References

1. Võ Văn Chi (2021), *Từ điển cây thuốc Việt Nam*, NXB Y học, Hà Nội, 377-378. **2.** Đỗ Tất Lợi (2004), *Cây thuốc và vị thuốc Việt Nam*, Y học, Hà Nội, 662. **3.** Nguyễn Thị Ánh Nguyệt, Nguyễn Tiến Chung (2023), Tác dụng chống viêm, giảm đau của dây gắm (*Gnetum montanum* Markgr.) trên động vật thực nghiệm, *Y dược Cổ truyền Việt Nam*, 1, 44-51. **4.** Sousa-Neto B. P., Cunha F. V. M., Nunes D. B., Gomes B. S., Amorim L. V., Lopes E. M., Gutierrez S.J. C., Almeida F. R. C., Arcanjo D. D. R., Souza M. F.V., Oliveira F. A. (2022), Anti-inflammatory and antioxidant effects of the indole-derived *N*-salicyloyltryptamine on peritonitis and joint disability induced by carrageenan in rodents, *Evidence-Based Complementary and Alternative Medicine*, 5524107. **5.** Fietz S., Bondzio A., Moschos A., Hertsch B., Einspanier R. (2008), Measurement of equine myeloperoxidase (MPO) activity in synovial fluid by a modified MPO assay and evaluation of joint diseases - An initial case study, *Research in Veterinary Science*. 84(3), 347-353. **6.** Ranasinghe P., Ranasinghe P., Abeysekera W. P., Premakumara G. A., Perera Y. S., Gurugama P., Gunatilake S. B. (2012), *In vitro* erythrocyte membrane stabilization properties of *Carica papaya* L. leaf extracts, *Pharmacognosy Research*, 4(4), 196-202. **7.** Nguyen T. H., Nachtergael A., Nguyen T. M., Cornet V., Duez P., Muller M., Ly Huong D. T., Kestemont P. (2020), Anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* Turcz based on *in vitro* and *in vivo* studies, *Journal of Ethnopharmacology*, 254, 112739. **8.** Oh Y. J., Jin S. E., Shin H. K., Ha H. (2023), Daeshiho-tang attenuates inflammatory response and oxidative stress in LPS-stimulated macrophages by regulating TLR4/MyD88, NF-κB, MAPK, and Nrf2/HO-1 pathways, *Scitific Reports*, 13: 18891. **9.** Barth C. R., Funchal G. A., Luft C., de Oliveira J. R., Porto B. N., Donadio M. V. (2016), Carrageenan-induced inflammation promotes ROS generation and neutrophil extracellular trap formation in a mouse model of peritonitis. *European Journal of Immunology*, 46(4), 964-970. **10.** Lê Thị Hồng Nhung, Nguyễn Tiến Chung (2022), Khảo sát hoạt tính kháng viêm và ức chế enzyme xanthine oxydase của một số loài thuộc chi dây gắm (*Gnetum*), *Khoa học và Công nghệ*. 58(3), 141-143.

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ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF THE ETHANOL EXTRACT FROM *CLERODENDRUM CYRTOPHYLLUM* TURCZ IN MOUSE AND RAT MODELS

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Summary

Anti-Nociceptive and Anti-Inflammatory Effects of the Ethanol Extract from *Clerodendrum cyrtophyllum* Turcz in Mouse and Rat Models

The present study aimed to evaluate the anti-nociceptive and anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* leaves (EE) using animal models. The analgesic activities were evaluated using two animal models: the hot-plate test and the acetic acid-induced writhing test. In the acetic acid-induced writhing test, the number of acetic acid-induced writhes was significantly decreased in the presence of EE at doses of 0.54, 1.08, and 2.16 g/kg, demonstrating strong peripheral antinociceptive activity. In the hot plate test, the hot plate pain threshold was significantly increased after 30, 60, and 90 min in the presence of EE at doses of 0.54 and 1.08 g/kg. The inhibitory effects of EE on the two animal models suggest that EE possesses peripheral and central analgesic activities. To evaluate the anti-inflammatory effect, we used two models: carrageenan-induced paw edema and cotton pellet-induced granuloma test. EE at doses of 0.32 g/kg and 0.64 g/kg remarkably inhibited edema of the paw induced by carrageenin in rats. These results indicate that EE exerts anti-inflammatory activity, especially in the acute phase of inflammation. EE at the dose of 0.64 g/kg effectively inhibited cotton pellet granuloma formation, suggesting that EE also displays anti-inflammatory activity in the chronic phase of inflammation. Taken together, the results consistently demonstrate that the raw ethanol extract of *C. cyrtophyllum* leaves presents potent anti-nociceptive and anti-inflammatory activities and may be useful for the treatment of various inflammatory diseases.

