JURNAL FARMASI SAINS DAN KOMUNITAS
Journal of Pharmaceutical Sciences & Community

p-ISSN 1693-5683; e-ISSN 2527-7146

Vol. 19, No. 2, November 2022, pp. 93-102

Research Article

Identification Bioactive Compound of Ethanol-Water Fraction of *Coleus atropurpureus* for Anti-rheumatic Rheumatism in CFA-induced Rats

Ipang Djunarko[1,4], Nanang Fakhrudin[2*], Arief Nurrochmad[3], Subagus Wahyuono[2]

[1] Postgraduate Program of Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 55281, Indonesia
[2] Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 55281, Indonesia
[3] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 55281, Indonesia
[4] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Sanata Dharma University Yogyakarta Campus III, 55282, Indonesia.

https://doi.org/10.24071/jpsc.004746

J. Pharm. Sci. Community, 2022, 19(2), 93-102

Article Info

Received: 09-06-2022
Revised: 29-06-2022
Accepted: 30-06-2022

***Corresponding author:**
Nanang Fakhrudin
email: nanangf@ugm.ac.id

Keywords:
Anti-rheumatic arthritis; *Coleus atropurpureus*; Complete Freund's Adjuvant

ABSTRACT

Nonsteroidal anti-inflammatory drugs are used for pain and to slow the progression of rheumatoid arthritis. Accordingly, the discovery of rheumatoid arthritis active compounds from the ethanol-water fraction *Coleus atropurpureus* (EWC) was conducted to characterize the isolated compounds as well as the anti-rheumatic effects of the EWC induced Complete Freund's Adjuvant (CFA). We conducted in vivo study in rats which were randomly divided into 5 groups. Group 1 was only given CFA as a negative control. Group 2 as positive control was orally exposed to diclofenac potassium (9 mg/BW). Three groups were given different EWCs orally as follows: 11.25 mg/BW, 22.5 mg/BW, and 45 mg/BW, respectively. Rheumatism rates were then compared with positive controls using a visual arthritic scoring system. The compounds identified by isolation of the EWC of *Coleus atropurpureus* predicted forskolin. The ethanol-water fraction *Coleus atropurpureus* did not act as an anti-rheumatic arthritis agent in CFA-induced rats.

INTRODUCTION

One of the drugs of choice for rheumatoid arthritis are non-steroidal anti-inflammatory drugs (NSAIDs), which can relieve inflammation and reduce pain by slowing the progression of the disease. Another group called Disease Modifying Anti-Rheumatic Drugs (DMARDs) acts through immunological processes to decrease hyperactive inflammation. Although the combination between the two groups could be more effective, however, they are rarely prescribed together due to the higher adverse side effects (Hall *et al*., 2017). Hence, due to the chronic arthritic nature, elderly patients tend to seek other alternative treatments. As one of the Indonesian herbal medicines, *Coleus atropurpureus* (*C. atropurpureus*) is known to have various health benefits including for inflammation disorders. However, strong scientific evidence is needed to confirm its anti-inflammatory activity. Flavonoids, saponins, polyphenols, and terpenoids were previously reported to be present in its leaves (Fakhrudin *et al*., 2020). The distinct content of phytochemicals in the fractions (ethanol extract, n-hexane, and ethanol-water) was detected by using thin layer chromatography (TLC) analysis. The n-hexane fraction mostly contained terpenoids, while the ethanol-water fraction was dominated by flavonoids (Djunarko *et al*., 2022). This study also

demonstrated that there are anti-inflammatory activities in the carrageenan model from all of the fractions. Previous research confirmed that eugenol and thymol as terpenoid compounds from *C. atropurpureus* exhibited analgesic, anti-irritant, antiparasitic, and antiseptic activities (Lenny *et al*., 2013). Isoflavones and flavones as flavonoids with substituents at C5, C7, and C4' were previously identified to be present in the leaves of *C. atropurpureus* (Verawati *et al*., 2016). Another study has shown that *C. atropurpureus* contains flavonoids and phenolics. These findings indicated the potential use of *C. atropurpureus* in anti-inflammatory study (Fakhrudin *et al*., 2016; Iqbal and Singh, 2019). Phenolics, flavonoids, and terpenoids are reported to be able to reactive oxygen species (ROS) after carrageenan induction (Heldin *et al*., 2016). Carrageenan generates ROS that induces initial tissue injury and tissue defects leading to inflammation (Raker *et al*., 2016).

In this present study, we conducted a deeper study to find a chemical entity from the ethanol-water fraction of *C. atropurpureus* that could be responsible for the potential activity as natural anti-inflammatory drug by *in vivo* evaluation using the Complete Freund's Adjuvant (CFA)-induced arthritis. The isolated compound was identified using spectroscopic methods including FTIR, ^{1}H-NMR, and ^{13}C-NMR.

METHODS

Material and Chemicals

Fresh leaves of *C. atropurpureus* were provided by the herbal garden of Sanata Dharma University (Yogyakarta, Indonesia). The plant was identified by the Department of Biology at the Faculty of Pharmacy, Sanata Dharma University with voucher specimen 481/LKTO/far-USD/05/13. The chemicals used for the isolation stage were n-hexane, ethanol, ethyl acetate (Merck, Germany), Cerium sulphate (Sigma-Aldrich, Germany), and silica gel F254 (Merck, Germany). During the *in vivo* study, the following material were used: carrageenan (Sigma-Aldrich, Germany) and potassium diclofenac (Novartis, Indonesia).

