

# EFFECT OF ETHANOLIC LEAF EXTRACT OF COCOR BEBEK (*BRYOPHYLLUM PINNATUM*) ON THE ATTENUATION OF THERMAL HYPERALGESIA

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## ABSTRACT

*Cocor bebek (Bryophyllum pinnatum)* has been proven as analgesics for relief of acute pain. Flavonoid is one of the compounds in *Bryophyllum pinnatum* which is potentially developed as analgesics for the treatment of chronic inflammatory pain. This study was conducted to determine the effect of ethanolic leaf extract of *Bryophyllum pinnatum* as attenuation of thermal hyperalgesia in rats with chronic inflammatory pain.

This study used a completely randomized design with complete Freund's Adjuvant/CFA-induced chronic inflammatory pain in wistar rats, at the first day. Thirty rats were divided into 5 (five) groups (n = 6), consisting of a positive control (ketamine 10 mg/kg bw), negative control (CMC Na 0.5%), and three treatment groups of ethanolic leaf extract of *Bryophyllum pinnatum* with doses of 200, 400 and 600 mg/kg bw. The extract was given twice a week for four weeks, at day-3 and day-6. The effect of the treatment on the attenuation of thermal hyperalgesia was investigated by using hot-plate at day-7, day-14, day-21 and day-28 to measure latency and subsequently, the percentage of the increase in latency was calculated. Analysis of Variance (ANOVA) was employed to analyze the data and continued by Least Significance Different test (LSD).

The ethanolic leaf extracts of *Bryophyllum pinnatum* at the doses of 200, 400, and 600 mg/kg bw had effect of the attenuation of thermal hyperalgesia by increasing latency by 3.18, 4.33 and 6.12 seconds, respectively. The optimum dosage as antihyperalgesia was 600 mg/kg bw, with latency was increased to twice than those of negative control group.

**Keywords:** *Thermal hyperalgesia, CFA, inflammatory pain, Bryophyllum pinnatum*

## INTRODUCTION

Hyperalgesia is a manifestation of inflammatory pain or neuropathic pain due to injury or persistent lesions that will activate and increase the sensitization of both peripheral and central nociceptive systems. The incidence of chronic pain reached 37.3% in developed countries and 41.1% in developing countries (Tsang *et al.*, 2008) and in Indonesia, approximately 25% of total patient visits in hospitals due to such a pain (Meilala, 2004). Treatment of chronic inflammatory pain has been using NMDA receptor-class antagonist agents, opiate analgesics or analgesic COX inhibitors (Huang *et al.*, 2004). However, long-term use of opiates can lead to addiction, lower body function, greater distress, neurological disability (Martell *et al.*, 2007) and induce hyperalgesia (Silverman, 2009; Sorensen & Sjogren, 2013).

An alternative that can be used for relieving chronic pain complaints is herbal medicine. One of the high-potential herbal medicine agent as analgesic drugs is cocor bebek (*Bryophyllum*

*pinnatum*). Secondary metabolite compounds contained in *Bryophyllum pinnatum* are mainly group of flavonoids, bufadienolida, and terpenoids (Muzitano *et al.*, 2006). Based on the study of flavonoids as the active substances of *Bryophyllum pinnatum*, they work as analgesic and anti-inflammatory (Parveen *et al.*, 2007). Several studies have shown the effect of *Bryophyllum pinnatum* extract in suppressing acute pain. Putranti *et al.* (2011) reported that methanol leaf extract of *Bryophyllum pinnatum* at dose of 150 mg/kg bw has 50.26% analgesic activity in acetic acid-induced mice. Similarly, Methylenchloride 300 mg/kg bw and leaf extract of *Bryophyllum pinnatum* at dose of 400 mg/kg bw also have analgesic activity (Biswas *et al.*, 2011; Ojewole, 2005).

Based on the data, it is necessary to investigate the effects of ethanolic leaf extract of *Bryophyllum pinnatum* on the attenuation of thermal hyperalgesia in chronic inflammatory pain inflammation model induced by Complete Freund's Adjuvant (CFA). Thus, the use of the leaf extract of *Bryophyllum pinnatum* for the treatment of chronic pain can be scientifically justified.

