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Research article

Anti-arthritic activity and phytochemical composition of "Cao Khai" (Aqueous extracts of *Coptosapelta flavescens* Korth.)

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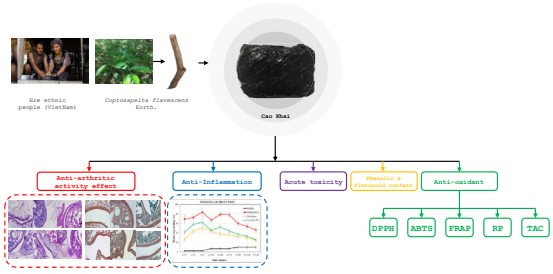
GRAPHICAL ABSTRACT



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ABSTRACT

For a long time, ethnic minorities in Ninh Thuan province have combined "Day Khai" (*Coptosapelta flavescens* Korth) with many other medicinal herbs, creating an esoteric remedy called "Cao Khai"-an aqueous extract from Day Khai. This study tested an aqueous extract from "Day Khai" for total phenolics, total flavonoids, antioxidant, and anti-inflammatory activity in an in vivo mice model. The aqueous extracts of raw materials *C. flavescens* collected in different regions were found to have phenolics, flavonoids, and antioxidant capacity in vitro according to DPPH, ABTS, RP, TAC, and FRAP methods. This study evaluated the effect of *C. flavescens* on arthritis of Complete Freund's adjuvant (CFA) induced-mice by observing changes in the degree of edema in the leg joints blood index and histology. The findings indicated that the "Cao Khai" had anti-inflammatory benefits and lowered the inflammatory symptoms in mice equivalent to Mobic medications ($p < 0.05$) while also limiting cartilage tissue damage after 14 days of usage. As a result, it is clear that "Cao Khai" can be considered a medicinal herb with tremendous potential for usage as an addition to illness therapy that should be protected and cultivated.

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1. Introduction

For a long time, the immune system has been shown to play a vital part in the human body because an overactive immune system is the source of illnesses caused by responses. Hypersensitivity or allergies can manifest in various ways, leading to responses against our cells and tissues — a condition known as an autoimmune disease (Choudhary et al., 2015). Rheumatoid Arthritis (RA) is a common inflammatory illness with a chronic history and varying degrees of complexity in joint, extra-articular, and systemic signs that affect millions of individuals (accounting for approximately 1% of the population) (Choudhary et al., 2015; Zhang et al., 2009; Tastekin et al., 2007). The primary symptoms of RA are discomfort, swelling, cartilage, and bone degeneration, which lead to irreversible impairment (Choudhary et al., 2015; Babushetty and Sultanpur, 2012). As a result, the fundamental goals of RA treatment are to alleviate pain, reduce inflammation, preserve the joint structure, and maintain and control immune system involvement (Longo et al., 2012; Bodkhe et al., 2019). There has been a revolution in the treatment of RA with the introduction of RA medicines to attenuate the RA symptoms; nevertheless, this category of medications still causes specific undesired side effects such as digestive difficulties, infections, and effects on the cardiovascular system... (Jadhav et al., 2007; Agrawal and Pal, 2013). When using conventional drugs, finding highly effective drugs with fewer side effects is a concerning problem. Steroid therapy, non-steroidal anti-inflammatory drugs (NSAIDs), interleukin-1 beta antagonists, and other treatments to control acute RA symptoms have only shown limited efficacy (Chitme and Patel, 2009).

Plants and herbs are rich sources of phytochemicals, which have been shown to help prevent, treat, or relieve various health problems. *Coptosapelta flavescens* Korth, namely Day Khai in Vietnamese, is a precious medicinal herb used by many ethnic groups in Southeast Asian countries to make skin creams, treat worms, colic, and nasal ulcers (Birgitta and Torsten, 2009; Line, 2005), reduce fevers, and defense against infection (Nursanti et al., 2018). C. *flavescens* extract was previously reported to suppress bacteria more efficiently than metronidazole (Hounkong et al., 2015), treat dysentery or malaria (Kongyen et al., 2014; Arnida et al., 2017, 2019), reduce blood pressure (Kosala et al., 2017), and exhibit anti-inflammatory activity (Kosala et al., 2018). According to the traditional folk experience from ethnic minorities in central Vietnam, C. *flavescens* root extract has been used to disinfect wounds, promote skin recovery, treat cold and flu symptoms, and aid in the treatment of gout (Institute of Medicine, 2003; Minh et al., 2014). This medicinal herb has been traditionally made into a product called "Cao Khai," - an aqueous extract from C. *flavescens* which is used as a traditional medicine remedy for bone and joint diseases, pain relief, anti-inflammatory, antibacterial, diarrhea treatment (Khanh, 2009). Furthermore, anthraquinones and saponins have been identified from the stems and roots of this medicinal plant. It has been hypothesized that C. *flavescens* contains a variety of different biological substances associated with its effects.

The problem with the use of naturally derived - medical products is a lack of scientific proof to back up the claims, often based exclusively on traditional knowledge. To our best knowledge, up to now, there have been several studies on phytochemical composition, biological activity, and anti-inflammatory ability of C. *flavescens* in different forms on *in vitro* and *in vivo* models. However, these studies have been concerned with the acute toxicity to elucidate the applicability of the C. *flavescens* extract in the pharmaceutical field, the behaviors of treated subjects related to biochemical and hematological properties during post-dosing period are less focused. Specifically, antioxidant activity was only conducted in basic methods (free radical scavenging ability). The antioxidant capacity of extracts from this plant species has not been thoroughly evaluated. Therefore, we, in this study, aimed to provide a detailed scientific data set on the quantification of phytochemical constituents, antioxidant activities, and cartilage-protective effects of the aqueous extract of C. *flavescens* on Complete Freund's adjuvant (CFA) induced-mice. The result is expected to give the comprehensive overview of its biological properties from C. *flavescens* for further studies to achieve the consistency and uniformity in the entire test chain on this extract.

2. Material and method

2.1. Material

The samples used in the study were an aqueous extract of C. *flavescens* (AECF) made from the raw material *Coptosapelta flavescens* Korth. Coptosapelta tomentosa Valeton ex K. Heyne is another synonym. The plant names are validated according to the http://www.theplantlist.org; the local name in Vietnam is typically called "Day Khai" and its extract is known as "Cao Khai" collected in September 2020 in Ninh Thuan province, Vietnam. In this study, each Cao Khai sample was differently named upon its district origin in Ninh Thuan province. "Cao Khai 1" (AECF1) was collected in Ninh Son district area (GPS: 11°35′42.0″N 108°39′10.7″E), "Cao Khai 2" (AECF2) was collected in Bac Ai (GPS: 11°56′03.4″N 108°47′59.2″E) district area. "Cao Khai 3″ was handcrafted and collected directly at a production facility of local people (Ninh Thuan province). Recorded photographs and video of the preparation process of Cao Khai are presented in the supplementary file.