Animal and Ethics

Wistar rats (male, 150-200 g) were obtained from the Imono Laboratory, Sanata Dharma University, Indonesia. The rats were housed in standard cages at 22± 3°C with 30-70% in relative humidity and in a 12 h dark-light cycle. Standard pellet diet and water were given ad libitum. The use of the animals was approved (number: KE/FK/0209/EC/2020) by the Medical and Health Research and Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada–Dr. Sardjito Public Hospital, Indonesia. All animals were acclimatized for two weeks prior to the *in vivo* study.

Extraction and Fractionation

The fresh leaves were rinsed off and then dried in the oven (50°C, 48 h). Dried leaves of *C. atropurpureus* (264 g) were macerated with ethanol (1:10). The macerate was filtered using vacuum filtration and was evaporated using a rotary evaporator (45^{o}C) to give 32.36 g of a crude extract. The mixture 1:2:1 of ethanol, water and n-hexane was added to the crude extract (15 g) and shaken intensively. Two separated layers were obtained; the upper layer was n-hexane fraction (8.84 g), and the lower fraction was ethanol-water fraction (5.88 g).

A 2.36 g of the ethanolic extract was fractionated using separating funnels in ethanol-water-n-hexane (1:2:1). The ethanol-water fraction was then dissolved in ethyl acetate followed by centrifuging them at 3,000 rpm for 10 min. The solubilized phase was then collected and dried up using a porcelain dish and prepared for a preparative-TLC. The sample was dissolved in chloroform-methanol (1:1) and then spotted on the silica F254 glass plate. The plate was developed under ethyl acetate 100% as the mobile phase and the spot was detected under ultraviolet (UV)254 and UV365, and further identified using cerium-sulphuric acid. The desired bands were then scratched and collected, followed by dissolving them in methanol. The isolate was then concentrated to have a mass of 11.6 mg and characterized using FTIR (Thermo Scientific Nicolet iS10) and -NMR (JEOL 1703). The deuterated solvent used in the NMR was $CDCl_3$.

In vivo studies of anti-arthritic rheumatoid activity

We divided twenty-five rats into five groups randomly. Three groups were orally administered different ethanol-water fractions of *C. atropurpureus* leaves (EWC) as follows: Group 1 (11.25 mg/BW); Group 2 (22.5 mg/BW); and Group 3 (45 mg/BW) (Djunarko *et al*., 2022). Furthermore, Group 4 was orally given diclofenac potassium (9 mg/BW) as the positive control, whereas Group 5 had no treatment as the negative control. Arthritis was induced through CFA injection according to Kumar *et al*. (2006). Briefly, the paw of the right hind limb of each rat was given 100 µL of CFA containing heat-killed

and dried *Mycobacterium tuberculosis* (strain H37Ra, ATCC25177) on day 0. Within those times, we measured the body weight using a digital balance every three days, sequentially. The arthritic level was then compared with the positive control using a visually arthritic scoring system.

The poor arthritic status was measured using a digital caliper showing the paw edema associating with primary and secondary lesions. The lesion was measured on the days 0, 1, 3, 6, 9, 12, 14, 16, 19, 21, 24, 26, and 28 after CFA injection (Bani *et al*., 2007; Singh *et al*., 2003). The edema thickness, area under curve (AUC), and percentage of inflammatory inhibition were calculated according to the previous study (Djunarko *et al*., 2022).

Table 1. The overview of the isolated compounds ^{1}H-NMR result (experimental) with the software prediction for forskolin structure

Proton Type	Prediction		Experimetal	
	δ (ppm)	Splitting Pattern	δ (ppm)	Splitting Patern
CH_3	1.11	s	1.08	s
CH_3	1.11	s	1.13	s
CH_3	1.16	s	1.23	s
CH_3	1.31	s	1.31	overlapped
CH_3	1.41	s	1.41	overlapped
CH_2	1.24/1.49	d/t	1.26/1.38	d
CH_2	1.47/1.72	d/t	1.48/ 1.69	low intensities
CH	1.47	d	1.47	overlapped
OH	2.0	s (br)	2.0	overlapped (br)
CH_3	2.01	s	2.02	overlap
CH_2	2.45/2.70	s	2.28/2.32	overlap
CH	3.15	t	2.98	low intensity
CH	3.75	t	3.77	t
CH	4.30	d	4.29	d
CH_2	5.23/5.24	d	5.27/5.33	d (overlapped)
CH	5.89	t	5.36	t (overlapped)

Table 2. The overview of the isolated compounds ^{13}C-NMR result (experimental) with the software prediction for forskolin structure

Carbon Type	Predicted δ (ppm)	Experimental δ (ppm)
CH_3	9.0	low
CH_3	11.6	14.35
CH_3	21.0	22.92
CH_3	26.0	low
CH_3	26.2	low
CH_2	27.4	29.59
CC	27.5	29.92
CH_2	37.8	32.14
CC	43.0	low
CH	43.9	low
CH_2	51.2	low
COH	65.4	low
COH	70.1	low
CC	77.0	low
CH	79.3	low
CC	81.4	low
CC	82.,7	low
C=C	112.6	low
C=C	145.7	low
C=O	170.3	low
C=O	209.6	low

Table 3. Primary and secondary arthritic lesions at 28st day in CFA-induced arthritis in rats (n=5)

Treatment	AUC_{0-28} day (mm^2.h)	% Induction of arthritis in the CFA
CFA control	625.3 ± 56.8	0.00
Diclofenac	561.0 ± 50.8*	10.28
EWC (11.25 mg/BW)	793.7 ± 71.6**	-26.93
EWC (22.5 mg/BW)	830.6 ± 65.0**	-32.83
EWC (45 mg/BW)	789.2 ± 63.2**	-26.21

Data represented in mean ± SEM
∗*P* > .05, compared with control group.
∗∗*P* < .05, compared with control group.