## MATERIALS AND METHODS

### Materials

This study used 2-3 months wistar rats (150-200 g), leaves of *Bryophyllum pinnatum*, CMC-Na, ethanol 96%, distilled water, CFA Sigma F 5881 (1 mg/mL), and ketamine (100 mg/mL).

### Tools

Several tools were prepared including evaporator, B40 sieve, analytical balance, waterbath (Laboratory Equipment Sydney), injection syringe, blunt end syringe, hot-plate, stopwatch, and a set of glassware.

### Methods

This study is an experimental research by using a Completely Randomized Design (CRD). It is *in vivo* study on anti-hyperalgesia activity.

### Preparation of leaf extract of *Bryophyllum pinnatum*

The maceration method was employed. The simplicia was macerated using ethanol 96% for 3 x 24 hours. In each process of maceration, stirring was performed for 15 minutes. After three days of sieving to separate the liquid with the dregs, the generated macerate was evaporated using a rotary evaporator for  $\pm$  90 minutes at a temperature range of 70-80°C. Subsequently, it was evaporated over a waterbath while the vapor pressure was reduced by using the fan until a viscous extract was obtained.

### Evaluation of analgesic activity

Thirty wistar rats as the test subjects were randomly divided into five groups. They were induced with 0.1 ml CFA on day-1. It was injected into the left leg of all subjects. Twice a week for 4 (four) weeks, i.e. on day-3 and day-6, the subjects in each group will be treated with ethanol leaf extract of *Bryophyllum pinnatum* at dose of 200, 400 and 600 mg/kg bw and standard ketamine 10 mg/kg bw through subcutaneous (SC) administration. On day-7, day-14, day-21, and day-28, latency measurement was performed to evaluate thermal hyperalgesia using the hot-plate method. Observations were carried out by measuring the response time of rats to jump after being placed on a hot-plate with a temperature of  $52 \pm 0.2^\circ\text{C}$ . There were three replications and the mean value was determined as latency of hyperalgesia. To avoid

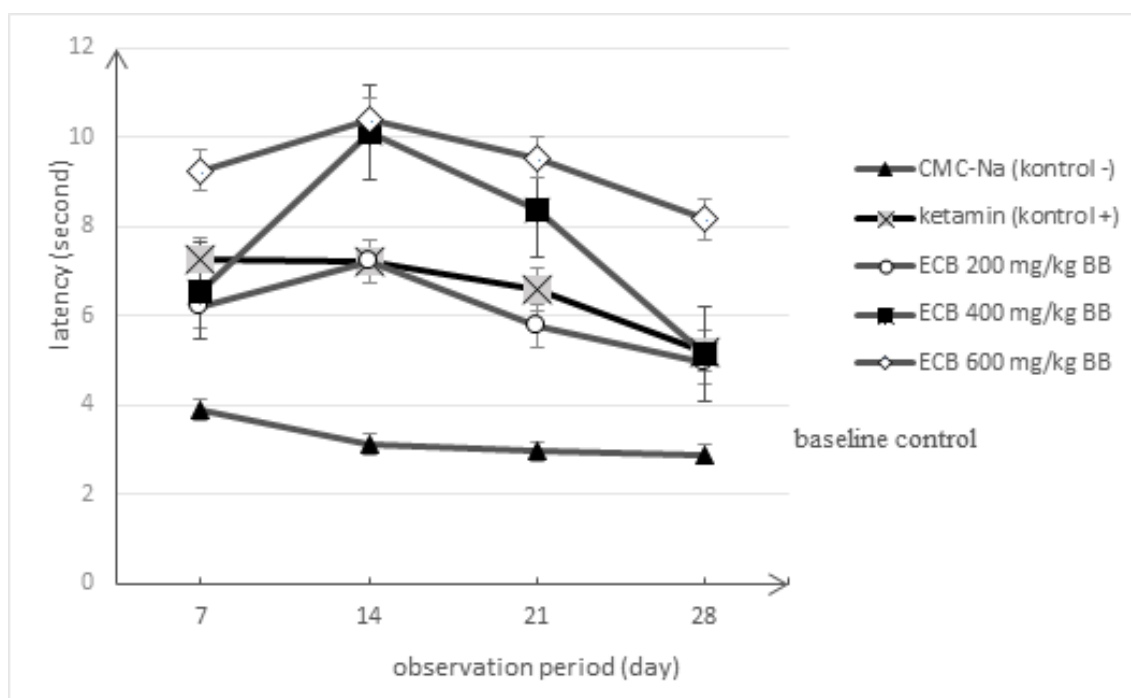
tissue injury, the maximum time limit was set on 60 seconds. The latency was obtained and calculated by the percentage increase in the time required.