Keywords: *Anti-nociceptive, Anti-inflammatory, Hot-plate test, Acetic acid induced writhing test, Carrageenan-induced paw edema, Cotton pellet induced granuloma test, Clerodendrum cyrtophyllum.*

1. Background

Inflammation is a common physiopathological symptom of many diseases including cancer, cardiovascular disease, diabetes, obesity, osteoporosis, rheumatoid arthritis, inflammatory bowel disease, and asthma. There are generally two types of inflammation: acute and chronic inflammation. Chronic inflammatory diseases are the most significant cause of death in the world. The World Health Organization (WHO) ranks chronic diseases as the greatest threat to human health [1].

Clerodendrum cyrtophyllum, a plant belonging to the Verbenaceae family, is widely

distributed in tropical countries such as Vietnam, China, India, Japan, Korea, and Thailand [2]. In Vietnam, it is used for treating migraine, hypertension, inflammation of the throat, and rheumatic arthritis. In several studies, phenolic acids and flavonoids have been isolated from various plant parts [3]. Total phenols and flavonoids displayed good antioxidant activity and potential anti-inflammatory activity in several experimental models. In our previous studies, EE displayed anti-inflammatory by inhibiting the production of NO and TNF-α in stimulated RAW 264.7 cells. In 2 models of inflammation, including cut tail-induced inflammation and copper sulfate-induced inflammation zebrafish model, the anti-inflammatory mechanism of EE was proved to involve reduced expression of pro-inflammatory genes, such as COX-2, PLA2, C3A, IL-1β, IL-8, TNF-α, and NF-kB, whereas it increased the expression of the anti-inflammatory IL-10 gene [4],[5]. To confirm the anti-inflammatory effects of the ethanol extract, in this study, we evaluated the anti-nociceptive and anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* in mice and rat models.

2. Object and research method

2.1. Chemicals

Carrageenan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acid acetic, prednisolone, and diclofenac were of the highest commercially available grade.

2.2. Laboratory animals

Male Sprague Dawley rats (180-200 g) and male Swiss albino mice (18-25 g) were obtained from the Laboratory Animal of Pharmacology Department, Ha Noi University of Pharmacy, Viet Nam. The animals were kept in polyacrylic cages containing six animals per cage and maintained under standard housing conditions (room temperature 24-27°C and humidity 60-65%) with a 12-h light and dark cycle. Food, in the form of dry pellets, and water were available ad libitum. Animals were acclimatized at least 1 week before the experiments were started.

2.3. Preparation of the ethanol extract (EE)

Plant collection: Leaves of *Clerodendrum cyrtophyllum* Turcz were collected from Northern provinces in Vietnam during the summer of 2021 by Dr Nguyen Thi Kim Thanh, from Vietnam National University **(VNU-BIOL)** Leaves are oval or long lanceolate, 5 - 13 cm x 3 - 7 cm, leave petiole 1 - 6 cm long. An official herbarium number (HNU 024106) was deposited at the Botanical Museum of Hanoi, University of Science. Leaves were cleaned and dried for 72 h to a constant weight using a hot air oven at 40°C and ground in a blender to fine powder (size smaller than 0.5 mm).