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e08933.

The extraction method of AECF1 and AECF2 were based on traditional knowledge of local people. After being harvested from the local forest, C. *flavescens* was preliminarily treated by removing excess leaves and cutting into small pieces (Figure 1a). The extracts were obtained by heating samples with water as the solvent at 100 °C for 48 h and the process was repeated in three times. The extract was achieved with the moisture content of 10%; the Cao Khai product is illustrated in Figure 1b. The product was ground and sieved through a mesh sieve (d = 0.5 mm) to obtain a sample with uniform size (Figure 1c). This sample was vacuum-packed and kept at -20 °C for future experiments.

The Oriental Medicine Association of Ninh Thuan province, Vietnam, identified and authenticated plant samples. A voucher specimen was deposited at Nguyen Tat Thanh University's Faculty of Pharmacy in Vietnam.

2.2. Reagents

All reagents were of analytical grade and were purchased from Sigma-Aldrich (USA). All solvents used were LC-MS grade and were purchased from Merck (USA).

2.3. Total polyphenols determination

The total phenol content was determined using the Folin–Ciocalteu reagent assay, following the method described by Singleton and Rossi

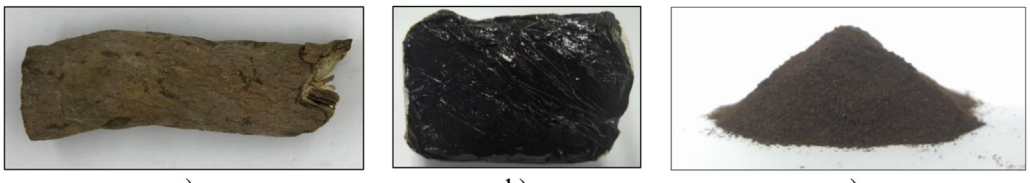

Figure 1. Raw material a) *Coptosapelta flavescens* Korth; b) An dried aqueous extract of C. *flavescens*, and c) C. *flavescens* powder.

(1965). The absorbance wavelength was read at 765 nm. Gallic acid was used as the standard. Polyphenols content was measured in milligrams of gallic acid equivalent per milligram of extract (μg GAE/mg extract).

2.4. Total flavonoids determination

The total flavonoids content in AECFs was determined using a colorimetric technique with minimal changes, as reported by Hossain and Rahman (2011). The absorbance was measured at 415 nm. Quercetin served as the standard. The total flavonoid content was given in micrograms of quercetin equivalent per milligram of extract (μg QE/mg extract).

2.5. Determination of total antioxidant capacity (TAC)

The phosphomolybdenum technique, as reported by Prieto et al. (1999), was used to determine the total antioxidant activity of AECF samples. AECF was mixed with the test solution (sulfuric acid, sodium phosphate, and ammonium molybdate), and the absorbance was measured at 695 nm.

2.6. Antioxidant activity determination using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant capacity of AECFs was determined by the modified Sharma and Bhat (2009) DPPH free radical neutralization method. The spectral absorbance of DPPH was measured at 517 nm.

2.7. Antioxidant activity determination using the ABTS free radical scavenging method

The antioxidant activity of AECFs was determined by the modified ABTS•+ decolorization method described by Nenadis et al. (2004). ABTS•+ is produced by the ABTS potassium persulfate reaction. The reaction mixture was measured for absorbance spectroscopy at 734 nm.

2.8. Antioxidant activity measurement using the ferric reducing/antioxidant power (FRAP) method

The antioxidant potential of AECFs was determined using a modified FRAP reduction (Benzie and Strain, 1996). The principle of this method is based on the reduction of the ferric-tripyridyltriazine complex. AECFs were reacted with FRAP solution, and the absorbance was determined at 593 nm.

2.9. Determination of antioxidant activity using the reducing power (RP) method

The reduction capacity of the AECFs was performed according to the method of Oyaizu (1986) with some adjustments. The reaction mixture consisting of AECFs, phosphate buffer, and $K_3Fe(CN)_6$ was prepared according to the instructions. The spectral absorbance of the reaction mixture was measured at 700 nm.

2.10. Experimental animals and approval

Swiss albino mice (6–8 weeks old and an average weight of 22 ± 2 g) were donated by Vietnam's Nha Trang Institute of Vaccines and Medical Biologicals. The mice were trained to dwell in conditions with a temperature of 25 ± 1 °C and relative humidity of 55 ± 5%. Mice were fed with standard pellets and tap water ad libitum. The use of animals and the location were authorized by the medical ethics council of Nguyen Tat Thanh University's Faculty of Pharmacy in Vietnam. The study was carried out in accordance with the "Science and Technology Program at the Level of the Department of Science and Technology of Ninh Thuan Province," which was approved by the director of the Department of Science and Technology of Ninh Thuan Province, Vietnam, on October 12, 2020, with the code 2/2020/HD-SKHCN. All tests were carried out in compliance with Vietnam's national rules on animal protection and experimental animal care, as outlined in the Law on Animal Health, 2015, Vietnam National Assembly, No. 79/2015/QH13, adopted on June 19, 2015.

2.11. Acute oral toxicity research

The study of acute oral toxicity was carried out according to the guidance of Decision 141 - Ministry of Health - Vietnam on the promulgation of professional documents "guideline for preclinical and clinical trials of traditional medicines and drugs from medicinal herbs" on October 27, 2015 (No. 141/QD-K2ĐT). The mice were starved overnight and simply given water. AECF was given orally at a dosage of 5000 mg/kg of body weight. The behavior and expression of mice were monitored within seven days after the oral treatment.

2.12. Anti-inflammatory activity testing procedure

The anti-inflammatory activity was evaluated by using published method of Winters et al. (1962). First, all mice were measured with average mice leg volume (V0) on a Plethysmometer. Mice were randomly assigned to 5 lots so that the difference in V0 was not statistically significant (08 mice/group).

Group I (Healthy control): distilled water, per os;
Group II (Inflammatory control): distilled water, per os;
Group III (Diclofenac control): 5 mg/kg diclofenac, per os;
Group IV (AECF): based on results of acute oral toxicity or human dose.