Table 4. Body and organ weights at 28st day in CFA-induced arthritis in rats (n=5).

Treatment	Body weight (g)	Thymus weight (g)	Spleen weight (g)
CFA control	198.6 ± 14.9	0.48 ± 0.10	0,63 ± 0.06
Diclofenac	232.0 ± 10.3*	0.47 ± 0.04*	0.88 ± 0.11*
EWC (11.25 mg/BW)	211.6 ± 12.8*	0.49 ± 0.04*	1.01 ± 0.09*
EWC (22.5 mg/BW)	217.2 ± 8.4*	0.53 ± 0.03*	1.12 ± 0.18*
EWC (45 mg/BW)	206.0 ± 5.7*	0.48 ± 0.04*	0.98 ± 0.13*

Data represented in mean ± SEM
∗*P* > .05, compared with control group.

Table 5. Alterations in hematological parameters and CRP in CFA-induced arthritis in rats

Treatment	RBC ($\times 10^6/mm^3$)	WBC ($\times 10^6/mm^3$)	Hb (mg%)	CRP (mg/dL)
CFA control	7.6 ± 0.4	11.2 ± 4.3	12.4 ±0.6	<0,2
Diclofenac	8.6 ± 0.4*	18.6 ± 4.7*	13.9 ± 0.7*	<0,2*
EWC (11.25 mg/BW)	8.0 ± 0.6*	14.4 ± 2.5*	13.0 ± 1.1*	<0,2*
EWC (22.5 mg/BW)	5.8 ± 1.5*	10.2 ± 1.7*	11.6 ± 1.3*	<0,2*
EWC (45 mg/BW)	7.6 ± 0.4*	12.8 ± 2.1*	12.1 ± 1.0*	<0,2*

Data represented in mean ± SEM (n = 5). RBC: red blood cell, WBC: white blood cell, Hb: hemoglobin, CRP: C-reactive protein
∗*P* > .05, compared with control group

Table 6. Changes in various pain test scores in CFA-induced arthritis in rat.

Treatment	Flexion pain test score	Mobility score	Stance score
CFA control	2 (2, 2)	2 (2, 2)	2 (2, 2)
Diclofenac	2 (2, 2)*	2 (2, 2)*	2 (2, 2)*
EWC (11.25 mg/BW)	2 (1, 2)*	2 (2, 2)*	2 (2, 2)*
EWC (22.5 mg/BW)	1 (1, 2)*	2 (2, 2)*	2 (2, 2)*
EWC (45 mg/BW)	1 (1, 2)*	2 (2, 2)*	2 (2, 2)*

Data represented in median (minimum, maximum), n = 5.
∗*P* > .05, compared with control group
∗∗*P* < .05, compared with control group

The arthritis score for evaluation of the pain associated with the arthritis was verified by a blinded observer using the visual arthritis scoring systems (Kumar *et al*., 2006, Laird *et al*., 2001). The arthritis score ranged from 0 to 4; wherein 0 indicates the least but definite swelling, while 4 represents the maximum swelling. This scoring system involves observations of all four paws giving a separate score for each limb. Hematological parameters and serum c-reactive protein (CRP) level were evaluated through routine laboratory methods. The animals were euthanized at the end of our study. We weighed the thymus and spleen of all the animals (Cui *et al*., 2019).

Statistical Analysis

The results are presented as the mean ± standard error of the mean (SEM) or median (minimum, maximum) for univariate analysis. Statistical differences between the controls and the treatments were evaluated by one-way ANOVA followed by Dunnett's multiple comparisons test and by the Kruskal-Wallis test followed by Dunn's multiple comparison test for normal data and for scored data, respectively. Arthritic activity was analyzed using Kolmogorov-Smirnov test and Mann-Whitney ($p < 0.05$).

RESULTS AND DISCUSSION

The EWC was produced in 5.88 g of yield appeared as thick gel, with leafy odor, and light brown-green color. Further isolation was able to collect a single band from the TLC. We approached the structure of the isolated compound using FTIR revealing some representative vibrated bands at the fundamental region. As seen in Figure 1a, the FTIR spectrum of the isolated compound demonstrates an -OH bonded stretching vibration at 3,399.69 cm-1, Sp3 -CH stretching asymmetric vibration at 2,924.54 and 2854.33 cm-1, and -C=O stretching vibration at 1735.59 cm-1. Looking at this FTIR spectrum, it may lead to the compound's identity of *C. atropurpureus* itself, i.e., coleonol or also named as forskolin (Figure 1b). After comparing with the FTIR spectrum of forskolin (Figure 1c), we found a high similarity on both spectra. The forskolin FTIR spectrum also shows -OH bonded stretching, Sp3 -CH stretching asymmetric, and -C=O stretching vibrations at the closed region with the isolated compound FTIR spectrum. Further support that the isolated compound could be forskolin was performed by the high similarity of their fingerprint region.

