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Phytochemical screening, anti-oxidant and anti-inflammatory activities of ethyl acetate extract of *Combretum punctatum* var. *squamosum*

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The objective of this study was to carry out the phytochemical screening of the ethyl acetate extract of *Combretum punctatum* var. *squamosum* and to investigate anti-inflammatory and anti-oxidant activities. The phytochemical investigation indicated the presence of flavonoids and steroids in the extract. Anti-inflammatory activity was studied by *in vitro* inhibition of albumin denaturation and HRBC membrane stabilization methods; *in vivo* cotton pellet induced granuloma and carrageenan-induced paw oedema studies. The anti-oxidant activity was studied by lipid peroxidation and DPPH radical scavenging activity assays.The ethyl acetate extract at different concentrations showed a significant percent inhibition of albumin denaturation and percent inhibition of haemolysis when compared with the standard. The extract at 200 and 400 mg/kg b.w. significantly reduced cotton pellet granuloma (P <0.001) and carrageenan paw oedema (P <0.05) when compared with the standard in rats.The extract at different concentrations also showed a significant percent inhibition of lipid peroxidation and DPPH when compared with the standard.Thus, the result indicates that the ethyl acetate extract exhibited significantly good anti-inflammatory and anti-oxidant activities.

Keywords: Anti-inflammatory, Anti-oxidant, Ethnomedicine, Leihruisen, Phytoconstituents.

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Introduction

The search for drugs in nature to liberate diseases dates from the far past and plants have served as a valuable source of drugs ever since ancient times. The whole lot was based on little knowledge on either the causes for the illnesses or which plant and how it could be utilized as a cure during those times[1]. In recent times, herbal medicines were used to cure sickness based on the theory that plants contain natural substances that can improve health and ease illnesses[2]. These traditional plant medicines were used with no proper investigation on the constituents and thetherapeutic activities of the plants. Therefore, research on plants has been a focus all over the world until today[3].

Combretum punctatum var. *squamosum* (Roxb.Ex. G. Don), belonging to the family Combretaceae, locally known as 'Leihruisen' in Mizoram, is a deciduous climber found mainly in South-east Asian countries like India, Burma, Bangladesh, Bhutan, Nepal, Philippines, Thailand, Vietnam, and China[4]. Traditionally, the juice of the fresh leaves is applied to wounds and cuts to stop bleeding; the broths of boiled leaves are taken as medicine for diarrhoea and cholera[5]. However, there is no proper investigation of the plant on its phytoconstituents and pharmacological activity. This study aimed at phytochemical screening and the evaluation of anti-inflammatory and antioxidant activity of the plant**.**

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Materials and Methods

Collection of plant material

The leaves of *C. punctatum* var. *squamosum* were collected from Khanpui village, Aizawl District, Mizoram, India during April 2018. The plant was identified by the Botanical Survey of India, Kolkata. A voucher specimen (IBTH-1) is being preserved in the Department of Pharmacy, RIPANS for further reference. The leaves were cleaned and dried under shade to remove moisture with occasional shifting and then powdered with a mechanical grinder. It was then subjected to successive extraction of phytoconstituents using Soxhlet apparatus.

Extraction of phytoconstituents

The dried powdered leaves of the plant were defatted with petroleum ether and the defatted

powdered material thus obtained was further extracted with chloroform and ethyl acetate in a Soxhlet apparatus. The extraction was carried out exhaustively and the solvents were recovered by simple distillation. The extract was concentrated and dried to yield free-flowing powder.

Phytochemical analysis

Preliminary phytochemical analysis of the ethyl acetate extract of *C. punctatum* var. *squamosum* for the presence of phytochemical constituents like alkaloids, carbohydrates, glycosides, steroids, flavonoids, phenols, tannins, proteins and amino acids, fats, and fixed oil were qualitatively carried out[6,7].

