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ANTI-INFLAMMATORY ACTIVITY OF CEP-CEPAN LEAVES (*Castanopsis costata* (*Blume*) A.DC)

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A B S T R A C T

Inflammation causes discomfort, suffering and lower productivity of the victims. Synthetic anti-inflammatory drugs are not readily available and have adverse side effects. Alternative herbal medicines possess bioactive compounds that are safer and efficient in the management of various diseases and disorders. The present study evaluated for the anti-inflammatory activity of ethanol extracts of *Castanopsis costata* in rats to scientifically validate their traditional use among the Karo communities in Medan, Nort Sumatera. The plant samples were collected with the help of local herbalists in Namo Keling Village, Sumatera Utara and transported to Phytochemistry and Pharmacology laboratories, Tjut Nyak Dhien University, for cleaning, air drying, milling, and extraction. Wistar male albino rats of divided into six groups of 5 animals each; normal control, negative control, positive control and three experimental groups. The anti-inflammatory activity was tested using carrageenan-induced hind paw edema method. The anti-inflammatory activity of the extracts was compared to reference drug diclofenac. The leaf extract of *C.costata* reduced inflamed hind paw diameter of rats by between 26.09%-100% with a dose of 125 mg/kg BW during six days. The diclofenac reduced inflamed hind paw diameter by between 39.75%-100% with a dose of 4.5 mg/kg BW during six days. The qualitative phytochemical screening indicated the presence of alkaloids, flavonoids, glycosides, glycoside anthraquinones, steroids, tannins, and triterpenoids. The present study demonstrated potent anti-inflammatory activities of ethanol extracts of *C. costata* leaves in a dose-dependent manner, which supports their traditional use. The present study recommends the ethnomedicinal use of *C.costata* in the management of inflammation.

INTRODUCTION

Inflammation is a normal biological process in response to tissue injury, microbial pathogen infection and chemical irritation. This biological process also involves the innate and adaptive immune systems. At a damaged site, inflammation is initiated by migration of immune cells from blood vessels and release of mediators, followed by recruitment of inflammatory cells and release of reactive oxygen species (ROS), reactive nitrogen species (RNS) and proinflammatory cytokines to eliminate foreign pathogens, resolving the infection and repairing injured tissues. (Medzhitov, 2008; Pan, *et al*., 2009).Thus, the main function of inflammation is beneficial for a host's defense. In general, normal inflammation is rapid and self-limiting, but the aberrant resolution and prolonged inflammation cause various chronic disorders (Calder, *et al*., 2009).

Inflammatory process has two phases: acute and chronic. The acute inflammation occurs a few minutes after tissue damage. It is characterized by an increase in permeability

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of blood vessels, extravasation of fluid and proteins and accumulation of white blood cells for a short period (Posadas, *et al*., 2004). The primary mediators of acute inflammation include histamine, serotonin, and COX-2 (Ravi, *et al*., 2009). The failure of the management of acute inflammation and an autoimmune response to a self-antigen lead to chronic inflammation and disease (Recio, *et al*., 2012). Chronic inflammation is mediated by inflammatory mediators such as PGE2, nitric oxide, and lipoxygenases. Chronic inflammation may results in ailments such as chronic peptic ulcers, rheumatoid arthritis, systemic lupus, asthma, chronic periodontitis, and cancer (Nordqvist, C., 2015).

During the inflammatory response, the PGE2 are at low levels in tissues with no inflammation and increase immediately in acute inflammation. As immune cells infiltrate the tissues, further increases in PGE2 levels is observed (Tilley, *et al*., 2001). The non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen, indomethacin, ibuprofen, diclofenac, and ketoprofen are the most commonly used conventional medicinal products in the treatment of inflammation (Warden, SJ., 2010). The NSAIDs inhibit the expression of cyclooxygenase 2 (COX-2) enzyme responsible for the production of PGE2 which induces pyrexia (Vane and Botting, 1987). However, the prolonged use of NSAIDs is linked with severe effects on the gastrointestinal tract, kidney, and cardiovascular system (Traversa, *et al*., 1995).