Before testing, mice were fasted for 12 h. After drinking distilled water, diclofenac, and test samples for 1 h, mice in groups 2 to 5 were injected with 0.025 ml of 1% carrageenan suspension into the sole of the right hind foot to cause paw inflammation. Healthy control mice group were injected with 0.9% NaCl solution. After that, all mice were placed in cages with supports to avoid foot infections. The paw volume of treated mice was measured at 1, 3, 5, 24, 48, 72, 96, 120, 144 h (Vt) after inflammation, respectively. The formula for calculating the degree of mice paw edema X (%) is described as followed:

$$X\ (\%) = (Vt - V0)/V0 \times 100$$

Where:
Vt: leg volume at time t after inflammation (ml)
Vo: leg volume at the time before inflammation (ml)
After measuring foot volume at 24, 48, 72, 96, 120, and 144 h, mice were given distilled water, diclofenac, or sample once a day.

The control and eucalyptus groups were examined for their relative anti-inflammatory effect via the ability to reduce mice paw edema (I %) which was calculated by the formula:

$$I\ (\%) = (X1 - X2) / (X1 - X3) * 100\%$$

Where:
X1: Paw edema of the control group
X2: Leg edema of the group using AECF or the leg edema of the control group.
X3: Leg edema of the healthy control.

2.13. Freund's adjuvant-induced arthritis

Ankle arthritis was induced by injecting Complete Freund's adjuvant (CFA, 50 μL) into a footpad of the left hind paw of mice. The animals were separated into six groups (six mice/group):

Group I – Healthy control;
Group II – Arthritic control;
Group III – Mobic 0.125 mg/kg, per os;

Group IV – AECF based on results of acute oral toxicity or human dose, per os;

Group V – Dosage less than twice that of group IV, per os;

Group VI – Dosage less than twice that of group V, per os.

On day 0 and day 28, the anti-arthritic activity of AECF was assessed using the following parameters: paw volume, joint diameter, pain threshold, thermal hyperalgesia, tactile allodynia, and body weight (Kumar et al., 2006). The joint diameter was measured by using a digital Vernier caliper (Mitutoyo, Japan) on day 0 before FCA injections and again on day 28. The difference in joint diameter was calculated by subtracting the final and starting joint diameters. On day 28, the animals were sedated with ether, and blood was collected through the retro-orbital puncture to assess various biochemical and hematological parameters.

Ankle joints were retrieved by dissection of the ankle area, which included the removal of skin and muscle. In a 10% formalin solution, the ankle joint was maintained. Dehydrated samples were rinsed with xylol after being dehydrated with various amounts of alcohol. Samples were paraffin-embedded then cut into 4–6 uM slices and stained with Hematoxylin and Eosin. Slices of the ankle joint were examined under a microscope to assess ankle joint damage and the anti-inflammatory efficacy of AECF. The slices were dyed with Safranin O, and the morphology of the cartilage tissue was evaluated following treatment to examine the ability to protect the cartilage tissue. Hematology and blood biochemical assays were performed on mice blood to assess the amounts of leukocytes, total red blood cells, RF, CRP, AST, ALT, and ALP.

2.14. Statistical analysis

The findings were statistically processed using Statgraphics Centurions 18.1.12 and Microsoft Excel 365. The analysis of variance (ANOVA) and the LSD test were used to determine the difference between treatments. The results are shown as mean SEM (standard error of mean). $P < 0.05$ is considered to be significant.

3. Result

3.1. Contents of total polyphenols and flavonoids compounds

Table 1 shows the total polyphenols and flavonoid content of "Cao Khai" obtained from various sources. There were statistically significant variations in the polyphenols and flavonoids content of the "Cao Khai" samples. This discrepancy might be attributed to the difference in Cao Khai origin and the applied extraction procedure. When compared to the other samples, the AECF1 sample exhibited the greatest flavonoid content (62.02 ± 0.96 μg QE/mg). In terms of polyphenols content, the AECF2 sample had the highest value of 49.67 ± 0.05 μg GAE/mg. Polyphenols are one of the most significant components, accounting for a large percent of all plants. Plants having a total polyphenols content of more than 20 μg GAE/mg are speculated to possess high antioxidant activity, according to Kähkönen et al. (1999). The results showed that the highest polyphenols content was recorded in the AECF2 sample (49.67 μgGAE/mg). The polyphenols content of the AECF1 and AECF2 samples were both higher than that of the AECF3 sample, which was purchased at the local market. This may be due to differences in raw material origin, collecting time, and extraction conditions. In general, the polyphenols content of the aqueous extract of "Cao Khai" was higher than that of other medicinal root-derived materials such as Angelica sinensis (32.79 μgGAE/mg), *Condonopsis javanica* (2.9 μgGAE/mg) (Ng et al., 2020; Pham et al., 2020). Current findings indicated that C. *flavescens* possessed significant amounts of polyphenols and flavonoids. Although the active component levels in each AECF vary due to differences in provenance, this product has the potential to be a rich source of natural antioxidants.

3.2. Antioxidant potential

Many investigations have revealed that superoxide, hydroxyl, or nitric oxide free radicals are the cause or precursor of DNA, lipid, and protein damage (Jao and Ko, 2002; Kanatt et al., 2007; Srinivasa et al., 2010). The antioxidant activity (DPPH•, ABTS•+, RP, FRAP, and TAC) of AECF2 was tested by measuring its capacity to neutralize or diminish free radicals formed in the reagents. Table 1 shows the antioxidant capacity of the "Cao Khai" samples tested using the DPPH, ABTS•+, RP, FRAP, and TAC techniques. The free radical scavenging capabilities of DPPH• and ABTS•+ have been demonstrated to be involved in the donor acceptance of hydrogen atoms and electrons (Kaviarasan et al., 2007). The current investigation found that AECF1 had more vigorous DPPH free radical scavenging activity than the others, with an EC_{50} value of 56.03 ± 0.31 μg/mL but it was still inferior to the vitamin C standard (EC_{50} = 5.67 ± 0.02 μg/mL). Although the AECF1 and AECF2 have not been proved to be capable of scavenging ABTS•+ free radicals, they both fared better than AECF3. This implied that AECF1 might include a significant number of hydrogen donors, lowering free radical generation and decolorization in the DPPH• and ABTS•+ tests. The previous study also found that the "Cao Khai" obtained from a local market in Ninh Thuan province had strong DPPH• and ABTS•+ scavenging activities, with a low EC_{50} value. Moreover, the EC_{50} value obtained from the ABTS•+ technique was more significant than that in our previous work (Nguyen et al., 2020).