Animal care and handling

The animal care and handling were carried out according to the guidelines issued by the World Health Organization, Geneva, Switzerland and the Indian National Science Academy (INSA), New Delhi, India. Usually, 6 to 8 weeks old healthy Swiss albino rats of both sexes weighing 100-180 g were picked out from an inbred colony maintained under the controlled conditions of temperature (25±2°C), humidity (55-60%) and 12 hours of light and dark cycle, respectively. The animals were housed in a sterile polypropylene cage containing wood powder (procured locally) as bedding material. The animals had free access to standard rodent diet and water. All animal experiments were carried out according to NIH, USA and Indian National Science Academy, New Delhi, India guidelines, after getting the approval of the Institutional Animal Ethics Committee of RIPANS, Aizawl - IAEC/RIPANS/42, Mizoram, India.

Acute toxicity test

The acute toxicity study of all extracts was performed as per the Organization for Economic Co-operation and Development (OECD-425) guidelines. The animals fasted overnight before oral administration of ethyl acetate extract of *C. punctatum* var. *squamosum* (2000 mg/kg body weight b.w.) in a single dose. After the extract was administered, food was withheld for a further 3-4 hours. One animal was dosed at the test dose. If the animal died, the main test was conducted to determine the LD50. But if the animal survived, four additional animals were dosed sequentially so that a total of five animals were tested. However, if three animals died, the limit test was terminated and the main test was performed. Animals were observed continuously during the first 30 minutes after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and mortality. The LD50 is greater than 2000 mg/kg b.w. if three or more animals survived. The LD50 is less than the test dose (2000 mg/kg b.w.) when three or more animals die[8].

***In-vitro* antioxidant activity study of ethyl acetate extract**

Lipid peroxidation inhibition assay

In this assay, a modified thiobarbituric acid-reactive species (TBARS) was used to measure the lipid peroxide formed, and egg yolk homogenate was used as a lipid source[9,10]. The reaction mixture contains egg homogenate (0.5 mL of 10% v/v) and 0.1 mL of extract in a test tube and made up to 1 mL with distilled water. 0.05 mL of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 minutes. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% sodium dodecyl sulphate and 0.05 mL 20% trichloroacetic acid (TCA) were added and the resulting mixture was vortexed and then heated at 95 °C for 60 minutes. It was cooled, and 5.0 mL of butan-1-ol was added to each tube and centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532 nm. In this study α-tocopherol was used as standard. The percentage of inhibition was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Extract})}{\text{Absorbance of Control}} \times 100$$

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of *C. punctatum* var. *squamosum* and the standard were estimated based on the DPPH free radical scavenging effect according to the method described by Blois (1958)[11] with slight modifications[12]. In this study, butylatedhydroxy toluene (BHT) was used as a reference standard. Exactly 3 mL of the extract and standard prepared in various concentrations (0.1, 0.5, 1, 2, 3, 4, and 5 μg/mL) was mixed with 0.5 mL of DPPH solution

(0.1 mM). The extract and standard were incubated for 30 minutes at 37°C. Absorbance was measured at 517 nm using an ultraviolet-visible (UV-Vis) spectrophotometer. The percentage of scavenging effect of DPPH free radical was calculated using the following equation.

$$\%\ \text{DPPH radical scavenging} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Extract})}{\text{Absorbance of Control}} \times 100$$

In-vitro anti-inflammatory activity study of ethyl acetate extract

Inhibition of albumin denaturation

The anti-inflammatory activity of ethyl acetate extract of *C. punctatum* var. s*quamosum* was studied by using inhibition of albumin denaturation method according to Mizushima *et al.*[13], and Sakat *et al.*[14], with minor changes[15]. Diclofenac sodium was used as the reference standard. The reaction mixture consists of test extract and standard prepared in various concentrations (100, 200, 300, 400, and 500 µg/mL) and 1 % aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min. After cooling the samples the turbidity was measured at 660 nm (UV-Visible Spectrophotometer). The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