Preparation of total extract: Given that polar (aqueous) extracts are used in traditional practice, we selected ethanol to extract polar secondary metabolites, minimizing the extraction of mineral salts and polysaccharides. Hundred g dried leaves powder were extracted with 1000 mL 95% ethanol, at 60°C in a water bath with 360 rpm agitation for 4 h. The extract was then filtered and collected. The residue was extracted for another two times using the same procedure. The collected ethanol extracts were then combined, concentrated on a rotary evaporator (40°C) under reduced pressure, and lyophilized to obtain the crude extract. The yield of extract was 11.3% relative to the dried leaves powder. The crude extract was stored at -20°C until use.

After the extraction, the EE was analysed for total phenol and flavonoid. The concentration of total phenolic compounds and flavonoids in the extract was 23.3 ± 1.5 GAE mg/g and 2.97 ± 0.01 QE mg/g expressed in dry weight of leaves material, respectively.

Preparation of test samples

The dry ethanol extract was dispersed in distilled water to suitable concentrations. Dosages in this study were calculated based on the traditional decoction dosage of dry leaves *C. cyrtophyllum* in humans. The human dose is 20 g dry leaves per day (equivalent to 0.4 g/kg body weight)

The dose for mice was extrapolated from the human dose according to the formula of Nair and Jacob for dose conversion between species [6]

AED (g/kg) = Human dose (g/kg) × Km ratio

Where Km ratio of mice = 12; Human dose = 0.4 g/kg

In this study, we evaluated anti-nociceptive properties of EE in mice using 3 doses: 4.8 g/kg (conversion dose from human), 9.6 g/kg (2 times conversion dose from human), and 19.2 g/kg (3 times conversion dose from human). The doses calculated according to ethanol extract are 0.54, 1.08, and 2.16 g/kg.

The dose for rats was extrapolated from the human dose according to the formula of Nair and Jacob [6]

AED (g/kg) = Human dose (g/kg) × Km ratio

Where Km ratio of rat = 7; Human dose = 0.4 g/kg

Doses for rats: 2.8 g/kg, 5.6 g/kg. The doses calculated according to ethanol extract are 0.32 and 0.64 g/kg.

2.4. Evaluation of antinociceptive activity of EE

2.4.1. Abdominal constriction test:

Peripheral antinociceptive activity of EE was evaluated using the abdominal constriction test described by [7] with slight modifications. Acetic acid 0.6% (v/v) solution in distilled water was used to induce pain in the peritoneal cavity of mice. Mice were randomly divided into 5 groups, 9-12 mice per group. Acetic acid (10 mL/kg, i.p.) was administered for 1 h after oral administration of distilled water, diclofenac (20 mg/kg), EE (0.54, 1.08, and 2.16 g/kg). The animals react with characteristic stretching behavior which is called writhing. Writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The number of abdominal constrictions was counted cumulatively over 30 min. Antinociceptive activity was indicated by a reduction in the mean of the number of abdominal constrictions in the test groups compared with the control group.

2.4.2. Hot plate test:

Central analgesic activity was evaluated by the hot plate test according to the method previously reported [8]. Mice were individually placed on the hot plate at 55 ±1°C (hot plate analgesia meter) before drug treatment. The time in seconds between reaching the platform until the animal either hind leg flinching, paw licking, and jumping was recorded as the response latency. Mice exhibiting latency time between 8 and 30 seconds were chosen. Mice then were randomly divided into 5 groups, 12 mice per group. The latency was recorded after 30, 60, and 90 min following oral administration of EE (0.54, 1.08, and 2.16 g/kg), normal saline (10 mL/kg), and subcutaneous administration of morphine (10 mg/kg). The prolongation of the latency times was taken as an analgesic response.

2.5. Evaluation of the anti-inflammatory activity of EE

2.5.1. Carrageenan-induced paw edema in rat test:

The anti-inflammatory effect was evaluated by carrageenan-induced rat paw edema according to the method of Winter et al. [9]. Edema was induced by injection of 100 µl a 1% suspension of carrageenan in sterile saline solution into the plantar side of the left hind paws of the rats. Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hind paw was determined prior to the administration of any drug. Vehicle, EE at doses of 0.32 and 0.64 g/kg or diclofenac 10 mg/kg (reference drug) were administered orally 1 h prior to injection of carrageenan. The paw volume was measured at 1, 3, and 5 h following the injection of carrageenan. Edema was expressed as the increase in paw volume (mL) after carrageenan injection relative to the preinjection value for each animal. The degree of swelling was evaluated by measuring the increase in paw volume.