### Data analysis

The data of latency and the percentage of latency increase were analyzed using Analysis of Variance (ANOVA) at 95% confidence intervals and continued with Least Significance Different (LSD).

## RESULTS AND DISCUSSION

The evaluation of analgesic activity aimed to determine the attenuation of thermal hyperalgesia as the effect of *Bryophyllum pinnatum*'s leaf extract on Complete Freund's Adjuvant/CFA-induced chronic inflammatory pain Wistar rats, and to reveal the effective dose to achieve the optimum attenuation of thermal hyperalgesia.



**Fig. 1 Profile of latency of thermal hyperalgesia in the rats after the treatment of ethanolic leaf extract of *Bryophyllum pinnatum* in day-7, day-14, day-21 and day-28.**

(a)  $p < 0.05$  significant different with negative control; (b)  $p < 0.05$  significant different with positive control; (Error bar: Standar Error). ECB= Ethanolic leaf extract of *Bryophyllum pinnatum*.

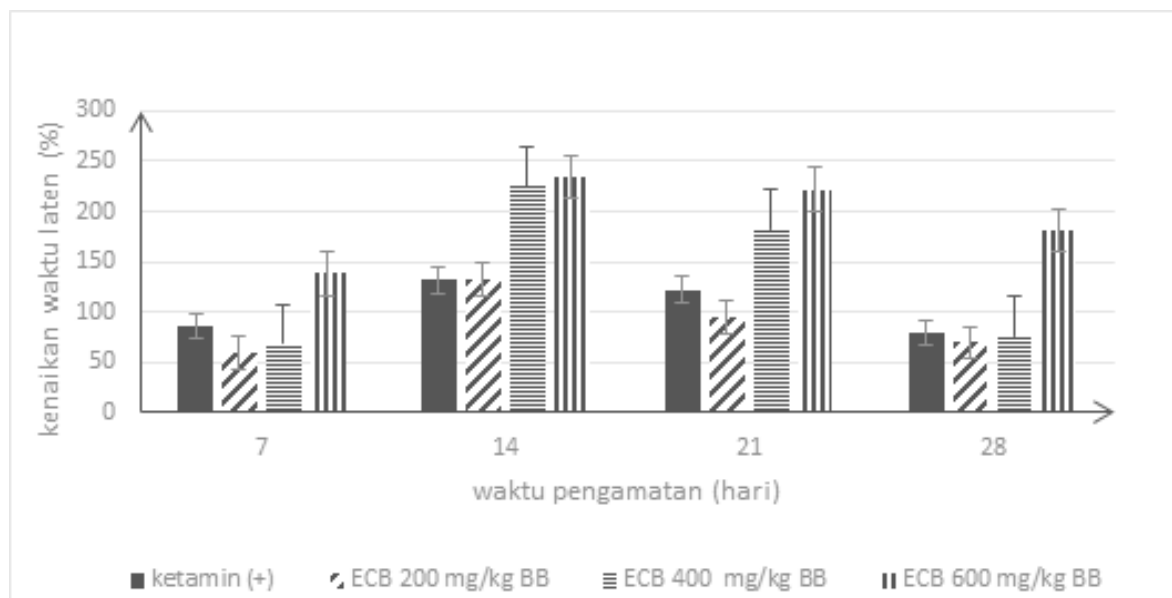
The results of observations on latency on day-7, day-14, day-21 and day-28 are presented in Fig. 1. Based on the figure, the latency of CFA baseline control was 3.21 seconds. All of the extract and ketamine treatment groups had a longer time, approximately 3.21 seconds, in compared to baseline controls. It indicates the positive control (ketamine) and the extract treatment groups had an analgesic effect as shown by the increased latency of the rats on heat stimulation or the attenuation of thermal hyperalgesia. Ethanolic leaf extract of *Bryophyllum pinnatum* with the doses of 200 mg/kg bw, 400 mg/kg bw and 600 mg/kg bw, and ketamine, can increase the mean latency of 3.18; 4.33 and 6.12, and 3.35 seconds, respectively.

