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## ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC LEAF EXTRACT OF *Colebrookea oppositifolia*

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### ABSTRACT

The objective of this study was to evaluate the anti-inflammatory activity of methanolic leaf extract of *Colebrookea oppositifolia* by using carrageenan induced hind paw-oedema model. *Colebrookea oppositifolia* (Lamiaceae), claimed to be used traditionally in the treatment of various ailments including wound healing, fever, epilepsy, indigestion, pneumonia, typhoid. The methanolic leaf extract of *Colebrookea oppositifolia* at the doses of 200, 400 and 800 mg/kg b.w., produced dose dependent significant reduction ( $p < 0.05 - 0.001$ ) in carrageenan-induced rat maximal paw oedema by  $26.92 \pm 1.32\%$ ,  $41.09 \pm 1.52\%$ ,  $51.27 \pm 0.88\%$  and the standard drug Indomethacin at dose  $1.3 \times 10^{-5}$  moles/kg reduced oedema by  $64.00 \pm 4.96\%$  respectively and the total (AUC) paw oedema by  $29.13 \pm 2.92\%$ ,  $36.31 \pm 2.54\%$ ,  $54.25 \pm 1.67\%$  and Indomethacin at dose  $1.3 \times 10^{-5}$  moles/kg reduced total(AUC)paw oedema by  $69.07 \pm 1.10\%$  respectively. The results suggested that the plant *Colebrookea oppositifolia* possessing *in-vivo* anti-inflammatory activity.

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### Key Words

*Colebrookea oppositifolia*,  
carrageenan, anti-inflammatory  
activity, Indomethacin.

## INTRODUCTION

The family Lamiaceae is also called as Labitae. The species of this family are mainly herbs or shrubs of various sizes, rarely trees. Lamiaceae is characterized by aromatic plants which have been widely used since ancient times. The family is known for culinary herbs such as basil, mint, oregano, rosemary, sage and thyme and are used traditionally in the treatment of various ailments including wound healing, fever, epilepsy, indigestion, pneumonia, typhoid<sup>1,2,3,4</sup>. However, literature survey indicated no published reports on anti-inflammatory activity on this plant. Hence, a detailed study was carried out on the methanolic leaf extract of *Colebrookea oppositifolia* for evaluating the anti-inflammatory activity by using carrageenan induced hind-paw-oedema model.

## MATERIAL AND METHODS

### Chemicals

All the chemicals and reagents used were of analytical grade. Carrageenan (Sigma), Indomethacin (Bayer AG), Sodiumcarboxymethylcellulose (Aldrich).

### Plant Material

The plant material was collected in November 2009 from Sunnapugadda, Srikakulam District, Andhra Pradesh, India. The Voucher specimen (BG/AVR/CO-11-09) was deposited in the herbarium, A.U College of Pharmaceutical Sciences, Andhra University.

### Methods

#### Preparation of Extract

The freshly collected leaves of the plant were shade dried and powdered. The powdered materials were then subjected to triple maceration with methanol: water (70:30). The extract thus obtained was concentrated under vacuum at temperature of 43°C by using rotary evaporator (Buchi), dried completely, weighed and stored in a desiccator.

#### Preliminary Phytochemical Screening

The methanolic leaf extract of *Colebrookea oppositifolia* on preliminary phytochemical screening showed the presence of sterols, Glycosides, Tanins, saponins, flavonoids. Literature review revealed the presence of Flavonoid

aglycones – chrysin, negletein, ladanein, Flavonoid glycosides – negletein 6-glucoside; 5,7,2' – trihydroxyflavone 2'- glucoside, Phenylethanoid – verbascoside (also called acetoside or kusagin).