HRBC membrane stabilization method

The anti-inflammatory activity of ethyl acetate extract of *C. punctatum* var. s*quamosum* was studied by using stabilization method of human red blood cell (HRBC) membrane by hypotonicity induced membrane lysis[16,17]. Blood was collected (2 mL) from healthy volunteers and was mixed with an equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl in distilled water) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline solution and a 10% v/v suspension was prepared with normal saline and kept at 4ºC undisturbed before use. Different concentrations of ethyl acetate extract of *C. punctatum* var. *squamosum* in normal saline and diclofenac sodium as standard (50, 100, 200, 500 and 1,000 µg/0.5 mL) and control (distilled water instead of hypo saline to produce 100% hemolysis) were separately mixed with 1 mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of 10 % HRBC suspension was added. All the assay mixtures were incubated at 37ºC for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula

$$\text{Percentage inhibition of haemolysis} = \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Control}} \times 100$$

In vivo anti-inflammatory activity study

Carrageenan induced inflammation

Rats were divided into four groups of four animals in each group. The test groups received different doses of the ethyl acetate extract of *C. punctatum* var. *squamosum* (200 and 400 mg/kg b.w.), the standard group receives Diclofenac sodium (40 mg/kg b.w.) while the normal group received the vehicle and served as control and was not induced for paw oedema. Paw oedema was induced in the test and standard groups by injecting 0.1 mL of 1% w/v carrageenan suspended in 1% CMC into sub-planter tissues of the left hind paw of each rat. The paw thickness was measured before injecting the carrageenan and after 1, 2, 3, 4, 5, 6 hours using a vernier calliper. The anti-inflammatory activity was calculated as percent inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group[18,19].The percentage inhibition of oedema was calculated using the formula

$$\%\ \text{inhibition} = \frac{\text{To} - \text{Tt}}{\text{To}} \times 100$$

Where Tt is the thickness of the paw of rats given test extract at corresponding time and To is the paw thickness of rats of the control group at the same time.

All the values were expressed as Mean±S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test, P <0.001 was considered significant.

Cotton pellet induced granuloma

In this study, the anti-inflammatory effect was evaluated by cotton pellet granuloma model (sub-acute) using the method adopted by D'Arcy (1960)[20]. Rats were divided into four groups of four animals in

each group. The test groups received different doses of the ethyl acetate extract of *C. punctatum* var. *squamosum* (200 and 400 mg/kg b.w.), the standard group receives Diclofenac sodium (40 mg/kg b.w.) while the normal group received the vehicle and served as control and cotton pellets were not induced. Under light ether anaesthesia by using blunted forceps, a subcutaneous tunnel was made and sterilized cotton pellets (10±0.5 mg) were implanted in the axilla and groin region of each animal in the test and standard groups. After recovering from anaesthesia, the animals were treated orally with vehicle control (saline 10 mL/kg), standard drug and test drug and were administered for seven consecutive days. On the eighth day, the animals were sacrificed by excessive anaesthesia and the cotton pellets were removed surgically. Pellets were separated from extraneous tissue and weighed immediately for wet weight and then dried at 60°C until the weight become constant[21,22]. The percentage inhibition of the wet weight and dry weight of the granuloma were calculated. The percent inhibition increase in the weight of the cotton pellets was calculated by:

$$\% \text{ inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where Wc is granulation weight in the control group and Wt is the granulation weight in the treated group

All the values were expressed as Mean±S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunnett Multiple comparison test, $P < 0.001$ was considered significant.

Table 1 — Preliminary phytochemical screening of ethyl acetate extract of *C. punctatum* var. *squamosum*

S.No.	Phytochemical test	Ethyl acetate extract
1	Alkaloids	-ve
2	Glycosides	-ve
3	Tannins	-ve
4	Flavonoids	+ve
5	Steroids	+ve
6	Saponins	-ve
7	Proteins and amino acids	-ve
8	Fats and fixed oil	-ve
9	Carbohydrates	-ve

[Key: + indicates presence, - indicates absence]

Results and Discussion

The preliminary phytochemical analysis of ethyl acetate extract of *C. punctatum* var. *squamosum* revealed the presence of flavonoids and steroids as shown in Table 1, which may be liable to contribute toward the biological activities of the plant.