The total antioxidant activity (TAC) of the extract was calculated using the reduction of Mo(VI) to Mo(V) by antioxidant chemicals and the creation of a blue phosphate/Mo(V) complex following a phosphomolybdenum method (Cardeñosa et al., 2002). The findings indicate that "Cao Khai" showed the ability to reduce Mo(VI) to Mo(V), although the efficiency is not favorably compared with vitamin C standard. At the level of statistical significance $p < 0.05$, AECF1 achieved the EC_{50} = 111.15 ± 1.28 μg/mL, which was more efficient than that of AECF2 and AECF3. One of the essential aspects to consider the antioxidant activity of one compound is its reducing ability (Huyut et al., 2017). This study used two methods: FRAP and RP, to determine the reducing activity of the sample. By locking iron or copper ions in a complex, which does not allow these ions to exist in free form, leading to the inhibiton of free radical formation process. Because it no longer exists in its free form, it can no longer accelerate the formation of free radicals (Benzie and Strain, 1996). Three samples of "Cao Khai" were analyzed, and the results revealed that their antioxidant potential ranged from 15.90 to 43.11 μg/mL. AECF1 had the greatest Fe^{3+} reducing capacity (15.90 ± 0.56 μg/mL), followed by

Table 1. Cao Khai's total polyphenols content, flavonoids content, and anti-oxidant activity.

Sample	Polyphenols (μgGAE/mg)	Flavonoids (μgQE/mg)	EC_{50} (μg/mL)				
			DPPH	ABTS	RP	TAC	FRAP
AECF1	37.00 ± 0.06^{b}	62.02 ± 0.96^{a}	56.03 ± 0.31^{c}	54.13 ± 0.18^{b}	44.86 ± 0.47^{c}	111.15 ± 1.28^{c}	15.90 ± 0.56^{c}
AECF2	49.67 ± 0.05^{a}	5.940 ± 0.52^{c}	65.21 ± 0.22^{b}	51.27 ± 0.25^{c}	50.14 ± 0.11^{b}	137.80 ± 1.21^{b}	28.93 ± 0.24^{b}
AECF 3	24.15 ± 0.25^{c}	10.22 ± 0.33^{b}	88.72 ± 0.43^{a}	106.48 ± 0.18^{a}	93.34 ± 0.04^{a}	175.69 ± 1.16^{a}	43.11 ± 0.60^{a}
Vitamin C			5.67 ± 0.02^{d}	3.70 ± 0.02^{d}	1.97 ± 0.00^{d}	15.41 ± 0.05^{d}	1.56 ± 0.01^{d}

Note: Values are expressed as mean ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P < 0.05$). Similarly hereinafter.

AECF2 (28.93 ± 0.24 μg/mL), and finally AECF3 (43.11 ± 0.60 μg/mL). Similarly, the AECF1 had a rather high EC_{50} value of 44.86 ± 0.47 μg/mL using the RP technique. The previous findings in examining polyphenols and flavonoids in "Cao Khai" also indicated that AECF showed high potential for use as a natural source of antioxidants.

3.3. Acute oral toxicity research

To confirm the safety of "Cao Khai" in test mice, the AECF1 sample (chosen for its most significant activity) was tested for its acute toxicity. After 72 h of receiving a single dosage of 5000 mg/kg body weight, all mice showed the normal status, with no symptoms of convulsions, diarrhea, ruffled fur, or shortness of breath. As the oral dosage of 5000 mg/kg was not lethal to mice, the LD_{50} value was estimated to be above 5000 mg/kg of body weight. Based on these results and local practice, a dosage of 400 mg/kg body weight was used for pharmacological testing.

3.4. Anti-inflammatory study

The findings of mice paw swelling in Figure 2 indicated that the values in the healthy control group, after 1 h, 3 h, 5 h, 24 h, 48 h, 96 h, 120 h, and 144 h differed significantly from the other groups ($p < 0.05$). At a 400 mg/kg of dosage, there was a reduction in edema of the mice's feet compared to the inflammatory control group during post-dosing period, except for the time of 5 h ($p < 0.05$). At 3 h and 5 h after causing inflammation, the swelling in using "Cao Khai" increased from 41.28% to 58.11% and 62.45%, respectively. This might be explained that the time period from 3 h to 5 h was the time when carrageenan exerted the highest inflammatory effect. At all the time points of the survey, the AECF1 sample at a dose of 400 mg/kg could reduce mice paw edema, although the decrease is not comparable to that of diclofenac 5 mg/kg. Thus, at an oral dosage of 400 mg/kg, AECF1 displayed anti-inflammatory activity, with a later onset than diclofenac at a dosage of 5 mg/kg. This finding was consistent with prior research on C. *flavescens*'s anti-inflammatory properties (Kosala et al., 2018, 2019). C. *flavescens* has been revealed to contain a wide range of polyphenols, saponins, anthraquinones, and terpenoids and has been proven to promote better anti-inflammatory properties than Indomethacin (Huyut et al., 2017). Food polyphenols have been shown to have anti-inflammatory activity by inhibiting Cyclooxygenase 2 induced by 12-O-tetradecanoylphorbol-13-acetate, stimulant-induced tumorigenesis in mice skin, and COX activity in macrophages based on the induction of lipopolysaccharides (Yoon and Baek, 2005; Hounkong et al., 2014; Su et al., 2016).

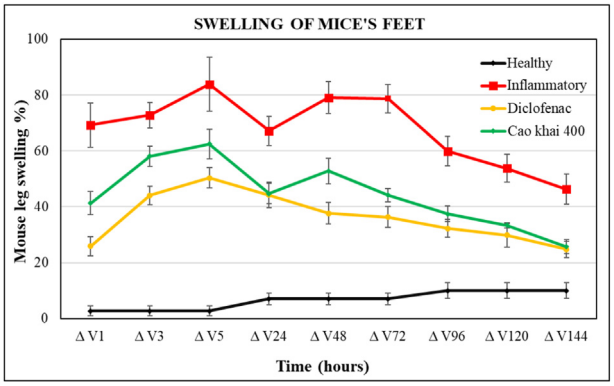

Figure 2. Swelling degree of the mice's paw of the experimental batches over time.