The demand for herbal medicine is increasing due to the growing recognition of natural products having fewer side effects, easily available, better cultural acceptability and being comparatively affordable (Kamboj, VP., 2000). *Castanopnis costata*, commonly known as 'cep-cepan', belongs to the Fagaceae family and is widely distributed in Thailand, Borneo, Malaysia, and Sumatera. This plant is used traditionally to alleviate various disease symptoms such as pain, fever, and inflammation, and the pharmacological activities of some extracts of these plants have been studied *in vitro* or *in vivo* without identifying the bioactive components (Alkandahri, *et al*., 2016; Salim, *et al*., 2017).*C. costata*are generally rich in flavonoids (Alkandahri, *et al*., 2016; Salim, *et al*., 2017);a large group of polyphenolic compounds, which are ubiquitously expressed in plants. These polyphenolic compounds are a subgroup of chemically related polyphenols that possess a basic 15-carbon skeleton and can be represented as C6-C3-C6, consisting of two benzene rings (C6) joined by a linear three carbon chain (C3) (Pan, *et al*., 2009).

C. costata are considered to be good sources of antioxidants due to the presence of high concentrations of polyphenolic compounds (Alkandahri, *et al*., 2016). Antioxidants have the ability to dismutate reactive oxygen species (ROS) which are produced by the oxidation processes in various cells. Oxidative stress, caused by the accumulation of ROS in animal tissues, is a major cause of cell damage or death and is considered an instrumental process that leads to various cancers and other diseases (Valko, *et al*., 2006).In addition, ROS in low concentrations acts as significant cell signaling molecules and regulates the biological conditions of cytokines, hormones and growth factors. High levels of free radicals, however, overcome the normal cellular antioxidant defences and end up being cytotoxic to the biological system (Fang, FC., 2004). These cumulative ROS are associated with a number of diseases including chronic inflammatory diseases (Mirshafiey and Mohsenzadegan, 2008; Nam, *et al*., 2008; Wang, *et al*., 2012). ROS have also been reported to be involved in the activation of NF-κB by pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α (Fang FC, 2004). Given the importance of activated NF-κBin inflammatory disease progression, suppression of this protein directly or through inhibition of ROS or pro-inflammatory cytokines preferably by antioxidants, remain therapeutically important because of the ability of the latter to combat pathogenic chain reactions initiated by free radicals (Kapewangolo, *et al*., 2015). Therefore, the aim of this study was to investigate whether the extracts of *C. costata* showed anti-inflammatory activity and to analyze some of its chemical constituents.

MATERIALS AND METHODS

Plant Determination

Fresh leaves (3.5 kg) of *C. costata* were collected during July (2016) with the help of local herbalists in Namu Keling Village, Sumatera Utara and transported to Phytochemistry and Pharmacology laboratories, Tjut Nyak Dhien University, for cleaning, air drying, milling, and extraction. Plant identification was done in the Jatinangor Herbarium, Department of Biology, Faculty of Mathematics and Science, Padjadjaran University.

Extraction

Cold extraction was obtained by immersing powder of simplicia (500 g) in an ethanol 96% at room temperature for 5 x 24 hours. Separation of residue and filtrate were done every 1 x 24 hours.The filtrate was collected and concentrated using a rotary evaporator at 58^0C.

Animal

Wistaralbino male rats weighing 150-200 g were used for the *in vivo* anti-inflammatory studies. The experimental animals were kept in the standard cages in the animal house maintained under standard laboratory condition of an ambient temperature of 25°C with 12 hours daylight and 12 hours darkness cycles. The experimental animals were fed on standard rodent pellets and provided with water ad libitum.