The increase in paw volume was calculated using the following formula:

$$\text{Increase paw volume (\%)} = \frac{(Ct-Co)}{Co} x\ 100$$

Where Ct = thickness of paw after carrageenan injection.

Co = thickness of paw before carrageenan injection.

2.5.2. Cotton pellet-induced Granuloma test:

The effect of EE on the chronic and proliferative phases of inflammation was assessed using a cotton pellet-induced granuloma tissue formation rat model. Rats were anesthetized and then a sterilized cotton pellet that weighed 20 ± 2 mg was implanted subcutaneously, in the axilla region of each rat. After the implantation of the cotton pellet, each treatment group of rats ($n = 9$ per group) was administered vehicle, prednisolone (2.5 mg/kg), EE at doses of 0.32 g/kg and 0.64 g/kg, once daily for seven consecutive days. On the eighth day, the animals were euthanized. The cotton pellets were removed and made free from extraneous tissues. The wet weights of the cotton pellets were determined. The pellets then were dried at 60°C for 24 h. Results are expressed as the difference between the initial implanted cotton pellet weight and the final dry mass of the cotton pellet and fibrovascular tissue.

Measure of wet weight = wet weight of pellet - initial weight of the pellet

Measure of dry weight = constant dry weight - initial weight of the pellet

2.6. Data presentation and statistical analyses

Data analyses were performed using SPSS 16.0. In the case of data with a normal distribution, data are shown as means ± S.E.M.; one-way analysis of variance ANOVA followed with LSD post hoc test were used for statistical comparisons to determine treatment differences. A probability level of $p < 0.05$ was considered significant.

When data were not normally distributed, data were shown as median (min - max), and the non-parametric Kruskal-Wallis one-way analysis of

variance by ranks was performed, followed by a Mann-Whitney test to determine significant differences between the experimental groups. Differences were considered statistically significant when p-values were < 0.05.

3. Results

3.1. Antinociceptive activity

3.1.1 Peripheral antinociceptive activity:

As shown in Table 1, intraperitoneal injection of acetic acid-induced the writhing in control mice with 81 writhes in 30 min. Diclofenac 20 mg/kg and EE at doses of 0.54, 1.08, and 2.16 g/kg significantly inhibited the number of writhes in comparison with normal control. The number of writhes in 30 min are 47, 46.5, 61, and 32.5, respectively. There was no significant difference in the number of writhes between diclofenac and EE at effective doses at all evaluation time points (p>0.05).

Table 1. Effect of EE on acetic acid-induced writhing response in mice

Group	Dose	n	Number of writhes on time (5 min)						
			0-5 min	5-10 min	10-15 min	15-20 min	20-25 min	25-30 min	30 min
Control		12	4 (0-19)	17.5 (0-36)	13.5 (9-30)	15.5 (12-31)	17.5 (12-23)	12.5 (6-23)	81 (52-147)
Diclofenac	**20 mg/kg**	9	7 (0-16)	12* (0-17)	7*** (0-15)	5*** (0-10)	8*** (0-12)	3 *** (0-16)	47** (0-65)
EE (g/kg)	**0.54**	12	1 (0-7)	11 (4-24)	7.5* (1-24)	9.5*** (1-13)	9*** (0-13)	7*** (2-12)	46.5*** (8-79)
	1.08	12	5 (0-12)	15 (0-24)	11 (0-18)	9*** (0-21)	11.5** (0-23)	12 (0-20)	61 (0-98)
	2.16	12	2 (0-11)	9*** (0-17)	6.5*** (0-17)	7.5*** (0-14)	4.5*** (0-11)	4.5*** (0-10)	32.5*** (0-77)

*(The values are expressed as the median (min-max) *p < 0.05, **p < 0.01, and ***p < 0.001 indicate statistically significant differences compared to the control group, EE: ethanol extract).*