After performing acute toxicity test using OECD 425 guidelines, the ethyl acetate extracts of leaves of *C. punctatum*, even at a high dose of 2000 mg/kg b.w., did not cause any untoward symptomatic behavior in the test animals as seen in Table No 2.

The biological membrane contains polyunsaturated lipid which is prone to the oxidative reaction of free radicals, which leads to lipid peroxidation. The lipid peroxidation products like malondialdehyde (MDA), 4-hydroxyl 2-nonenal (4-HNE), and some other alkanes form adducts with significant irreversible effects on cellular functions and biochemistry when they react with cell macromolecules[23,24]. The formation of adducts thus leads to membrane permeability, oxidative nucleic acid damage, and eventually to mutation and cancer[25,26]. In this study, the antioxidant activity was tested for lipid peroxidation inhibition. The lipid peroxidation inhibitory effect of the ethyl acetate extract of *C. punctatum* var. *squamosum* and the standard were shown in Table 3. The percentage inhibition of lipid peroxidation of the extract and the standard α-tocopherol at a concentration of 100 µg/mL was 76.93 and 86.21% respectively. The EC50 value calculated was found to be 22.324 µg/mL for the standard compound and 48.23 µg/mL for the ethyl acetate extract.

DPPH radical is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract[27]. The results of DPPH radical scavenging activity of ethyl acetate extract of *C. punctatum* var. *squamosum* and the standard were shown in Table 4. In this study, the extract

Table 2 — Observations for the presence of symptomatic behaviour in acute toxicity study of ethyl acetate extract of *C. punctatum* var. *squamosum*

Treatment	Tremors	Convulsion	Lethargy	Sleep	Coma	Diarrhoea	Salivation	Mortality
Control (0.3% Sodium Carboxy Methyl Cellulose)	-	-	-	-	-	-	-	-
Ethyl acetate extract (2000 mg/kg b.w.)	-	-	-	-	-	-	-	-

[Key: (-) indicates absence]

significantly reduced the DPPH radical with increasing concentrations of the plant extract. This may indicate an increased ability to donate hydrogen ions which results in a lighter solution which is proportional to the number of electrons gained[28]. The percentage inhibition of the DPPH radical by the ethyl acetate extract of *C. punctatum* var. *squamosum* and standard BHT at 5 µg/mL was 78.9 and 79.13% respectively. The EC50 value for both the extract and BHT was calculated and found to be 1.298 µg/mL for the extract while it was 0.104 µg/mL for the standard BHT.

Inflammation is a physiological response that protects the body from the injury of the tissues. Acute inflammation occurs very rapidly, and the course of action can last for few or several minutes to several days, with exudation of fluid and plasma proteins as its main features.When the acute inflammatory occurs repeatedly or continuously, chronic inflammation occurs with the process lasting for several weeks to months and even years[29]. Denaturation of proteins is a well-documented cause of inflammation in many disease conditions[30]. Proteins are denatured when exposed to stresses like heat, strong acid or base and results in the loss of their biological functions. When bovine serum albumin (BSA) is heated it undergoes denaturation and expresses antigens associated with type III hypersensitive reactions which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematous[31]. Thus, the *in vitro* anti-denaturation (stabilization) effect of heat-treated (immunogenic) bovine serum albumin assay is being used for detecting a wide range of anti-inflammatory compounds. NSAIDs (Non-Steroidal Anti-inflammatory Drugs) have been widely used for treating inflammation. They act by inhibiting the production of endogenous prostaglandins and also preventing the denaturation of proteins[32]. The anti-inflammatory effect of ethyl acetate extract of *C. punctatum* var. *squamosum* was observed by evaluating the inhibitory effect on albumin denaturation. Maximum inhibition of 61.63% by the extract was observed at 500 µg/mL. Diclofenac sodium, a standard anti-inflammatory drug showed the maximum inhibition of 90% at the concentration of 500 µg/mL (Table 5). The EC50 value was found to be 361.5 µg/mL for the ethyl acetate extract while for the standard compound diclofenac sodium, it was found to be 129.66 µg/mL.