In Vivo Anti-inflammatory Activity (Winter, etal., 1962)

Thirty Wistar albino ratsof either sex were divided randomly into six groups of five rats each and treated as follows; Group I (normal control) was not induced with inflammation but received 0,5% Na. CMC. Group II (negative control) was induced with inflammation and received 0,5% DMSO. Group III (positive control) was induced with inflammation and received diclofenac (reference drug) at a dose of 4,5 mg/kg body weight. Groups IV, V and VI (experimental groups) were induced with inflammation and received the extracts at the dose levels of 62.5 mg/kg, 125 mg/kg and 250 mg/kg body weight. This design is summarized in Table 1.

Table 1 Treatment protocol for evaluation of anti-inflammatory activities of ethanol extracts of *C. costata*in Wistar albino rats; Carrageenan = 1%, Na. CMC = 0,5%w/v

Group	Status	Treatment
I	Normal control	Na. CMC (p.o)
II	Negative control	Carrageenan + Na. CMC(p.o)
III	Positive control	Carrageenan + 4.5 mg/kg BW diclofenac (p.o)
IV	Experimental group A	Carrageenan + 62.5 mg/kg BW extract (p.o)
V	Experimental group B	Carrageenan + 125 mg/kg BW extract (p.o)
VI	Experimental group C	Carrageenan + 250 mg/kg BW extract (p.o)

The anti-inflammatory activity of the extracts was assessed using carrageenan-induced right paw edema in rats. Acute inflammation was induced by subplantar injection of 0.05 ml 1% carrageenan in normal saline 30 minutes after treatment. The change in paw diameter was measured using a*plethysmometer* 30 minutes before injection of carrageenan and at 1, 2, 3, 4, 5 and 6 hours after induction of inflammation. The average feet swelling in test as well as standard groups were compared with that of control and the % inhibition of paw edema volume was calculated using the formula:

$$\text{Inflammation (\%)} = \frac{Ct - Tt}{Ct} \times 100\%$$

Where:

Ct = Paw diameter at t hour after carrageenan administration.

Tt = Paw diameter before carrageenan-induced.

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100\%$$

A = % Average inflammation of negative control at time t.

B = % Average inflammation of treated group at time t.

Data Analyses

The data was subjected to descriptive statistics and expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to determine whether there was any significant difference between the means of different groups. This was followed by Turkey's tests to separate means and obtain the specific significant differences among the various treatment groups. The values of $p<0.05$ were considered significant. The data was presented in graphs.

RESULTS

The Extraction and Phytochemical Screening

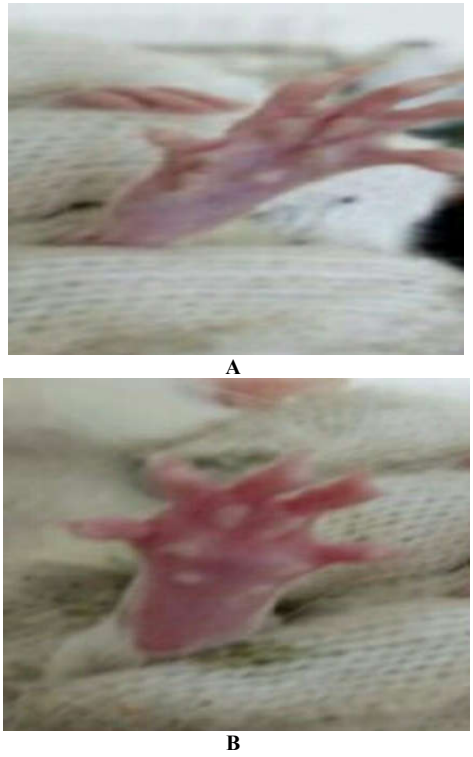
The maceration resulted 7.0 g of viscous ethanol extract of *C. Costata* leaves. Phytochemical screening showed that this extract consisted of alkaloids, flavonoids, glycosides, glycoside anthraquinones, tannins, and triterpenoids. The result is presented in the Table 2.