3.5. Effect of the Cao Khai on ankle joint edema

CFA-induced arthritis is a widely used chronic test model because the clinical and pathological changes of the trial can be compared with those observed in rheumatoid arthritis in human (Singh et al., 2003). In the CFA model, chronic inflammation is expressed through an increase in the volume of the injected foot (Jalalpure et al., 2011). In the arthritic control group, the results increased the mean joint diameter from 2.72 ± 0.09 mm to 5.85 ± 0.41 mm after 28 days of CFA injection. This increase is a common phenomenon in rheumatoid arthritis (Garg and Azim, 2021). Secondary lesions occur in arthritic mices due to cell-mediated immunity, and their increase suggests immunostimulant action (Pandey et al., 2012). After 28 days, the ankle joint diameter gradually decreased in all AECF- treated groups and the Mobic groups. The difference in joint diameter between all AECF- treated groups and Mobic control group on day 28 was statistically significant ($p < 0.05$) compared to the arthritic control group. However, the difference between the AECF-treated group at a dosage of 400 mg/kg and the Mobic control groups was not statistically significant ($p < 0.05$). The findings also revealed that, in reducing joint swelling, the treatment of AECF at two dosages of 200 and 400 mg/kg showed the same efficacy with the Mobic treatment (Table 2). The secondary response is the expression of cell-mediated immunity in adjuvant arthritis, and 'AECF1′ demonstrated an increase of this response, implying immunomodulatory function of AECF1. CFA-induced polyarthritis has been shown to be associated with an immune-mediated inflammatory response, and the only subject documented to develop polyarthritis following CFA treatment is mice (Nielen, 2006).

After 28 days, all CFA-treated mice had a substantial ($p < 0.05$) increase in paw volume compared to the healthy control group. Mice paw volume after 28 days of using AECF1 at the dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg was significantly alleviated. Simultaneously, at the significance level, it was discovered that the AECF1 treatment at 400 mg/kg efficiently decreased the mice leg volume and could be comparable to the Mobic control group at 0.125 mg/kg dosage. The ability of "Cao Khai" to inhibit mice paw edema can be understood due to its inherent immunosuppressive activity. Furthermore, the inhibitory effect of "AECF1″ on leg edema was found to be comparable to that of Mobic

Table 2. Ankle joint diameter (mm) and leg volume (cm^3/ml) groups of mice.

Group	Diameter of the ankle joint (mm)		Paw volume (cm^3/ml)	
	Day			
	0	28	0	28
Healthy control	2.61 ± 0.09^{a}	2.67 ± 0.11^{a}	0.22 ± 0.01^{a}	0.25 ± 0.01^{ab}
Arthritic control	2.72 ± 0.09^{a}	5.85 ± 0.41^{c}	0.23 ± 0.01^{a}	0.38 ± 0.02^{d}
100 mg/kg	2.69 ± 0.07^{a}	3.81 ± 0.23^{d}	0.22 ± 0.03^{a}	0.30 ± 0.03^{c}
200 mg/kg	2.75 ± 0.06^{a}	3.28 ± 0.15^{e}	0.23 ± 0.02^{a}	0.29 ± 0.03^{bc}
400 mg/kg	2.66 ± 0.12^{a}	$2.99 \pm 0.15^{a,b}$	0.21 ± 0.01^{a}	0.24 ± 0.01^{a}
Mobic 0.125 mg/kg	2.67 ± 0.10^{a}	3.11 ± 0.20^{b}	0.23 ± 0.01^{a}	0.25 ± 0.03^{ab}

Note: Values are expressed as mean ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P < 0.05$). Similarly hereinafter.

drugs. This result suggested a potent inhibitory effect by "AECF1″ of cell-mediated immunity in rheumatic diseases.

3.6. Variation of hematology profile

Information about the pathophysiology of arthritis is presented in Table 3. The research findings revealed an increase in RF, CRP, and WBC index in the arthritis-induced group compared to the healthy control group. After 28 days of administration, treatments with AECF1 and Mobic lowered RF, CRP, and total white blood cells parameters to near-normal levels. The rise in RF, CRP, and WBC in the arthritis group was caused by the immune system's activity against inflammatory substances (Bihani et al., 2014). The RBC index in the arthritis group, on the other hand, was found to noticeably decrease as compared to the healthy group. Meanwhile, the RBC index was recovered to the same level as the healthy control group when mice were treated with AECF1 and the Mobic control group after 28 days. A slight increase in WBC count has been documented in the arthritic group, owing to increased colony-stimulating factors mediated by IL-1β. The body counteracts the inflammatory activity through leukocyte infiltration that damages the tissue and lysosomal membranes (Kumar et al., 2011). These leukocytes release components of their lysosomes, such as bactericidal enzymes or protease, causing further tissue damage and inflammation (Paramita et al., 2017). Injured lysosome tissue or gills trigger the release of phospholipase A2 (PLA2) that mediates the hydrolysis of phospholipids to lysophospholipids and free fatty acids, which are considered precursors of inflammatory mediators. The present results showed that the use of "AECF1″ tended to reduce the WBC value nearly to normal level.

Some biochemical blood parameters, such as AST, ALT, and ALP, increased significantly in arthritic mice, which was connected to ankle joint tissue destruction and was consistent with earlier findings. Meanwhile, treatments with AECF1 and Mobic was reported to alleviate these values. On day 28 of therapy, treatment at a dosage of 400 mg/kg was considerably similar to positive control. Serum AST and ALT have been shown in many studies to play essential roles in the production of chemical mediators in inflammation. The inhibitory action of AECF1 may be ascribed to the presence of anti-oxidants. Plants are rich in antioxidants, which inhibit lipid oxidation (Yokota et al., 2006). In this study, C. *flavescens* was noted to be rich in polyphenols and flavonoids. Previous reports have also revealed the role of these phytochemical components in treating inflammation and joint problems via the inhibition of pro-inflammatory cytokines such as TNF-α, IL-1β (Kuraoka-Oliveira et al., 2020; Sarkaki et al., 2015). Our results showed that AEFC could reduce foot swelling and regulate hematological parameters in the CFA-induced arthritis mice. These results indicated that the polyphenols and flavonoids in AECF play a possitive role in the anti-arthritic effect.

3.7. Histological analysis

HE was used for dyeing the ankle joint tissue. The results revealed that after 28 days (Figure 3), the ankle joints of mice injected with CFA were not damaged. However, an inflammatory reaction developed, with

Table 3. Hematological and biochemical indicators of the blood of groups of mice.