Further approach to characterize the isolated compounds were done by NMR. The ^{1}H-NMR shows some similar patterns with the ^{1}H-NMR spectrum of forskolin predicted by ChemDraw Ultra 8.0 software. They are CH_3 protons showed at 1.08, 1.13, 1.23, 1.31, and 1.41 ppm. Further proton signals such as CH_2, CH, OH, and =CH can be overviewed in Table 1 describing the individual chemical shifts and the splitting patterns. Unfortunately, some proton signals show very weak intensities due to the small amount of the sample (Figure 2). The ^{13}C-NMR spectrum also shows some similarity with its software's prediction. The carbon signals at 14.35 and 22.92 ppm were indicated as CH_3 carbons. Furthermore, the carbon signals at 29.59, 29.92, and 32.14 ppm were indicated as CH_2, CC, and CH_2 carbons, respectively. Unfortunately, many carbon signals were not observed due to its less intensity in the electromagnetic irradiation performance (Figure 3). The predicted as well as the experimental ^{13}C-NMR of the forskolin can be overviewed in Table 2. Previously, it had been reported that forskolin signatures have been found in another species of coleus, *Coleus forskholii* (Bhowal and Mehta, 2017).

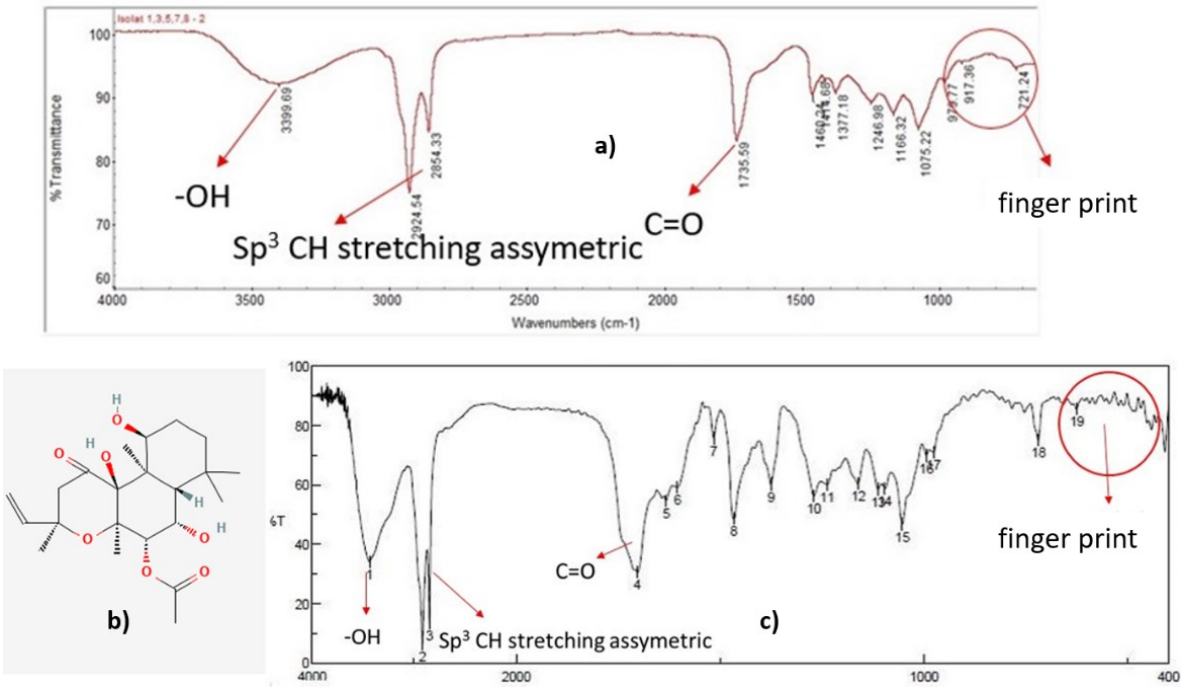

Figure 1. The presentation of a) FTIR spectrum of the isolated compound from *C. atropurpureus*, b) forskolin structure, and c) the FTIR spectrum of forskolin from Saritha *et al.* (2015).