Table 2 Phytochemical screening of ethanol extractof *C. costata* leaves

No	Secondary metabolite	Reagent	Observation	Result
1	Alkaloids	Dragendorff Bouchardat Mayer	(+) Light brown sediment (+) Dark brown (+) Muddy and white	(+) Alkaloids
2	Flavonoids	Zn + HCl (p) Mg + HCl (p)	(+) Red	(+) Flavonoids
3	Glycosides Non sugar	Molish Fehling A+B Anhydride acetic acid + sulfuric acid (LB)	(+) Purple ring (+) Brown-red sediment (+) Brown-purple	(+) Glycosides
4	Saponins	Hot water + HCl	(-) Bubble	(-) Saponins
5	Glycoside Anthraquinones	NaOH	(+) Purple-red in NaOH layer	(+) Glycoside Anthraquinones
6	Tannins	FeCl3 1%	(+) Yellow	(+) Tannins
7	Triterpenoids	Anhydride acetic acid + sulfuric acid (LB)	(+) Purple-brown	(+) Triterpenoids

Anti-inflammatory Activity of Ethanol Extract of C. Costata Leaves on Carrageenan-Induced Inflammation in Wistar Albino Rats

The ethanol extract of *C. Costata* leaves showed significant anti-inflammatory activity on carrageenan-induced paw edema, which was demonstrated by the reduction in inflamed hind paw diameter after extract administration (Figure 2).



A

B

Figure 1 A) Before of carrageenan-induced paw edema; B) After of carrageenan-induced paw edema.

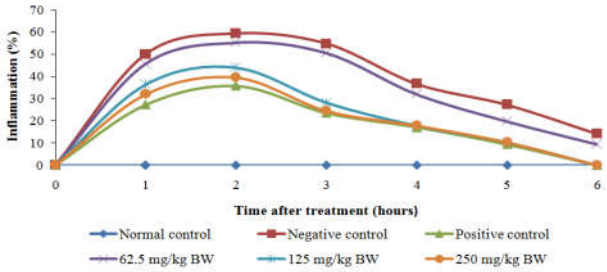


Figure 2 Percentage of inflammation after administration of ethanol extract *C. costata* leaves.

In the first hour after treatment, the leaf extract of *C.costata* at the dose level of 62.5, 125, 250 mg/kg and

the diclofenac (reference drug) at the dosage of 4.5 mg/kg body weight showed anti-inflammatory effect by reducing hind paw diameter by 8.59%, 27.10%, 35.88% and 45.27% respectively (Figure 3). In the sixth hour after treatment, the extract at the dose levels of 62.5 mg/kg, 125 mg/kg, and 250 mg/kg body weight, as well as the diclofenac (reference drug) reduced the inflamed hind paw diameter by 33.33%, 100%, 100% and 100% respectively (Figure 3). The anti-inflammatory activity of the extract at the dosage of 125 mg/kg and 250 mg/kg body weight showed no significant difference and were comparable to diclofenac (reference drug).

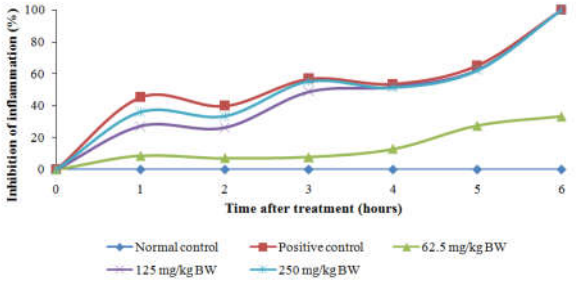


Figure 3 Inhibition of inflammation after administration of ethanol extract *c.costata* leaves.

DISCUSSION

The present study evaluated for the anti-inflammatoryactivity of ethanolextract of *C. costata*leaves on carrageenan-induced paw edema in rats. Carrageenan, dextran, arachidonic acid, dextran, histamine, serotonin and formalin-induced paw edema; cotton pellet induced granuloma; Freund's adjuvants are the standard agents for causing acute, sub-acute and chronic inflammation respectively in animal models (Ismail, *et al*., 1997; Mujumdar and Misar, 2004; Lai, *et al*., 2009; Kolawole, *et al*., 2013).