Day		Group					
		Healthy control	Arthritic control	100 mg/kg	200 mg/kg	400 mg/kg	Mobic
28	RF (U/ml)	1.76 ± 0.35^{a}	7.63 ± 0.45^{b}	7.73 ± 0.35^{c}	5.06 ± 0.35^{c}	3.73 ± 0.49^{d}	2.63 ± 0.57^{e}
	CRP (mg/dL)	1.27 ± 0.24^{a}	4.38 ± 0.33^{d}	2.83 ± 0.30^{c}	2.50 ± 0.20^{c}	1.93 ± 0.24^{b}	1.82 ± 0.12^{b}
	RBC (10^{6} TB/μL)	7.63 ± 0.42^{a}	5.26 ± 0.5^{b}	6.91 ± 0.34^{a}	7.65 ± 0.55^{a}	7.67 ± 0.42^{a}	7.11 ± 0.49^{a}
	WBC (10^{3}TB/μL)	3.97 ± 0.31^{a}	7.23 ± 0.57^{d}	5.77 ± 0.31^{c}	4.80 ± 0.46^{b}	4.03 ± 0.47^{ab}	3.67 ± 0.45^{a}
	AST (U/L)	178.06 ± 15.40^{a}	305.86 ± 15.20^{b}	200.43 ± 15.16^{c}	182.33 ± 17.04^{a}	184.70 ± 14.64^{a}	172.36 ± 13.22^{a}
	ALT (U/L)	65.03 ± 9.83^{a}	116.60 ± 12.21^{b}	76.33 ± 7.15^{a}	68.06 ± 3.31^{a}	66.23 ± 10.56^{a}	64.71 ± 6.30^{a}
	ALP (U/L)	134.06 ± 10.43^{ab}	235.66 ± 21.14^{b}	153.13 ± 17.82^{b}	142.50 ± 9.76^{ab}	137.60 11.50^{ab}	126.76 12.90^{a}

Note: Values are expressed as mean ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference ($P < 0.05$). Similarly hereinafter.

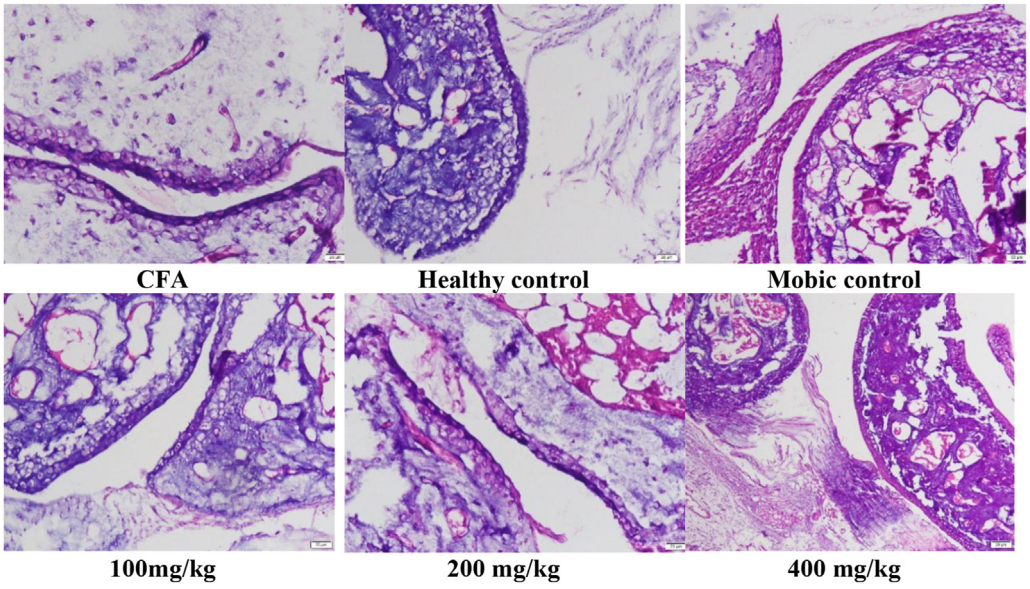

Figure 3. Hematoxylin and Eosin staining results.

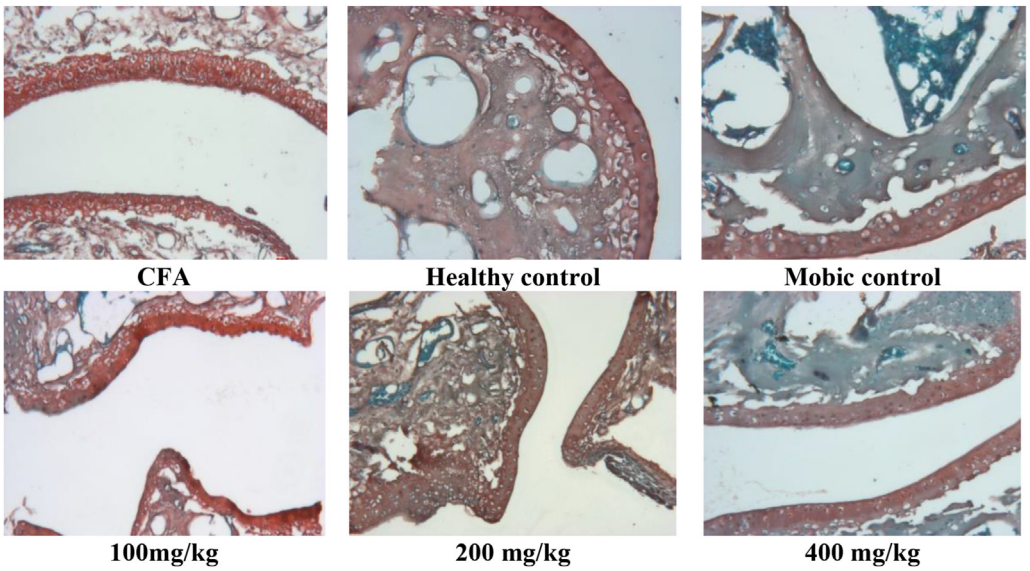

Figure 4. Safranin O staining results.

mononuclear cell infiltration into the joint capsule. The ankle joint was intact in both the healthy control and Mobic control samples, and the joint capsule was devoid of mononuclear cells. Histological investigation of the batch utilizing AECF1 revealed a variation in the anti-inflammatory activity of the three dosages. The joint surface was less injured in mice treated with AECF at three dosages, although mononuclear cells occurred at a higher density in the joint capsule of mice treated at 400 mg/kg oral dosage than in the others. This conclusion was consistent with the results of measuring the joint diameter of mice at three different concentrations.

3.8. Cartilage preservation and regeneration capacities

The Safranin O staining of the control mice on the 28th day (Figure 4) revealed that the articular cartilage was thick and undamaged, with a high proteoglycan content. The CFA-induced arthritis mice showed the thinner articular cartilage and low proteoglycan content. Meanwhile, the articular cartilage in the positive control mice was fairly well protected, illustrated by a small portion of the articular cartilage surface with a lighter red color. Besides, the loss of proteoglycan in positive control mice was attenuated during the red-orange process, leading to an improved proteoglycan content. In terms of AECF1 treated mice, the results indicated that the proteoglycan content was improved and the articular cartilage appeared to be protected (light red and thin articular cartilage compared to the positive control). However, this protective action was considerably less effective than the Mobic control group. The AECF1 therapy at a higher dosage could possibly promote more protective effect on articular cartilage than those at low dosages.