The highest increase of latency occurred in day-14 in the extract treatment groups, and began to decrease until day-28 (Fig. 1). The effects of ethanolic leaf extract of *Bryophyllum*

*pinnatum* in the attenuation of thermal hyperalgesia at the peak time in day-14 (Luo *et al.*, 2004). Thus, it confirms that the administration of ethanolic leaf extract of *Bryophyllum pinnatum* has been able to delay the development of hyperalgesia optimally. Further decrease in latency until day-28 is possibly due to the continually exposure to hot-plate, hence inhibiting the recovery of hyperalgesia-related inflammation.

Treatment of ethanolic leaf extract of *Bryophyllum pinnatum* (ECB) 200 mg/kg bw showed insignificant difference in compared with positive control, ECB 400 mg/kg bw showed significant difference in day-14 and day-21, and ECB 600 mg/kg bw showed significant difference in day-7, day-14, day-21 and day-28 (Fig. 1).

Ethanolic leaf extract of *Bryophyllum pinnatum* 600 mg/kg bw has the longest latency (Fig. 1). It also provides the most optimal effect on the attenuation of thermal hyperalgesia, followed by the ethanolic leaf extract 400 mg/kg bw, 200 mg/kg bw, positive control, and finally, 200 mg/kg bw. It indicates that the increased dose of ethanolic leaf extract of *Bryophyllum pinnatum* will increase the latency. Thus, it implies the occurrence of dose dependent effect, in which the higher the dose, the higher the analgesic effect or the attenuation of thermal hyperalgesia.



**Fig. 2 Percentage of the increase of latency on the treatment of ethanolic leaf extract of (*Bryophyllum pinnatum*) in compared with ketamin (positive control); (\*)  $p < 0.05$  significant different with positive control; (Error bar: Standar Error). ECB = Ethanolic leaf extract of *Bryophyllum pinnatum*.**

All extract treatments proved to be effective in the attenuation of thermal hyperalgesia based on the increase in latency from day-7 to day-28 with the peak latency occurred on day-14. The ethanolic leaf extract of *Bryophyllum pinnatum* 600 mg/kg bw is the optimal antihyperalgesia, which generates significant increase in latency in compared with positive control starting in day-7 (Fig. 2). The higher the percentage of the increase in latency, the greater the analgesic effect. The results affirm that the ethanolic leaf extract of *Bryophyllum pinnatum* 600 mg/kg bw has the greatest analgesic effect as indicated by the latency is increased to twice than those of negative control group (Fig. 2), followed by the ethanolic leaf extract of *Bryophyllum pinnatum* 400 mg/kg bw (increased to 1.5 times) and 200 mg/kg bw (increased to 1-time).

The potential of leaf extract of *Bryophyllum pinnatum* in inhibiting the pain is due to the flavonoid compounds that serve as anti-inflammatory and analgesics. Previous studies divulged the leaf extract of *Bryophyllum pinnatum* is effective as a peripheral and central analgesic since it is able to relieve the pain response induced by acetic acid and formalin (Nguelefack *et al.*, 2006) and *hot-plate* (Ojewole, 2005). Peripheral pain is caused by E2 prostaglandin release modulation, nerve growth factor (NGF), bradykinin and histamine (Meliala and Pinzon, 2007).