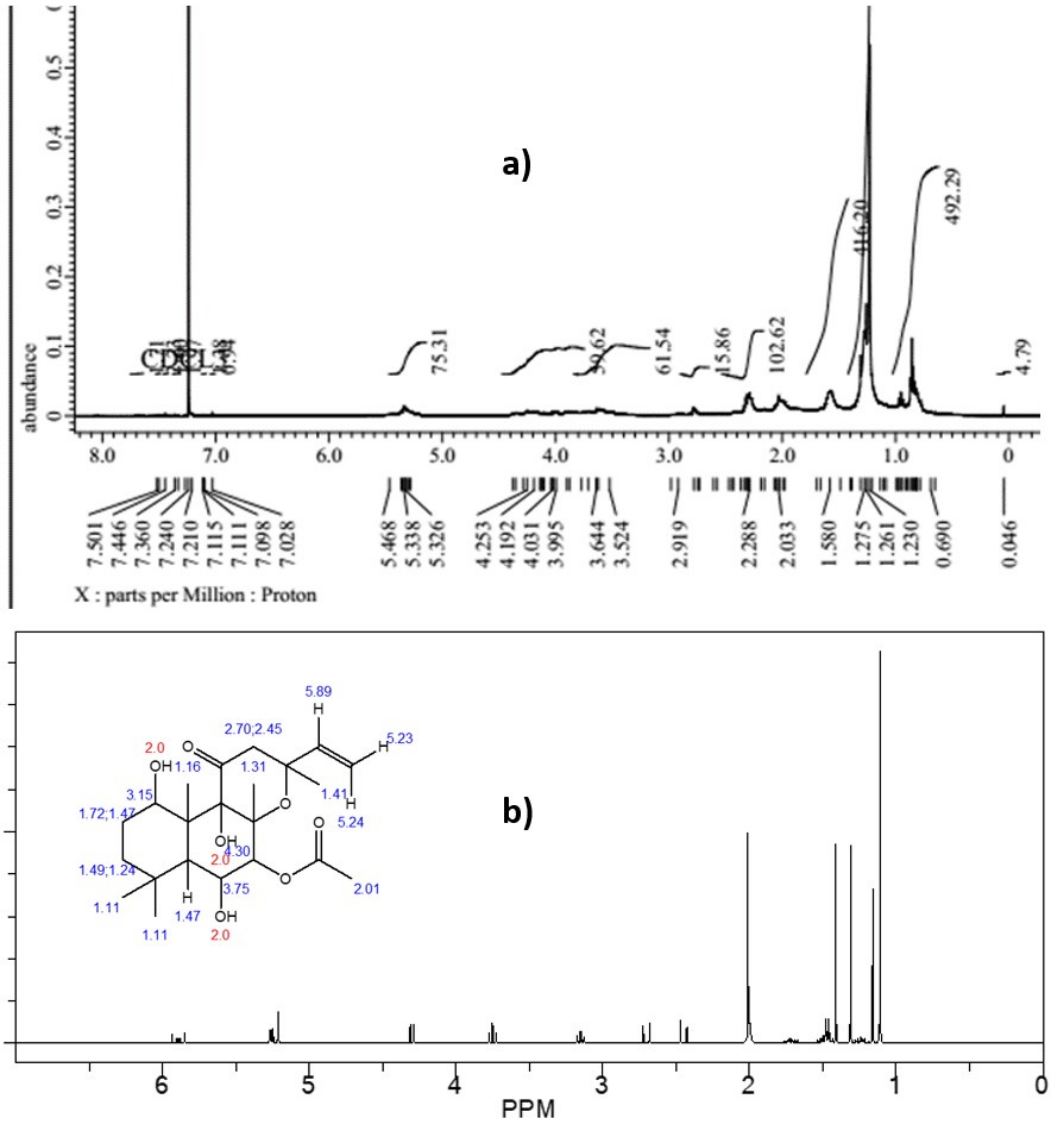

Figure 2. The ^{1}H-NMR spectra of a) the isolated compound from *C. atropurpureus*, and b) forskolin predicted by software.

Observations of CFA-induced foot edema in rats and arthritis scores were recorded at days 0, 1, 3, 6, 9, 12, 14, 16, 19, 21, 24, 26, and 28 after adjuvant injection. The control group showed arthritis progression through increasing claw thickness in CFA-injected areas, thus indicating arthritic lesions in primary or secondary responses. Weight loss and arthritis scores changing demonstrated arthritis induction in the CFA-treated control group. Assessments performed on days 0 to 28 indicated that the treatment group, either diclofenac or EWC treatment, could reduce adjuvant-induced primary and secondary lesions significantly compared to the CFA control. Notably, the secondary lesion reduction was not significantly different in the diclofenac and EWC-treated groups at either dose 11.25; 22.5; or 45 mg/BW (Table 3).

The CFA control group gained less weight than the group given EWC and diclofenac on day 28 (Table 4). However, the effect on body weight was statistically distinct and not significantly different between the treatment groups. Spleen and thymus weights at day 28 were not reduced significantly in the EWC-treated group.

The EWC treatment could improve CFA-induced hematological disorders (Table 5). However, the changes between the treatment groups were statistically different but not

significant. High serum CRP levels (<0.2 mg/dL) as a marker of systemic inflammation was observed in the CFA control groups. However, in all of the doses of EWC and diclofenac treatments were not able to reduce the increasing serum CRP levels. The effect of EWC was not statistically significant between the treatment groups.

The flexion pain test scores, mobility scores, and attitude scores which were pain score parameters, did not change significantly in the mice treated with diclofenac and EWC doses 11.25; 22.5; or 45 mg/BW. The results indicated that EWC was not able to reduce the pain associated with adjuvant-induced arthritis (Table 6).

Based on the spectrometric characterization, the isolated compound from ethanol-water fraction of *C. atropurpureus* leaves approaches forskolin. A study by Chiadak *et al*. (2016) reported that lipopolysaccharide-induced modulation of MCP-1 and GPR120 in 3T3-L1 adipocytes through an inhibition of NFκB were inhibited by forskolin. Another research by Karthika *et al*. (2016) further supported the capability of forskolin isolated from *Solena amplexicaulis* as an anti-inflammatory agent.

CFA-induced arthritis is a chronic animal model that is the most widely used in rheumatoid arthritis experimental models (Noh *et al*., 2021; Patel *et al*., 2021). The increasing volume of the injected leg indicates chronic inflammation in the CFA model. However, the inhibitory effect of EWC (11.25; 22.5; 45 mg/BW) on the injected leg volume was not significantly different from that of diclofenac 9 mg/BW.