During inflammation, lyses of lysosome srelease their component enzymes which lead to a variety of disorders. The beneficial effects of non-steroidal anti-inflammatory drugs (NSAIDs) are the results of inhibition of the released lysosomal enzymes or stabilization of the lysosomal membranes[33]. The HRBC membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the erythrocyte membrane is similar to that of the lysosomal membrane and its stabilization indicates that the extract may well stabilize lysosomal membranes[34,35]. Table 6 shows that the ethyl acetate extract at different concentration range has shown significant percentage inhibition of haemolysis and has maximum inhibition of 59% at 500 µg/mL compared with the standard diclofenac sodium

Table 3 — Effect of ethyl acetate extract of *C. punctatum* var. *squamosum* on lipid peroxidation

S. No	Concentration (µg/mL)	% Inhibition of standard α-tocopherol (Mean)	% Inhibition of ethyl acetate extract (Mean)
1	10	59.18	30.23
2	20	65.67	24.70
3	40	71.62	54.26
4	60	77.83	61.03
5	80	81.62	64.56
6	100	86.21	76.93

Table 4 — DPPH radical scavenging activity of ethyl acetate extract of *C. punctatum* var. *squamosum*

S. No	Concentration (µg/mL)	% Inhibition of BHT (Mean)	% Inhibition of ethyl acetate extract (Mean)
1	0.1	46.26	33.50
2	0.5	51.32	45.30
3	1.0	58.74	57.30
4	2.0	65.34	61.50
5	3.0	69.61	72.10
6	4.0	74.46	74.20
7	5.0	79.13	78.90

Table 5 — Effect of ethyl acetate extract of *C. punctatum* var. *squamosum* on albumin denaturation

S. No	Concentration (µg/mL)	% Inhibition of diclofenac (Mean)	% Inhibition of ethyl acetate extracts (Mean)
1	100	66.66	27.63
2	200	80.00	31.60
3	300	83.00	41.36
4	400	86.66	58.56
5	500	90.00	61.63

which shows maximum inhibition of 91.66% at 500 µg/mL. The EC50 value for the ethyl acetate extract was found to be 371.2 µg/mL while it was 162.7 µg/mL for the standard compound.

The *in vivo* anti-inflammatory activity was assessed on ethyl acetate extract of the plant at two different doses (200 and 400 mg b.w.) in rat models using cotton pellet induced granuloma and carrageenan-induced paw oedema. It was observed that the ethyl acetate extract at dose 200 mg/kg and 400 mg/kg b.w. produced a significant anti-inflammatory activity (P <0.001) by reducing the dry weight and wet weight granuloma but lower than the standard drug diclofenac sodium. The percent inhibition of granuloma formation exhibited by a high dose of the extract was found to be almost comparable with the standard drug diclofenac at a dose of 10 mg/kg b.w. (Table 7). A reduction in paw oedema induced by carrageenan was also observed in Table 8 for both the standard diclofenac sodium at a dose of 10 mg/kg b.w. and two different doses (200 and 400 mg/kg b.w.) of ethyl acetate extracts (P <0.001) when compared with control. It was found that the paw-oedema inhibition exhibited by the high dose (400 mg/kg b.w.) ethyl acetate extract was much higher than that exhibited by the standard diclofenac at a dose of 10 mg/kg b.w. The anti-inflammatory activity of the ethyl acetate extract of *C. punctatum* var. *squamosum* leaves, as well as the standard drug, was found to be dose-dependent in both the *in vivo* models.