#### Carrageenan Induced Paw-inflammation Model

Anti-inflammatory activity was evaluated in 1% carrageenan induced rat paw oedema model<sup>[7,8]</sup>. The methanolic leaf extract of *Colebrookea oppositifolia* was tested at three different doses (200 mg, 400 mg and 800 mg/kg, b.w.). Inflammation was induced in the right hind paw of each rat by subplantar injection of 1% carrageenan suspension (0.1ml). The left hind paw of the rat was injected 0.1ml of saline. Group-A received drug vehicle 1% sodium.CMC. Group-B received standard drug Indomethacin of dose 1.3×10<sup>-5</sup> moles/kg. Group-C, Group-D and Group-E orally received methanolic extract of *Colebrookea oppositifolia* at the doses of 200mg, 400mg and 800 mg/kg body weight, respectively. Two hours after administration of doses, each rat was injected with saline subcutaneously into the sub-plantar tissue of the left hind paw and with 1% carrageenan in saline subcutaneously into the sub-plantar tissue of right hind paw. The paw thickness of each rat was measured using Zeitlin's apparatus<sup>[9-12]</sup> before carrageenan injection and every hour up to 6hrs after carrageenan injection. The percentage inhibition of paw edema was calculated by using the following formula and given in the table-1.

$$\% \text{ Increase in paw thickness} = \frac{Y_t - Y_0}{Y_0} \times 100$$

$Y_t$  = Paw thickness at time t (1, 2, 3, 4, 5 and 6th) after injection

$Y_0$  = Paw thickness at 0 hr (before injection).

#### Statistical Analysis of Data

Data obtained from animal experiments were expressed as the mean standard error (±S.E.M.). Statistical differences between the treated and the control groups were evaluated by ANOVA and Students–Newman–Keuls post hoc tests.  $p < 0.05$  was considered to be significant (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

## RESULTS AND DISCUSSION

The methanolic extract of leaves of *Colebrookea oppositifolia* at the doses of 200, 400 and 800 mg/kg b.w., produced dose dependent significant reduction ( $p < 0.05$ – $0.001$ ) in carrageenan-induced rat maximal paw oedema (Fig.1.1) by  $26.92 \pm 1.32\%$ ,  $41.09 \pm 1.52\%$ ,  $51.27 \pm 0.88\%$  and Indomethacin at dose  $1.3 \times 10^{-5}$  moles/kg reduced oedema by  $64.00 \pm 4.96\%$ , respectively and the total (AUC) paw oedema (Fig.1.2) by  $29.13 \pm 2.92\%$ ,  $36.31 \pm 2.54\%$ ,  $54.25 \pm 1.67\%$  and

**Table 1:** Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with different doses of methanolic leaf extract of *Colebrookea oppositifolia* and Indomethacin.

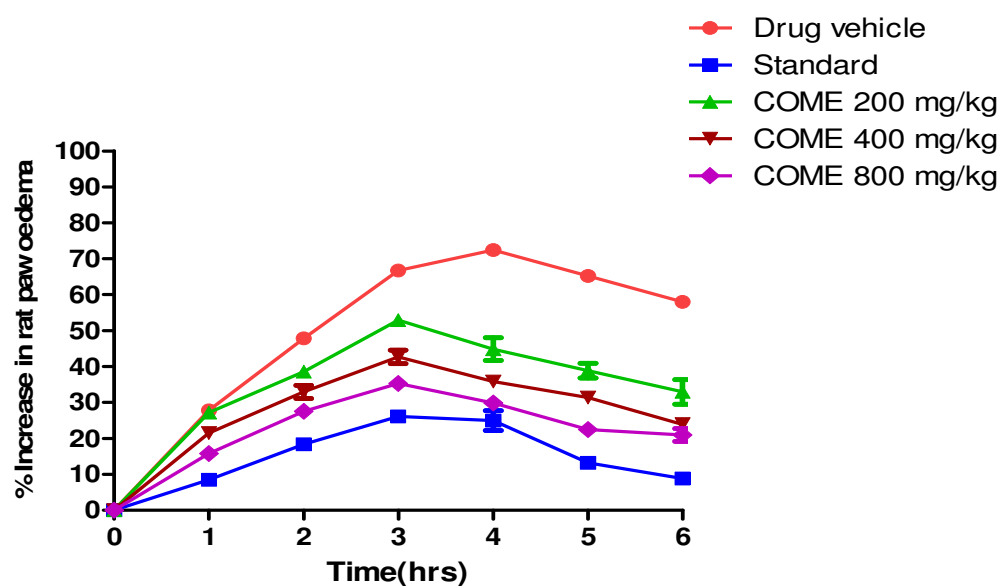
Indomethacin at dose  $1.3 \times 10^{-5}$  moles/kg reduced total (AUC) paw oedema by  $69.07 \pm 1.1\%$  respectively.