4. Conclusion

This study demonstrated a high content of antioxidants (polyphenols and flavonoids) associated with high antioxidant activities of Cao Khai – an aqueous extract from *Coptosapelta flavescens* Korth and successfully assessed its anti-inflammatory properties. The high content of antioxidants in Cao Khai suggested its potential as a strong antioxidant material and its ability to neutralize and reduce many oxidizing compounds. *In vivo* results also revealed that a dosage of 400 mg/kg body weight of AECF1 was found to exhibit the most effective anti-inflammatory activity and alleviate inflammatory symptoms. Furthermore, histological results revealed that the administration of the AECF1 product was observed to attenuate cartilage tissue damage in treated mice during arthritis after 14 days. Current results justify the folkloric usage of Cao Khai and establish the basis for further research on bioactivities of the "Cao Khai" traditional ingredient in Vietnam.

Declarations

Author contribution statement

Tri Nhut Pham: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tien Dung Le: Conceived and designed the experiments; Performed the experiments.

Long Giang Bach: Conceived and designed the experiments.

Thi Bach Tuyet Nguyen and Thi Phuong Lien Nguyen: Performed the experiments; Analyzed and interpreted the data.

Xuan Tuyen Nguyen and Trong Doan Phan: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

Agrawal, J., Pal, A., 2013. Nyctanthes arbor-tristis Linn—a critical ethnopharmacological review. J. Ethnopharmacol. 146, 645–658.

Arnida, A., Sutomo, S., Rosyidah, L., 2019. Aktivitas penghambatan polimerisasi hem Dari fraksi etil asetat daun manuran, Coptosapelta tomentosa Valeton ex K.Heyne (Rubiaceae). IJPF 6, 309–314.

Arnida, Ningsih, Rini Noor Mala, Sumoto, 2017. Heme Polymeritation Inhibition Activity of Ethanol Extract of Manuran (Coptosapelta Tomentosa Valeton Ex K.Heyne) Leaf from Kotabaru South Kalimantan. MIPS 3 Unhas Makassar ID: 951678864.

Babushetty, V., Sultanpur, M.C., 2012. Evaluation of anti-arthritis activity of Asystasia dalzelliana leaves. Int. J. Pharmaceut. Biol. Archive 3, 377–382.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239, 70–76.

Bihani, G.V., Rojatkar, S.R., Bodhankar, S.L., 2014. Anti-arthritic activity of methanol extract of Cyathocline purpurea (whole plant) in Freund's complete adjuvant-induced arthritis in rats. Biomed. Aging Pathol. 4, 197–206.

Birgitta, Bremer, Torsten, Eriksson, 2009. Three time of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. Int. Plant Sci. 170 (6), 766–793.

Bodkhe, R., Balakrishnan, B., Taneja, V., 2019. The role of microbiome in rheumatoid arthritis treatment. Therapeut. Adv. Musculoskelet. 11, 1759720X1984463.

Cardeñosa, R., Mohamed, R., Pineda, M., Aguilar, M., 2002. On-line HPLC detection of tocopherols and other anti-oxidants through the formation of a phosphomolybdenum complex. J. Agric. Food Chem. 50, 3390–3395.

Chitme, H.R., Patel, N.P., 2009. Antiarthritis activity of aristolochia bracteata extract in experimental animals. TONPJ 2, 6–15.

Choudhary, M., Kumar, V., Malhotra, H., Singh, S., 2015. Medicinal plants with potential anti-arthritic activity. J. Intercult. Ethnopharmacol. 4, 147.

Garg, U., Azim, Y., 2021. Challenges and opportunities of pharmaceutical cocrystals: a focused review on non-steroidal anti-inflammatory drugs. RSC Med. Chem. 12, 705–721.

Hossain, M.A., Rahman, S.M.M., 2011. Total phenolics, flavonoids and anti-oxidant activity of tropical fruit pineapple. Food Res. Int. 44, 672–676.

Hounkong, K., Sawangjaroen, N., Kongyen, W., Rukachaisirikul, V., Wootipoom, N., 2015. Mechanisms of 1-hydroxy-2-hydroxymethylanthraquinone from *Coptosapelta flavescens* as an anti-giardial activity. Acta Trop. 146, 11–16.

Hounkong, K., Sawangjaroen, N., Kongyen, W., Rukachaisirikul, V., Voravuthikunchai, S.P., Phongpaichit, S., 2014. Anti-intestinal protozoan activities of 1-hydroxy-2-hydroxymethylanthraquinone from *Coptosapelta flavescens*. Asian Pac. J. Trop. Dis. 4, 457–462.

Huyut, Z., Beydemir, Ş., Gülçin, İ., 2017. Anti-oxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochem. Res. Int. 2017, 1–10.

Institute of Medicine, 2003. Medicinal Plants and Medicinal Animals in Vietnam,, vol. I. Science and Technology Publishing House, pp. 645–646.

Jadhav, R.B., Patil, C.R., Surana, S.J., Bhatnagar, S.P., Patil, M.J., 2007. Rheumatoid arthritis and herbal drugs: current status and future prospects. In: Govil, J.N., Singh, V.K. (Eds.), Phytopharmacology and Therapeutic Values II, vol. 20. Studium Press LLC, Houston, TX, U.S.A., pp. 277–300

Jalalpure, S.S., Mandavkar, Y.D., Khalure, P.R., Shinde, G.S., Shelar, P.A., Shah, A.S., 2011. Anti-arthritic activity of various extracts of Mesua ferrea Linn. seed. J. Ethnopharmacol. 138 (3), 700–704.

Jao, C.H., Ko, W.C., 2002. 1, 1- Dipheny1-2 picryl hydrazyl (DPPH) radical scavenging by protein hydrolysates from tuna cooking juice. Fish. Sci. 68, 430–435.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.-P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Anti-oxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47, 3954–3962.

Kanatt, S.R., Chander, R., Sharma, A., 2007. Anti-oxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. Food Chem. 100, 451–458.

Kaviarasan, S., Naik, G.H., Gangabhagirathi, R., Anuradha, C.V., Priyadarsini, K.I., 2007. In vitro studies on antiradical and anti-oxidant activities of fenugreek (Trigonella foenum graecum) seeds. Food Chem. 103, 31–37.

Khanh, T.C., 2009. Herbal medicine named "Day Khai". Med. Health 383, 15.

Kongyen, W., Rukachaisirikul, V., Phongpaichit, S., Sawangjaroen, N., Songsing, P., Madardam, H., 2014. Anthraquinone and naphthoquinone derivatives from the roots of *Coptosapelta flavescens*. Nat. Product Commun. 9, 1934578X1400900.