*Bryophyllum pinnatum* contains flavonoids—which have a peripheral analgesic effect since it can inhibit cyclooxygenase enzymes thereby suppressing the release of prostaglandins (Tanko *et al.*, 2012). Filho *et al.* (2008) reaffirmed that quercetin as one of the main components of flavonoids in *Bryophyllum pinnatum* can inhibit pain through the tachykinin receptor pathway by suppressing the release of P substance. Takeda *et al.* (2012) explained that inflammation by the induction of CFA may trigger the release of Substance P that causes central sensitization.

Furthermore, Lu *et al.* (2013) reaffirmed that quercetin also has an effect on glutamate neurotransmitters. It inhibits pain due to the induction of glutamate by 68.2% (Filho *et al.*, 2008). It suppresses the release of glutamate by inhibiting the depolarization of neurons by decreasing Ca<sup>2+</sup> influx as a result of inhibition of voltage dependent Ca-channels in presinap as well as inhibiting phosphorylation in CCP and PKA which will reduce signal transduction activity (Lu *et al.*, 2013). Likewise, Severini *et al.* (2003) asserted that tachykinins and glutamate receptor (NMDA receptor) have a linkage in which the activation of NMDA receptors modulates the tachykinins pathway in central sensitization. The release of glutamate will stimulate the activation of NMDA receptors thereby stimulating the release of Substance P and the activation of NK1 receptors.

Ethanollic leaf extract of *Bryophyllum pinnatum* can be linked to the attenuation of thermal hyperalgesia through the increase of latency of animal model to heat stimulant with optimum dose of 600 mg/kg bw. As an antihyperalgesia agent, the leaf extract of *Bryophyllum pinnatum* inhibits the development of central sensitization. Inflammation accompanied by hyperalgesia is the result of neuron sensitization due to increased release of neuropeptide substance P and amino acid excitatory in the form of glutamate (Severini *et al.*, 2003). However, this study has not been indecisive in determining the analgesic activity of ethanollic leaf extract of *Bryophyllum pinnatum* through inhibition of glutamate neurotransmitters. Hence, further studies to reveal the target of central sensitization inhibition by inducing glutamate that causes neurogenic pain in which glutamate induction modulates NMDA and non-NMDA receptors and the release of nitric oxide (NO).

## CONCLUSION

The ethanollic leaf extract of *Bryopyllum pinnatum* affects the attenuation of thermal hyperalgesia by increasing the latency by 3.18; 4.33 and 6.12 seconds at doses of 200, 400, and 600 mg/kg bw, respectively. The higher the given dose, the longer the generated latency. Based on the percentage of the increase of latency, the ethanollic leaf extract of *Bryophyllum pinnatum* dose 600 mg/kg bw has the most optimal antihyperalgesia effect as indicated by the increase of mean latency to approximately twice in compared with those of negative control group.