## CONCLUSION

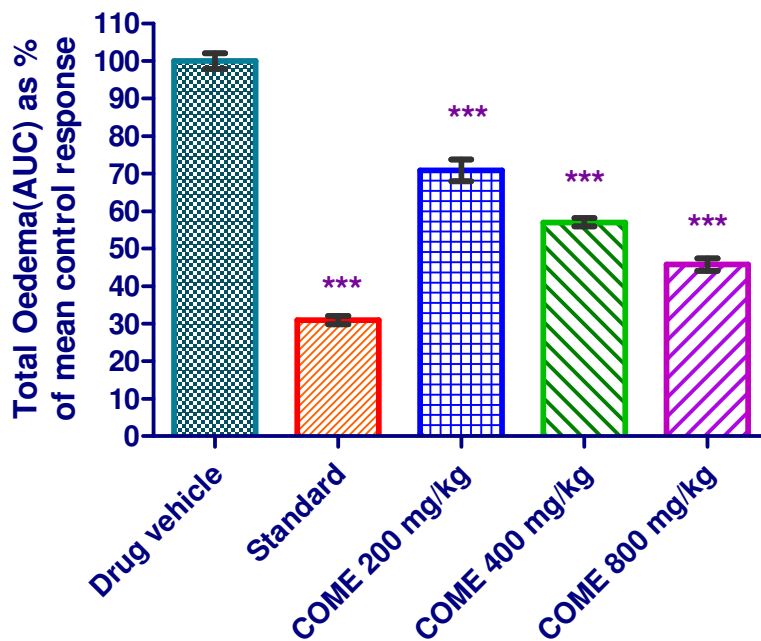
The results showed that the methanolic leaf extract of *Colebrookea oppositifolia* possesses anti-inflammatory activity. The preliminary phytochemical examination suggested that the plant having sterols, Glycosides, Tanins, saponins, flavonoids. The anti-inflammatory activity of *Colebrookea oppositifolia* may be due to the presence of these flavonoid glycosides,

Test sample	Percentage inhibition of the maximal paw oedema during 6th hr.	Percentage inhibitions of total AUC paw oedema during 6th hr.
Control (1% sodium CMC)	$0.0 \pm 0.99$	$0.0 \pm 1.96$
Indomethacin ( $1.3 \times 10^{-5}$ moles/kg., b.w)	$64.00 \pm 4.96^{***}$	$69.09 \pm 1.1^{***}$
COME 200 mg/kg., b.w	$26.92 \pm 1.32^{***}$	$29.13 \pm 2.92^{***}$
COME 400 mg/kg., b.w	$41.09 \pm 1.52^{***}$	$36.31 \pm 2.54^{***}$
COME 800 mg/kg., b.w	$51.27 \pm 0.88^{***}$	$54.25 \pm 1.67^{***}$

Data represent mean  $\pm$  S.E.M. (standard error mean) ( $n = 6$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . COME- Methanolic leaf extract of *Colebrookea oppositifolia*



**Fig.1:** Effect of the crude extract of *Colebrookea oppositifolia* 200mg, 400mg, 800mg/kg b.w., along with Indomethacin ( $1.3 \times 10^{-5}$  moles/kg body wt.) on the maximal paw oedema in carrageenan induced rats



**Fig.2:** Effect of the crude extract of *Colebrookea oppositifolia* 200mg, 400mg, 800mg/kg b.w., along with Indomethacin ( $1.3 \times 10^{-5}$  moles/kg body wt.) on the total paw in carrageenan induced rats.

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