Table 6 — Effect of ethyl acetate extract of *C. punctatum* var. *squamosum* on HRBC membrane stabilization

S. No	Concentration (µg/mL)	% Inhibitionof haemolysis by standard diclofenac sodium	% Inhibition of haemolysis by ethyl acetate extract
1	100	57.37	30.28
2	200	69.23	36.06
3	300	77.88	43.00
4	400	87.50	54.57
5	500	91.66	59.00

Table 7 — Effect of ethyl acetate extract *of C. punctatum* var. *squamosum* on cotton pellets induced granuloma

Groups	Treatment	Dose (mg/kg)	Mean wet weight of cotton (g)	% Inhibition in wet weight	Mean dry weight (g)	% Inhibition in dry weight
1	Control (Normal saline)	-	78.87±1.17	-	24.70±0.86	-
2	Standard (Diclofenac)	10	54.90±1.25	30.39	13.57±0.69	45.06
3	Ethyl acetate extract	200	68.32±0.88	13.38	18.55±0.65	24.89
4	Ethyl acetate extract	400	55.42±0.98	29.73	14.95±0.97	39.48

Each value represents Mean±S.E.M., n=4; p value <0.001 compared with control

Table 8 — Effect of ethyl acetate extract *of C. punctatum* var. *squamosum* on carrageenan-induced paw oedema

Groups	Treatment	Initial paw thickness	Change in paw thickness in mm (Mean±SEM) value+ % Inhibition of paw oedema					
			1 h	2h	3h	4h	5h	6h
1	Control (1% carrageenan + Normal saline)	3.21±0.01	5.32±0.01	5.41±0.01	5.41±0.02	5.40±0.01	5.23±0.01	5.10±0.01
2	Standard (1% carrageenan + 10 mg/kg Diclofenac)	3.20±0.02	4.14±0.01 (22.18%)	4.03±0.01 (25.51%)	3.92±0.06 (27.54%)	3.82±0.01 (29.26%)	3.67±0.01 (29.83%)	3.51±0.01 (31.18%)
3	Low dose Extract (1% carrageenan + 200 mg/kg ethyl acetate extract)	3.29±0.01	4.50±0.02 (15.42%)	4.05±0.26 (25.14%)	4.03±0.19 (25.60%)	3.88±0.15 (28.24%)	3.70±0.06 (29.25%)	3.65±0.05 (28.43%)
4	High dose Extract (1% carrageenan + 400 mg/kg ethyl acetate extract)	3.27±0.01	4.05±0.26 (23.87%)	3.83±0.23 (29.30%)	3.78±0.18 (30.22%)	3.425±0.10 (36.57%)	3.20±0.11 (38.81%)	3.10±0.09 (39.22%)

All values are expressed as Mean±SEM (n=4); P <0.001 when compared with control

Conclusion

The present study demonstrated that the ethyl acetate extract of *C. punctatum* var. *squamosum* possessed significant *in vitro* anti-oxidant property when compared with standard compounds like α-tocopherol and butylated hydroxytoluene even though the standard compounds were found to have better activities.The *in vitro* anti-inflammatory activity studies using protein denaturation and HRBC membrane stabilization models showed that the ethyl acetate extract exhibited a certain amount of anti-inflammatory activities but not as good as the standard diclofenac sodium. The acute toxicity study of the ethyl acetate extract of *C. punctatum* leaves using OECD 425 limit test showed that it is safe to be used at the doses required in each *in vivo* test and the LD50 value is higher than 2000 mg/kg b.w. The *in vivo* anti-inflammatory activity study of the extract using cotton-pellet induced granuloma model showed that the ethyl acetate extract could inhibit granuloma formation at both 200 and 400 mg/kg b.w., but the standard diclofenac exhibited better anti-inflammatory activity even at a low dose of 10 mg/kg b.w. However, in the *in vivo* acute carrageenan-induced inflammation model, it was found that the ethyl acetate extract, at a dose of 400 mg/kg b.w. exhibited better anti-inflammatory activity than the standard drug at a dose of 10 mg/kg b.w. Further investigation is required for identifying the unknown bioactive compounds and to establish their pharmacological properties involved in the activities.

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Conflict of Interest

The authors declare that there is no conflict of interest between the authors on any financial or personal interests.

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