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## REFERENCES

- Tsang A., Von KM., Lee S. Common Chronic Pain Conditions in Developed and Developing Countries: Gender and Age Differences and Comorbidity with Depression-Anxiety Disorders. *J. Pain*. 2008. 9(10): 883–891.
- Meliala L. *Terapi Rasional Nyeri: Tinjauan Khusus Nyeri Neuropatik*. Yogyakarta: Aditya Media. 2004.
- Huang C, Hu ZP, Long H, Shi Y, Han J, You Wan. Attenuation of Mechanical but not Thermal Hyperalgesia by Electroacupuncture with the Involvement of Opioids in Rat Model of Chronic Inflammatory Pain. *Brain Research Bulletin*. 2004. 63: 99–103.
- Martell BA, O'Connor PG, Kerns RD. Systematic Review: Opioid Treatment for Chronic Back Pain: Prevalence, Efficacy, and Association with Addiction. *Ann. Intern.Med.*2007. 146(2): 116–127.
- Silverman SM. Opioid Induced Hyperalgesia: Clinical Implication for the Pain Practitioner. *Pain Physician*. 2009. 12:679-684.
- Sorensen J, Sjogren J. Opioid-Induced Hyperalgesia. In Hanna M & Zylicz Z (Eds) *Cancer Pain*. London: *Springer*. 2013. p 131-142.
- Muzitano MF, Tinoco LW, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS. The Antileishmanial Activity Assessment of Unusual Flavonoids from *Kalanchoe pinnata*. *Phytochemistry*. 2006. 67: 2071-2077.
- Parveen Z, Yulin D, Muhammad KS, Rongji D, Waar A, and Yu HY. Antiinflammatory And Analgesic Activities Of Thesium Chinense Turoz Extracts And Its Major Flavonoid, Kaempferol And Kaempferol 3-O- Glucoside. 2007. Available at: <http://www.jstage.jst.go.jp>. Accessed in March, 24 2014.
- Putranti ZD, Baroroh HN, Arjadi F. Aktivitas Analgetik Ekstrak Metanol Daun Sosor Bebek (*Kalanchoe pinnata* Pers.) pada Mencit yang Diinduksi Asam Asetat. *Media Farmasi Indonesia*. 2011. 6 (2): 1-8.
- Biswas SK, Chowdury A, Das J, Hosen SMZ, Uddin R, Rahaman S. Literature Review on Pharmacological Potentials of *Kalanchoe pinnata* (Crassulaceae). *African Journal of Pharmacy and Pharmacology*. 2011. 5(10): 1258-1262.
- Ojewole JAO. Antinociceptive, Anti-inflammatory and Antidiabetic Effects of *Bryophyllum pinnatum* (Crassulaceae) Leaf Aqueous Extract. *J. Ethnopharmacology*. 2005. 99: 13-19.
- Luo H, Cheng J, Han JS, Wan Y. Chang of Vanilloid Receptor 1 Expression in Dorsal Root Ganglion and Spinal Dorsal Horn During Inflammatory Nociception Induced by Complete Freund's Adjuvant in Rats. *Neuro Report*. 2004. 15 (4): 655-658.
- Nguelefack TB, Nana P, Atsamo AD, Dimo T, Watcho P, Dongmo A, Tapondjou LA, Njamen D, Wansi SL, Kamanyi A. Analgesic and Anticonvulsant Effects of Extracts from the Leaves of *Kalanchoe crenata* (Andrews) Haworth (Crassulaceae). *Journal of Ethnopharmacology*. 2006. 106: 70-75.
- Meliala L and Pinzon R. Breakthrough in Management of Acute Pain. *Jurnal Kedokteran dan Farmasi Dexa Medica*. 2007.4 (20):81-97.

- Tanko Y, Mohammed A, Saleh MIA, Etta E, Bako IG, and Yerima M. Antinociceptive and Anti-Inflammatory Activities of Ethanol Extract of *Bryophyllum Pinnatum*. *IOSR Journal of Dental and Medical Sciences*. 2012. 3(1): 46-49.
- Filho AW, Filho VC, Olinger L, Souza M. Quercetin: Further Investigation of its Antinociceptive Properties and Mechanisms of Action. *Arch Pharm Res*. 2008. 31(6):713-21.
- Takeda M, Takahashi M, Matsumoto S. Suppression of Neurokinin-1 Receptor in Trigeminal Ganglia Attenuates Central Sensitization Following Inflammation. *Journal of The Peripheral Nervous System*. 2012. 17: 169-181.
- Lu C, Lin T, Wang S. Quercetin Inhibits Depolarization-Evoke Glutamate Release in Nerve Terminals from Rat Cerebral Cortex. *Neurotoxicology*. 2013. 39:1-9.
- Severini C, Ciotti MT, Mercanti D, Barbato C, Calissano P. A Tachykinin-Like Factor Increases Glutamate Toxicity in Rat Cerebellar Granule Cells. *Neuropharmacology*. 2003. 44:117-124.