3.1.2. Central analgesic activity:

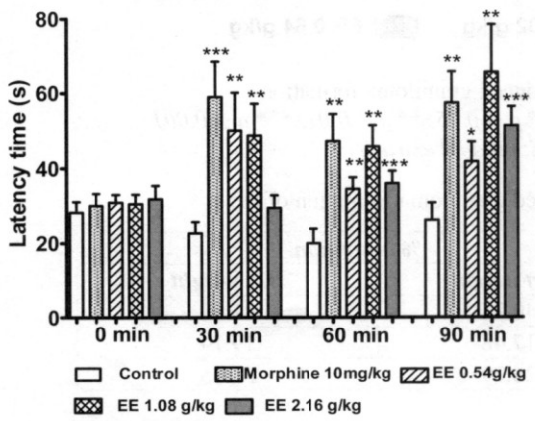

Fig. 1. Effects of EE on hot-plate latency in mice
*The values are expressed as the mean ±SEM, n=12 *p < 0.05, **p < 0.01, and ***p < 0.001 indicate statistically significant differences compared to the control group, EE: ethanol extract.*

As shown in Fig. 1, morphine, a centrally acting analgesic drug, significantly increased latency time at all time points. Similarly, in groups treated with EE at doses of 0.54 and 1.08 g/kg, the latency times were significantly increased after 30, 60, and 90 min of treatment. However, the increase was not dose-dependent. EE at a high dose of 2.16 g/kg only expressed analgesic activity at 60 and 90 min. There was no significant difference in increasing latency time between morphine and EE at effective doses after 30, 60, and 90 min.

3.2. Anti-inflammatory activity

3.2.1. Acute anti-inflammatory activity:

The inhibitory activity of oral administration of EE on carrageenan-induced hind paw edema in rats was demonstrated in Fig. 2. The injection of carrageenan resulted in hind paw edema within 1 h and the maximum effect was reached at 3 h. EE (0.32 g/kg) as well as diclofenac (10 mg/kg) significantly inhibited hind paw edema formation at all evaluation time points. The effect of EE at 0.32 g/kg was better than those at a higher dose (0.64 g/kg). No significant difference in paw edema was observed between the groups receiving diclofenac 10 mg/kg and EE 0.32 g/kg at 1 h, 3 h, and 5 h (p> 0.05).

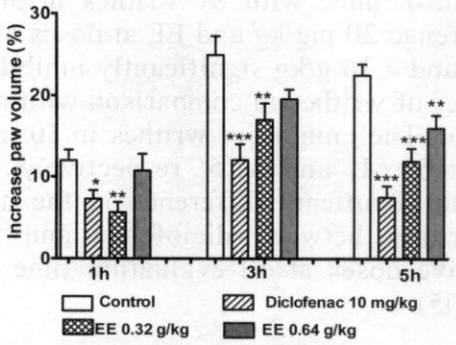

Fig. 2: Effect of EE on carrageenan-induced hind paw edema in rats
*The values are expressed as the mean ±SEM, n=9 *p < 0.05, **p < 0.01 and ***p < 0.001 indicate statistically significant differences compared to the control group, EE: ethanol extract.*

3.2.2. Chronic anti-inflammatory activity:

In the cotton pellet-induced granuloma, EE 0.64 g/kg was found to be effective at the exudative and granulation phases of inflammation. Prednisolone (2.5 mg/kg) and EE (0.64 g/kg) were found to inhibit exudate formation (wet weight) by 40.94 and 26.79% respectively and inhibit granuloma formation (dry weight) by 41.68 and 25.75% respectively (Fig. 3 and Table 2). There was no significant difference in the inhibition of cotton pellet-induced granuloma formation between prednisolone (2.5 mg/kg) and EE (0.64 g/kg) ($p > 0.05$).