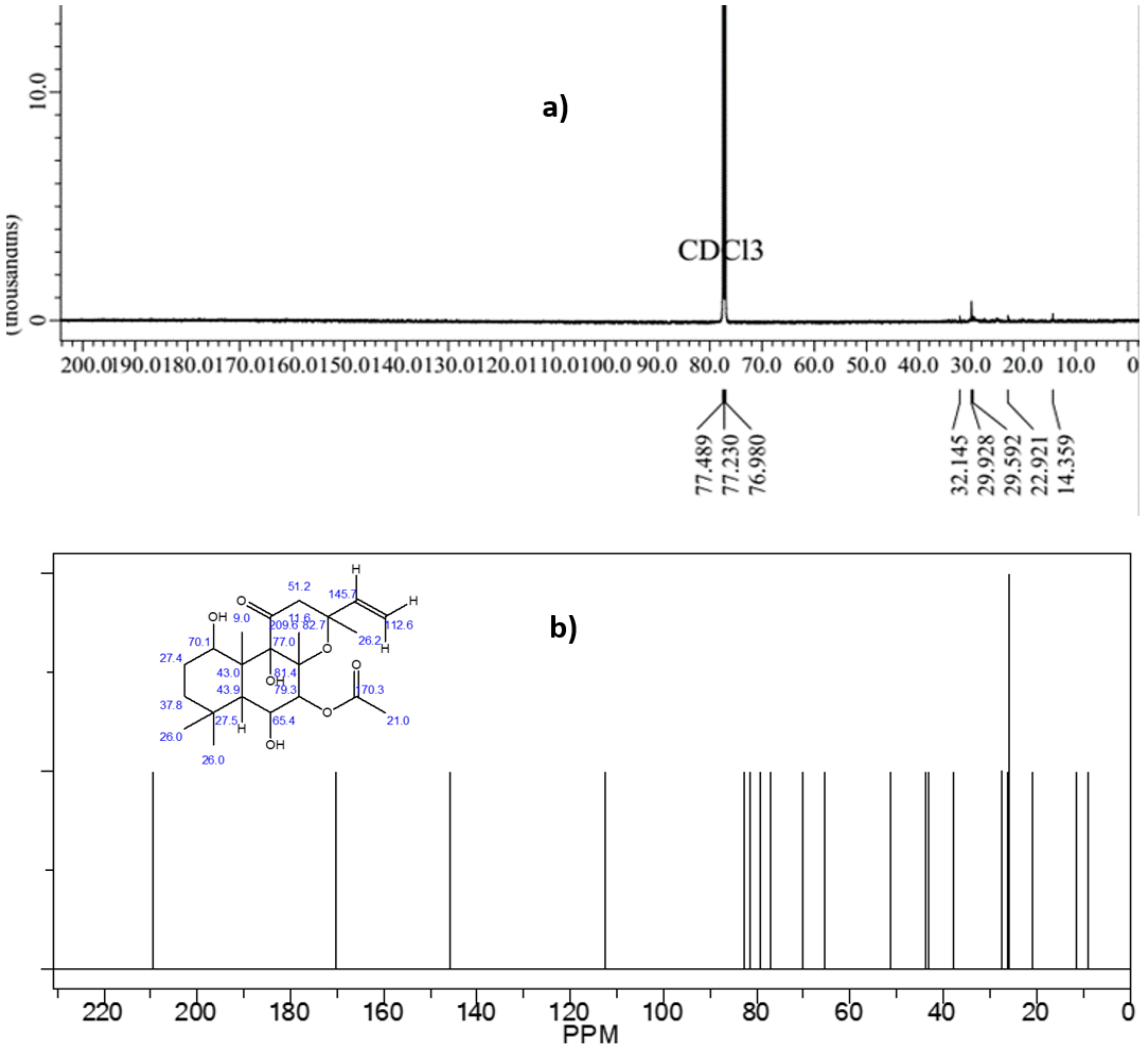

Figure 3. The ^{13}C-NMR spectra of a) the isolated compound from *C. atropurpureus*, and b) forskolin predicted by software.

An immune-mediated inflammatory reaction was related with CFA-induced arthritis (El-Tanbouly *et al*., 2022). In this model, edema at the depot site were initial reactions due to the irritant effect of the adjuvant, whereas late-phase arthritis and flares in the injected foot are considered immunological events (Biddle and Sofat, 2020; Yang *et al*., 2016). An un-injected leg swelling as secondary lesions is a manifestation of cell-mediated immunity. Its immunosuppressive activity was obtained by suppression of these secondary lesions (Bani *et al*., 2007; Singh *et al*., 2003). EWC was less effective at reducing secondary lesions than diclofenac. This revealed that EWC caused less intense suppression in cell-mediated immunity. Likewise, it decreases rheumatism scores and secondary leg swelling. The immunosuppressive effect of the anti-inflammatory drug was obtained by the selective reduction in arthritis scores (Choudhary *et al*., 2014; Faisal *et al*., 2018). The reduction in arthritis scores by EWC as observed in our study was less suggestive for its possible immunosuppressant activity. The insignificant reduction in thymus weight in the EWC-treated group further supports this observation.