Kosala, K., Ismail, S., Fikriah, I., Magdalena, A.R., 2017. In vitro exploration of vasodilation activity of the methanol extract of the *Coptosapelta flavescens* korth stem. JIMR-J. Islamic Med. Res. 1, 10–14.

Kosala, K., Sjarif, I., Ika, F., Endang, S., 2019. Anti-inflammatory activity study and secondary metabolites detection in *Coptosapelta Flavescens* korth root's water extract. EurAsia J. BioSci. 13, 2317–2320.

Kosala, K., Widodo, M.A., Santoso, S., Karyono, S., 2018. In vitro and in vivo anti-inflammatory activities of *Coptosapelta flavescens* korth root's methanol extract. J. Appl. Pharmaceut. Sci. 8, 42–48.

Kumar, V., Bhat, Z.A., Kumar, D., Bohra, P., Sheela, S., 2011. In-Vitro anti-inflammatory activity of leaf extracts of Basella Alba Linn. Int. J. Drug Dev. Res. 3, 176–179.

Kumar, V., Roy, S., Sehgal, R., Padhy, B.A., 2006. Comparative study on the efficacy of rofecoxib in monoarticular arthritis induced by latex of Calotropis procera and Freund's complete adjuvant. Inflammopharmacology 14, 17–21.

Kuraoka-Oliveira, Â.M., Radai, J.A.S., Leitão, M.M., Lima Cardoso, C.A., Silva-Filho, S.E., Leite Kassuya, C.A., 2020. Anti-inflammatory and anti-arthritic activity in extract from the leaves of Eriobotrya japonica. J. Ethnopharmacol. 249, 112418.

Line, K.W., 2005. Ethnobotanical study of medicinal plants used by the Jah Hut peoples in Malaysia Indian. J. Med. Sci. 59, 156–161.

Longo, D.L., Fauci, A.S., Kasper, D.L., Hauser, S.L., Jameson, J.L., Loscalzo, J., Harrison, T.R., 2012. Harrison's Principles of Internal Medicine. McGraw-Hill, New York.

Minh, V.V., Yen, N.T.K., Thoa, P.T.K., 2014. Medicinal plants used by the hre community in the Ba to district of central Vietnam. J. Med. Plants Stud. 2, 64–71.

Nenadis, N., Wang, L.-F., Tsimidou, M., Zhang, H.-Y., 2004. Estimation of scavenging activity of phenolic compounds using the ABTS •+ assay. J. Agric. Food Chem. 52, 4669–4674.

Ng, Z.X., Koick, Y.T.T., Yong, P.H., 2020. Comparative analyses on radical scavenging and cytotoxic activity of phenolic and flavonoid content from selected medicinal plants. Nat. Prod. Res. 1–6.

Nguyen, N.Q., Pham, T.N., Nguyen, V.T., Nguyen, M.T., Dung, L.T., Bach, L.G., 2020. Phytochemical screening and anti-oxidant potential of crude drug "Cao Khai" in Ninh Thuan province, Vietnam. IOP Conf. Ser. Mater. Sci. Eng. 991, 012016.

Nielen, M.M.J., 2006. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. Ann. Rheum. Dis. 65, 535–537.

Nursanti, Novriyanti, Dan Cory, Wulan, 2018. Various types of potential drug plants in Muhammad Sabki Urban forest Jambi city. Media Konservasi 23 (2), 169–177.

Oyaizu, M., 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet. 44, 307–315.

Pandey, A., Bani, S., Satti, N.K., Gupta, B.D., Suri, K.A., 2012. Anti-arthritic activity of agnuside mediated through the down-regulation of inflammatory mediators and cytokines. Inflamm. Res. 61, 293–304.

Paramita, S., Kosala, K., Dzulkifli, D., Saputri, D.I., Wijayanti, E., 2017. Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia. Biodiversitas 18, 1556–1561.

Pham, T.N., Nguyen, V.T., Toan, T.Q., Cang, M.H., Bach, L.G., Van Muoi, N., 2020. Effects of various processing parameters on polyphenols, flavonoids, and anti-oxidant activities of Codonopsis javanica root extract. Nat. Product Commun. 15, 1934578X2095327.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of anti-oxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.

Sarkaki, A., Farbood, Y., Gharib-Naseri, M.K., Badavi, M., Mansouri, M.T., Haghparast, A., Mirshekar, M.A., 2015. Gallic acid improved behavior, brain electrophysiology, and inflammation in a rat model of traumatic brain injury. Can. J. Physiol. Pharmacol. 93, 687–694.

Sharma, O.P., Bhat, T.K., 2009. DPPH anti-oxidant assay revisited. Food Chem. 113, 1202–1205.

Singh, B., Bani, S., Gupta, D.K., Chandan, B.K., Kaul, A., 2003. Anti-inflammatory activity of 'TAF' an active fraction from the plant Barleria prionitis Linn. J. Ethnopharmacol. 85, 187–193.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acidreagent. Am. J. Enol. Vitic. 16, 144–158.

Srinivasa Rao, K., Chaudhury, P.K., Pradhan, A., 2010. Evaluation of anti-oxidant activities and total phenolic content of Chromolaena odorata. Food Chem. Toxicol. 48, 729–732.

Su, Y.-D., Cheng, C.-H., Wen, Z.-H., Wu, Y.-C., Sung, P.-J., 2016. New anti-inflammatory sterols from a gorgonian Pinnigorgia sp. Bioorg. Med. Chem. Lett 26, 3060–3063.

Tastekin, N., Aydogdu, N., Dokmeci, D., Usta, U., Birtane, M., Erbas, H., Ture, M., 2007. Protective effects of l-carnitine and alpha-lipoic acid in rats with adjuvant arthritis. Pharmacol. Res. 56, 303–310.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Exp. Biol. Med. 111, 544–547.

Yokota, J., Takuma, D., Hamada, A., Onogawa, M., Yoshioka, S., Kusunose, M., Miyamura, M., Kyotani, S., Nishioka, Y., 2006. Scavenging of reactive oxygen species by Eriobotrya japonica seed extract. Biol. Pharm. Bull. 29, 467–471.

Yoon, J.-H., Baek, S.J., 2005. Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med. J. 46, 585.

Zhang, R.-X., Fan, A.Y., Zhou, A.-N., Moudgil, K.D., Ma, Z.-Z., Lee, D.Y.-W., Fong, H.H.S., Berman, B.M., Lao, L., 2009. Extract of the Chinese herbal formula Huo Luo Xiao Ling Dan inhibited adjuvant arthritis in rats. J. Ethnopharmacol. 121, 366–371.