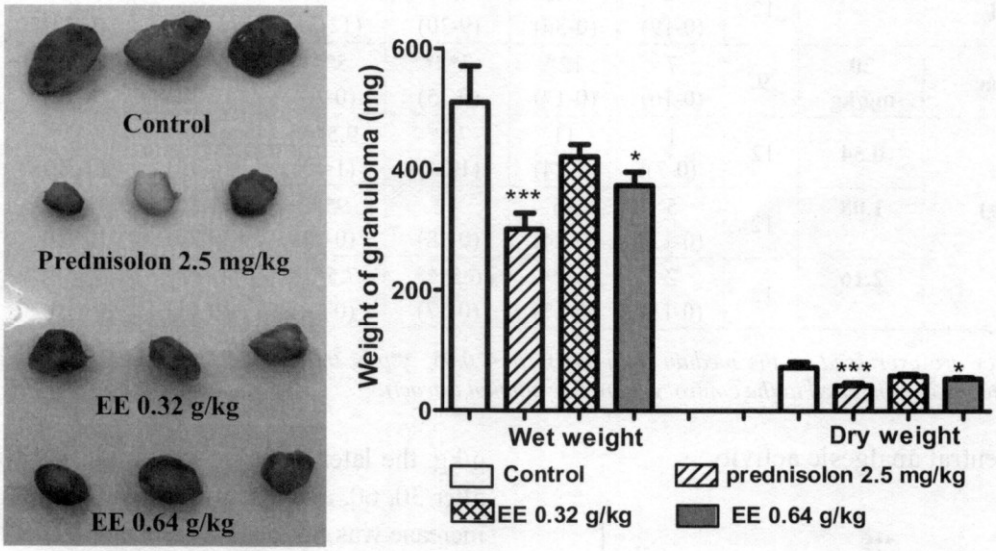

Fig. 3. Effect of EE on cotton pellet-induced granuloma formation.
*Each value is expressed as mean ±SEM (n = 9). * p < 0.05, ** p < 0.01, *** p < 0.001 compared to the control, EE: ethanol extract.*

Table 2. % Inhibition cotton pellet-induced granuloma formation of EE

Group	*% inhibition*	
	Wet weight	*Dry weight*
Prednisolone 2.5 mg/kg	40.94	41.68
EE 0.32 g/kg	17.48	19.14
EE 0.64 g/kg	26.79	25.75

4. Discussion

The anti-inflammatory and analgesic effects of EE were investigated in this study. The analgesic activities were evaluated using two animal models: the hot-plate test and the acetic acid-induced writhing test. The hot-plate test was selected to investigate central analgesic activity. Thermal stimulus-induced hyperalgesia is specific for centrally-mediated nociception and is thought to involve opioids. In this study, EE increased latency time after treatment. This result indicates that EE possesses analgesic activities related to non-inflammatory pain.

The acetic acid-induced writhing model was selected to evaluate peripheral analgesic effects. Acetic acid is an irritant. Intraperitoneal injection of acetic acid causes the release of prostaglandins, substance P which activates and sensitizes peripheral chemosensitive nociceptive receptors, provoking pain nerve endings and

leading to the induction of abdominal constrictions. In this study, EE reduced the number of acetic acid-induced writhing. This result indicated that EE is effective against chemically induced pain by acetic acid. These data indicated the extract possesses anti-nociceptive effects related to inflammatory pain. The analgesic activity of EE may be mediated through its anti-inflammatory effects. The inhibitory effects of EE on the two animal models suggest that they both have peripheral and central analgesic activities.

In evaluating the anti-inflammatory effect, it is important to estimate the activities of the extracts in the acute phase as well as in the chronic phase of inflammation. Paw edema induced by carrageenan was selected to evaluate acute anti-inflammatory activity. Injection of carrageenan into the plantar surface of the paw induces an inflammatory response by inducing pro-inflammatory mediator releases such as histamine, serotonin, and prostaglandins which in turn cause vascular changes, increase vascular permeability, and induce paw edema. In this study, the results showed that EE remarkably inhibited edema of the paw induced by carrageenan in rats. These results indicate that EE exerts anti-inflammatory activity, especially in the acute phase of inflammation.