The carrageenan-induced inflammation is described as a biphasic event in which various mediators operates to produce an inflammatory response (Gupta, *et al*., 2006). The first mediators detectable in the early phase (1 hour) include histamine, serotonin, and cyclooxygenase. On the other hand, the late phase (over 1 hour) is sustained by the production of PGE2 and it is mediated by bradykinin and leukotrienes (Ravi, *et al*., 2009; Unnisa and Parven, 2011). Inducible nitric oxide synthase (iNOS) and COX-2 enzyme are responsible for the production of an enormous amount of inflammatory mediators (Necas and Bartoksikova, 2013; Handy and Moore, 1998). Carrageenan-induced inflammation is also associated with enhanced levels of the endogenous pyrogenic cytokines such as tumor necrosis factor and interleukins (IL-1 and IL-6) which act as pro-inflammatory mediators (Cuzzocrea, *et al*., 1999).

The anti-inflammatory activity of ethanol extract of *C. costata* leaves, could be due to the presence of bioactive constituents that exhibit anti-inflammatory action. This could be through inhibition of inflammatory mediators such as prostaglandins, histamine, serotonin, and lysosome (Dina, *et al*., 2010). The qualitative phytochemical screening of ethanol extract of *C. costata* leaves indicated the presence of alkaloids, flavonoids, glycosides, glycoside anthraquinones, steroids, tannins, andtriterpenoids (Table 2). The presence of some these bioactive compounds such asalkaloids, flavonoids, terpenoids, and steroids have shown to exhibit anti-inflammatory activity in experimental animals (Bhaskar and Balakrishnan, 2009; Di Carlo, *et al*., 1999).

Flavonoids have been reported to inhibit pro-inflammatory mediators such as TNF-α and phospholipase A2 (Bhaskar and Balakrishnan, 2009). Furthermore, some flavonoids respond by blocking both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentration while at the lower level only the lipoxygenase pathway is blocked (Salminen, *et al*., 2008). Research findings have revealed that triterpenoids suppresses some function of macrophages, neutrophils and also inhibit nitric oxide (NO), NF-κB signaling and PGE2 production responsible for inflammation induction (Frantz, *et al*., 1994). The NF-κB can detect noxious stimuli, such as infectious agents, cellular injuries and free radicals, and then directs DNA to synthesize inflammatory cytokines. Thus, their inhibition leads to management of edema (Mencarelli, *et al*., 2009).

Steroids also attenuate inflammation by inhibiting phospholipase A2, which hydrolyzes arachidonic acid from membrane phospholipids and subsequent formation of prostanoids and leukotrienes via the cyclooxygenase and lipoxygenase pathways (Kamau, *et al*., 2016).Alkaloids have also been reported to inhibit pro-inflammatory mediators such asTNF-α, PGE2, MIP-1α, MIP-1α productionand mRNA expression of TNF-α, MIP-1α, COX-2. Suppression of κB phosphorylation, subsequent inhibition NF-κB transcriptional activity and reductions in mRNA expression (IL-6 and IL-8) and NF-κB translocation (Yoshikawa, *et al*., 2006; Zhou, *et al*., 2012).

CONCLUSION

The ethanol leaf extract of *C.costata* showed potent anti-inflammatory activity on carrageenan-induced paw edema in rats. The anti-inflammatory activity of leaf extract of *C.costata* demonstrated a dose-dependent response and were comparable to diclofenac (reference drug). The extracts were most active at the dose level of 125 mg/kg and 250 mg/kg body weight in the sixth hour of treatment. The extract of *C.costata* could be an alternative bio-resource for generating anti-inflammatory agent. However, further studies are necessary to elucidate the mechanism behind this effect and their active compounds. The present study, scientifically confirms and supports the traditional use of *C.costata* in the management of inflammation.

Conflict of Interest

No conflict of interest to declare.

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