CFA-induced arthritis in mice is correlated with an increase in plasma levels of CRP (He *et al*., 2022). The EWC did not significantly reduce the inflammation and autoimmune biomarkers in the EWC treatment groups. Another arthritis pathology that was obtained during this study included hematological parameters, changes in body weight, organ weight and percentage of inhibition of rat paw edema. The results showed that treatment with EWC and diclofenac avoided joint changes associated with arthritis which was indicated by a decrease in rat foot edema although the results were not statistically significant and EWC was concluded to have no potential as an anti-rheumatoid arthritis agent. The diclofenac and EWC treatment groups had a recovery in body weight of rats along with observations until the 28th day. Weight loss during inflammation is caused by a lack of nutrients absorption through the intestines and interestingly, the treatment using anti-inflammatory drugs normalizes this absorption disturbance (Maseda and Ricciotti, 2020). The results show that the occurrence of this recovery was statistically different, however, they were not significantly different from the CFA arthritis induction treatment group.

The EWC and diclofenac-treated groups led to a recovery in rat's body weight which may involve the increased intestinal nutrient absorption and followed by a reduction in the suffering caused by the arthritis. In arthritic conditions, the moderate increase in white blood cells counts was observed, due to IL-1B-mediated increases in each colony-stimulating factor. This study revealed that EWC and diclofenac treatments tended to normalize white blood cells counts. Other hematological changes such as a decreasing hemoglobin count and an increasing erythrocyte sedimentation rate (Singh *et al*., 2003) were also reversed by EWC and diclofenac treatments. It is proposed that the decrease in hemoglobin count during arthritis is caused by the decreased erythropoietin levels, bone marrow erythropoietin response, and the premature destruction of red blood cells.

The decreased spleen and the increased thymus weights are associated with the stimulation effect of the immune system (Chen *et al*., 2019). The decreasing in spleen and thymus weights are observed in EWC-treated mice suggesting changes in the cell population of these organs, which are associated with the immune function. Supposedly, diclofenac resulted in a reduction of the spleen and thymus weights, which could be attributed to its anti-proliferative action. However, it turned out that neither diclofenac nor EWC could show a similar effect on these organs. This finding shows that there is no immunosuppressant effect of both diclofenac and EWC, therefore evidence is needed to confirm the its immunosuppressant activity.

Arthritis is an inflammatory joint condition associated with hyperalgesia that is mediated by prostaglandins, other endogenous mediators and functional impairment (Zhang and Lee, 2018). Treatment of diseases such as arthritis is expected to overcome changes in some of the mediators and/or their effects to obtain clinical benefits. Evidence from this study proves that the plant-derived flavonoids EWC did not appear to exert valuable effects on various pathological manifestations of CFA-induced arthritis in rats. Therefore, this molecule may be less proven to have clinical value if further systematic investigation and development were conducted. On the other hand, the terpenoid content of the hexane fraction is also proven as an acute anti-inflammatory agent (Djunarko *et al*., 2022).

The activity of arthritis in our study was evaluated by arthritic scores and the dorsal flexion pain test visually. EWC was not effective to improve pain threshold and reduce flexion pain test score. Moreover, the functional impairment in arthritis were determined by its

mobility and stance scores. EWC treatment decreased the mobility score and improved the stance score, thus indicating pain reduction. It is proposed that the effects of endogenous pain mediators were affected by EWC significantly, even though actual quantification of the mediators of pain was not performed in this study (Kumar *et al*., 2006). Nowadays, drug development has been less relevant in many chronic diseases because of involving multiple organ systems and interdependent etiological factors. Moreover, drug discovery is now changing from single-target to multiple-target approaches (Ramsay *et al*., 2018). Treatment of diseases such as arthritis is expected to overcome changes in some of the mediators and/or their effects in order to obtain clinical benefits. It is evident from this study, prediction of forskolin compounds isolated from EWC seems to have a beneficial effect on several pathological manifestations related to CFA-induced arthritis in rats. Accordingly, this molecule has not been able to prove clinical effects thus, it requires further investigation especially in chronic inflammatory and pain conditions.

CONCLUSIONS

The compound identified by the isolation of the ethanol-water soluble fraction *Coleus atropurpureus* using FTIR, ^{1}H-NMR, and ^{13}C-NMR predicted forskolin. The ethanol-water fraction of *C. atropurpureus* did not act as an anti-rheumatoid arthritis agent in rats induced by Complete Freund's Adjuvant.

CONFLICT OF INTEREST

The authors affirm no conflict of interest in this study.

REFERENCES

Bani, S., Kaul, A., Khan, B., Gupta, V. K., Satti, N. K., Suri, K. A., and Qazi, G. N. 2007. Anti-arthritic activity of a biopolymeric fraction from *Euphorbia tirucalli*. *Journal of Ethnopharmacology*, 110(1), 92–98.

Bhowal, M., and Mehta, D. M. 2017. *Coleus forskholii*: phytochemical and pharmacological profile. *International Journal of Pharmaceutical Sciences and Research*, 8(9), 3599–3618.

Biddle, K., and Sofat, N. 2020. Rheumatoid arthritis: other perspectives towards a better practice. in IntechOpen, London.

Chen, C., Su, X., and Hu, Z. 2019. Immune promotive effect of bioactive peptides may be mediated by regulating the expression of SOCS1/miR-155. *Experimental and Therapeutic Medicine*, 18(3), 1850–1862.

Chiadak, J. D., Arsenijevic, T., Verstrepen, K., Gregoire, F., Bolaky, N., Delforge, V., Flamand, V., Perret, J., and Delporte, C. 2016. Forskolin inhibits lipopolysaccharide-induced modulation of MCP-1 and GPR120 in 3T3-L1 adipocytes through an inhibition of NFκB. *Mediators of Inflammation*, 2016, 1431789.