The cotton pellet granuloma formation test is one of the most commonly used methods in animal research to screen for chronic anti-inflammatory activity of the drugs. In the cotton pellet-induced granuloma formation, the responses can be divided into three phases. The first, transudative phase, 0-3 h after cotton pellet implantation, is defined as the leakage of fluid from blood vessels caused by an increase in vascular permeability. The second, exudative phase, 3-72 h, is defined as the leakage of protein from the bloodstream around granuloma caused by the intensive maintenance of vascular permeability change. The final, proliferative phase, 3-6 days, is defined as the production of granulomatous tissues, the appearance of collagen, mucopolysaccharide synthesis, and an increase in the number of fibroblasts around the cotton pellets occurs. EE at the dose of 0.64 g/kg effectively inhibited cotton pellet granuloma formation. EE decreased wet weight of the cotton pellet indicated that EE inhibited vascular permeability, and decreased exudation of fluid. EE significantly decreased the dry weight of the cotton pellets, and it decreased the amount of granulomatous tissue, suggesting that it has the capability of reducing the synthesis of mucopolysaccharides and collagen and the number of fibroblasts, which are natural proliferative events of granulation in tissue formation. These data indicate that EE displayed anti-inflammatory activity in the chronic phase of inflammation.

These results are similar to our published results in the zebrafish model where the ethanol extract of *Clerodendrum cyrtophyllum* displayed anti-inflammatory in both cut tail-induced inflammation and copper sulfate-induced inflammation zebrafish model [4],[5]. These results were also similar to the results published with other species of *Clerodendrum.* Das et al. (2010) found that the methanol extract of leaves of *Clerodendron infortunatum* Linn. had anti-inflammatory activity against carrageenan, histamine, and dextran-induced rat paw edema [10]. This activity might be due to inhibition of the production or action of inflammatory mediators. EE was proved to inhibit the production of NO and TNF-α in stimulated RAW 264.7 cells [5]. Furthermore, *in vivo* results in zebrafish confirmed that the ethanol extract from *C. cyrtophyllum* inhibited the inflammation via the downregulation of inflammatory genes expression of COX-2, PLA2, C3A, IL-1β, IL-8, TNF-α, and NF-kB, and upregulation of the anti-inflammatory cytokine IL-10 gene [4],[5]. This is a possible mechanism for the anti-inflammatory activity of EE.

5. Conclusion

In conclusion, based on the data presented here, it may be concluded that EE possesses both anti-nociceptive and anti-inflammatory activities. This novel finding provides a scientific basis for its ethnobotanical uses for alleviating pain and treating inflammatory disorders.

References

1. Dzoyem J. P., Mcgaw, L. J., Keute V., Bakowsky U., (2017), Anti-inflammatory and anti-nociceptive activities of African medicinal spices and vegetables, *Elsevirer Inc*, 239- 270. **2**. Kar P., Goyal A. K., Das A. P., Sen A., (2014), Antioxidant and pharmaceutical potential of *Clerodendrum* L: An overview, *International Journal of Green Pharmacy*, 8 (4), 210-216. **3.** Zhou J., Zheng X., Yang Q., Liang Z., Li D., Yang X., Xu J., (2013), Optimization of ultrasonic assisted extraction and radical-scavenging capacity of phenols and flavonoids from *Clerodendrum cyrtophyllum* Turz leaves, *PloS One*, 8(7), 1-8. **4.** Nguyen T. H., Le H. D., Kim T. N. T., The H. P., Nguyen T. M., Cornet V., Lambert J., Kestemont P. (2020), Anti-

