudatus* leaves was assessed by Carrageenan-induced hind paw edema method [15, 16] with slight modification using digital plethysmometer. Prior to any treatment, each mice was weighed properly; the doses of the extracts, standard drug, and control materials were adjusted and the initial paw volume was noted for each mice. Group 1 received 0.5 % Sodium CMC (10mL/kg) and served as normal control group. Groups 2- 5 received the respective extract (200mg/kg b.wt.) and group 6 received standard drug, diclofenac sodium (10mg/kg b.wt.) orally

30 min prior to administration of Carrageenan (0.1 mL of 1% w/v). Carrageenan was administered in subplantar region of right hind paw of each mice of every group. The paw volume was measured at 60, 120, 180 and 240 min after administration of extracts and standard drug. The anti-inflammatory activity was determined as the percent inhibition of the edema formed after two hours of Carrageenan administration.

The percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Mean paw edema volume of control} - \text{Mean paw edema volume of test}}{\text{Mean paw edema volume of control}}$$

### *2.3 Statistical Analysis*

The results were expressed by mean ± SEM. Statistical significance were analyzed by ANOVA followed by Dunnet "t" test, the statistical analysis was conducted with SPSS software (v.17, SPSS, USA) at significant levels of 0.05 and 0.01 [17].

## 3. RESULTS AND DISCUSSION

All the extracts were clearly distinguished by their physical data (Table 1). Aqueous extract had the highest percentage yield (22.4%) whereas petroleum ether extract had the lowest percentage yield (1.9%). Carrageenan induced hind paw edema method was used to evaluate the possible anti-inflammatory activity of various extracts of *C. caudatus* leaves. The results found that all the extracts of *C. caudatus* leaves (200 mg/kg) significantly ($P<0.05$ and $P<0.01$) inhibited Carrageenan induced paw edema at 3$^{rd}$ and 4$^{th}$ h (Table 2). Methanol extract followed by aqueous extract exhibited high significant *($P<0.01$)* anti-inflammatory activity. The % inhibition of paw edema by methanol and aqueous extracts are well comparable that elicited by Diclofenac sodium (10 mg/kg).

Anti-inflammatory activity of natural products in animal models was assessed by inducing the inflammatory mediators using external stimuli such as egg white, dextran, histamine, Carrageenan and formalin. Carrageenan induced inflammation is a suitable experimental model to establish the oral anti-inflammatory agents [18]. The development of edema induced by Carrageenan is believed to be biphasic event with involvement of different inflammatory mediators. The first phase is mediated by release of histamine, serotonin and kinins during the first 2 h after administration of Carrageenan, while the second phase (3–4 h after Carrageenan injection) is related to release of prostaglandins such as cyclooxygenase products and lipoxygenase products [3, 17, 19].

Di Rosa et al., (1971) reported that the metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins especially prostaglandin E 2 (products of the cyclooxygenase pathway) and leukotriene B4 (product of lipoxygenase pathway) [20].

The medicinal plants known to contain flavonoids are used in folk medicine, in some cases as anti-inflammatory agents [21]. The presence of flavanoids in *C. caudatus* [9] and its potent antioxidant activity are well reported [22]. The previous studies support the anti-inflammatory property of flavonoids [3, 17, 23]. By acting on both proliferative and exudative phases of inflammation, flavanoids exhibit their anti-inflammatory property [24, 25]. Certain flavanoids modulate the enzyme activities of arachidonic acid metabolizing enzymes such as phospholipase $A_2$, cyclooxygenase, lipoxygenase and nitric oxide synthase. An inhibition of these enzymes by flavonoids reduces the production of crucial inflammatory mediators such as arachidonic acid, prostaglandins, leukotriene, and nitric oxide. Thus, the inhibition of these enzymes exerted by flavonoids is definitely one of the important cellular mechanisms of anti-inflammation [23]. Diclofenac sodium is a cyclooxygenase inhibitor and inhibits the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against Carrageenan induced paw edema [26]. From the findings of the present study, the inhibition of Carrageenan induced paw edema by the extracts of *C. caudatus* leaves could be due to its inhibitory activity on the lipoxygenase enzyme.

## 4. CONCLUSION

The finding of this study concluded that *Cosmos caudatus* leaves has the potential to treat inflammation and acts as a good source as anti-inflammatory agents. The current study leads to the search for novel anti-inflammatory agents from natural products. Extensive scientific research on *C. caudatus* is needed to deal with the isolation of bioactive constituents, responsible for anti-inflammatory activity and assessing the possible mechanism of action. The continual efforts for the research on *C. caudatus* may be contribute in the next future to know the importance of *C. caudatus* for the development of a new class of anti-inflammatory agent based on the flavonoid molecule.

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**Table 1.** Colour, consistency, and percentage yield of various extracts of *C. caudatus*

| Extracts | Colour | Consistency | % yield |
|---|---|---|---|
| Petroleum ether | Yellowish green | Solid | 1.9% |
| Chloroform | Greenish brown | Semi solid | 5.2% |
| Methanol | Reddish brown | Semi solid | 14.5% |
| Aqueous | Reddish brown | Mucilaginous | 22.4% |

**Table 2.** Effect of various extracts of *C. caudatus* against Carrageenan induced paw edema

| Treatment | Paw edema Volume (ml) mean ± SEM | | | | |
|---|---|---|---|---|---|
| | **1st hr** | **2nd hr** | **3rd hr** | **4th hr** | **% inhibition at 4th hr** |
| Control | 1.25±0.080 | 2.15±0.061 | 2.38±0.053 | 2.66±0.095 | - |
| Petroleum ether extract | 1.14±0.098 | 2.05±0.081 | 1.96±0.064 | 1.17±0.055* | 56.02 |
| Chloroform extract | 1.25±0.075 | 2.12±0.267 | 1.80±0.170 | 1.08±0.035* | 59.40 |
| Methanol extract | 1.14±0.111 | 2.04±0.092 | 1.51±0.083* | 0.81±0.098** | 69.54 |
| Aqueous extract | 1.12±0.040 | 2.03±0.190 | 1.49±0.141* | 0.89±0.140** | 66.54 |
| Standard drug | 1.16±071 | 1.98±0.012 | 1.42±0.046* | 0.79±0.022** | 70.30 |

Values are mean ± S.E.M., *$P$<0.05, **$P$<0.01, statistically significant between control group, n=3