inflammatory and antioxidant properties of the ethanol extract of *Clerodendrum cyrtophyllum* Turz in copper sulfate-induced inflammation in zebrafish, *Antioxidants (Basel)*, 9(3), 192. **5**. Nguyen T. H., Nachtergael A., Cornet V., Kestemont P., (2020), Anti-inflammatory properties of the ethanol extract from *Clerodendrum cyrtophyllum* Turz based on *invitro* and *invivo* studies, *Journal Ethnopharmacology*, 254, 112739. **6**. Nair AB., Jacob S. (2016), A simple practice guide for dose conversion between animals and human, *Journal Basic and Clinical Pharmacy*, 7(2), 27-31. **7.** Zhang L., Hu J. J., Lin J. W., Fang W. S., Du G. H. (2009), Anti-inflammatory and analgesic effects of ethanol and aqueous extracts of *Pterocephalus hookeri* (C.B. Clarke) Höeck, *Journal Ethnopharmacology*, 123(3), 510-514. **8.** Ishola I. O., Agbaje O. E., Narender T., Adeyemi O. O., Shukla R., (2012), Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Cnestis ferruginea* Vahl ex DC. (Connaraceae) root, *Journal Ethnopharmacology*, 142(2), 383-389. **9**. Winter C. A., Risley E. A., Nuss G. W., (1962), Carrageenin induced edema in hind paw, *Experimental Biology Medicine*, 3(111), 544-547. **10**. Das S., Haldar P. K., Pramanik G., (2010), Evaluation of anti-inflammatory activity of *Clerodendron infortunatum* Linn. extract in rats, *Global Journal of Pharmacology*, 4(1), 4850.

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MICROPROPAGATION OF *STEPHANIA BRACHYANDRA* DIELS, A RARE MEDICINAL PLANT OF THE NORTH-WESTERN VIETNAM

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Summary

Micropropagation of *Stephania brachyandra* Diels, a Rare Medicinal Plant of the North-Western Vietnam

An effective protocol for micropropagation of *S. brachyandra*, a rare medicinal plant of the North-Western Vietnam has been established. 2-3 cm long *in vivo* nodal segments were efficiently disinfected with 0.1% $HgCl_2$ solution for 10 minutes and regenerated on basic MS solid medium augmented with 0.5 mg/L kinetin. The highest *in vitro* shoot multiplication coefficient of 8.2 was obtained on the medium containing full-strength MS combined with 1.0 mg/L kinetin. The best medium for *in vitro* rooting was the half-strength MS solid medium fortified with 0.25 mg/L IBA, giving a rooting percentage of 85.1%, and an average of 3.3 roots per shoot after 4 weeks of culture. The rooted plantlets were successfully acclimatized in TRIBAT substrate with 96.4% vigor rate and then transferred to the field in Tam Dao Research Center for Medicinal Materials, Vinh Phuc province, where they thrived well.

Keywords: *Stephania brachyandra, Micropropagation, Medicinal plant, North-Western Vietnam.*

1. Introduction

Stephania brachyandra is a medicinal plant belonging to the genus *Stephania*, having common characteristics of the genus, such as perennial plant with deciduous twining and large tuberous rootstock. It was distributed mainly on ravine sides in forests, at altitudes of about 1000 m [1]. Its tuber contains the main active ingredient L-tetrahydropamatin, which has been used in Vietnam for several clinical indications under the drug Rotundine. This is a kind of sedative to treat effectively neuropathic diseases today due to its few side effects. *S. brachyandra* is being overexploited, leading to the exhaustion of its natural resources, therefore, it was listed in the Viet Nam Red Data Book at Endangered level (EN), and the list of Group II in Decree No. 84/2021/ND-CP dated September 22nd, 2021 of the Vietnam Government on the management of endangered, rare and precious species of forest fauna and flora and observation of convention on international trade in endangered species of wild fauna and flora.

Because of the increasing demand for seedlings and medicinal materials of *S. brachyandra*, propagation of this endangered medicinal plant species for the conservation of genetic resources and to develop its production areas of medicinal materials is an urgent duty. Sexual propagation and asexual propagation using rootstocks could not provide enough seedlings for production in large-scale, because breeding materials are rare. Furthermore, it is difficult for its seeds to germinate in natural conditions. To achieve the highest rate of 90%, they require the optimal treatments such as soaking in tap water for 6 hours, 1% NPK containing substrate and 50% shading mode [2]. Micropropagation, which uses small pieces of the plant as explants under aseptic and controlled environmental conditions to produce a large number of consistently uniform and true-to-type plants, overcomes the downsides of above traditional propagation methods. Therefore, it is the most optimal method to propagate *S.*