Choudhary, M., Kumar, V., Gupta, P., and Singh, S. 2014. Investigation of antiarthritic potential of *Plumeria alba* L. leaves in acute and chronic models of arthritis. *BioMed Research International*, 2014, 474616.

Cui, X., Wang, R., Bian, P., Wu, Q., Seshadri, V. D. D., and Liu, L. 2019. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. *Artificial Cells, Nanomedicine, d Biotechnology*, 47(1), 3391–3398.

Djunarko, I., Fakhrudin, N., Nurrochmad, A., and Wahyuono, S. 2022. In vivo anti-inflammatory activity of *Coleus atropurpureus* leaves extract and fractions. *Tropical Journal of Natural Product Research*, 6(1), 40–43.

El-Tanbouly, G. S. and Abdelrahman, R. S. 2022. Novel anti-arthritic mechanisms of trans-cinnamaldehyde against complete Freund's adjuvant-induced arthritis in mice: involvement of NF-κB/TNF-α and IL-6/IL-23/ IL-17 pathways in the immuno-inflammatory responses. *Inflammopharmacology*, 1, 1–12.

Faisal, R., Ahmad, N., Fahad, Y. S., and Chiragh, S. 2018. Anti-arthritic effect of thymoquinone in comparison with methotrexate on pristane induced arthritis in female Sprague Dawley rats. *Journal of Ayub Medical College, Abbottabad : JAMC*, 30(1), 3–7.

Fakhrudin, N., Khairunnisa, S. Y., Azzahra, A., and Ajiningtyas, R. J. (2016). Study of Radical Scavenger Activity, Total Phenol and Flavonoid Contents of Artocarpus altilis Leaves Extracts. *International Journal of Pharmaceutical and Clinical Research*, 8(5), 352–356.

Fakhrudin, N., Pertiwi, K. K., Takubessi, M. I., Susiani, E. F., Nurrochmad, A., Widyarini, S., Sudarmanto, A., Nugroho, A. A., and Wahyuono, S. 2020. A Geranylated Chalcone with Antiplatelet Activity from The Leaves of Breadfruit (Artocarpus altilis). *Pharmacia*, 67(4), 173–180.

Hall, J. J., Bolina, M., Chatterley, T., and Jamali, F. 2017. Interaction Between Low-Dose Methotrexate and Nonsteroidal Anti-inflammatory Drugs, Penicillins, and Proton

Pump Inhibitors. *The Annals of Pharmacotherapy*, 51(2), 163–178.

He, Y., Zhou, M., Jian, Z., Fang, L., Huang, L., and Song, J. 2022. C-Reactive Protein Knockout Attenuates Temporomandibular Joint Inflammation in Rats. *Journal of Immunology Research*, 2022, 8613986.

Heldin, C. H., Lu, B., Evans, R., and Gutkind, J. S. 2016. Signals and Receptors. *Cold Spring Harbor Perspectives in Biology*, 8(4), a005900.

Iqbal, H., and Singh, D. 2019. Antioxidants: A Brief Review. *South Asian Res J Med Sci*, 1(2), 36–39.

Karthika, K., Jamuna, S., Abinaya, G., Venkatachalapathi, A., Thenmozhi, K., and Paulsamy, S. 2016. Evaluation of Anti-inflammatory and Antioxidant Properties of Crude Extract and Forskolin from Solena amplexicaulis Leaf. *Indian Journal of Pharmaceutical Sciences*, 78(3), 377–387.

Kumar, V. L., Roy, S., Sehgal, R., and Padhy, B. M. 2006. A Comparative Study on The Efficacy of Rofecoxib in Monoarticular Arthritis Induced by Latex of Calotropis procera and Freund's Complete Adjuvant. *Inflammopharmacology*, 14(1–2), 17–21.

Laird, J. M. A., Carter, A. J., Grauert, M., and Cervero, F. 2001. Analgesic Activity of A Novel Use-Dependent Sodium Channel Blocker, Crobenetine, in Mono-Arthritic Rats. *British Journal of Pharmacology*, 134(8), 1742.

Lenny, S., Barus, T., Marpaung, L., and Pandapotan Nasution, M. 2013. Structure Elucidation of Flavonoid Compound from The Leaves of Coleus Atropurpureus Benth Using 1d-and 2d-NMR Techniques (Elusidasi Struktur Sebatian Flavonoid Dari Daun Coleus Atropurpureus Benth Menggunakan Teknik 1D-Dan 2D-NMR). *The Malaysian Journal of Analytical Sciences*, 17, 255–261.

Maseda, D., and Ricciotti, E. 2020. NSAID–Gut Microbiota Interactions. *Frontiers in Pharmacology*, 11, 1153.

Noh, A. S. M., Chuan, T. D., Khir, N. A. M., Zin, A. A. M., Ghazali, A. K., Long, I., Ab Aziz, C. B., and Ismail, C. A. N. 2021. Effects of Different Doses of Complete Freund's Adjuvant on Nociceptive Behaviour and Inflammatory Parameters in Polyarthritic Rat Model Mimicking Rheumatoid Arthritis. *PLOS ONE*, 16(12), e0260423.

Patel, R., Kadri, S., Gohil, P., Deshpande, S., and Shah, G. 2021. Amelioration of Complete Freund's Adjuvant-induced Arthritis by Calotropis Procera Latex in Rats. *Future Journal of Pharmaceutical Sciences 2021,* 7(1), 1–11.

Raker, V. K., Becker, C., and Steinbrink, K. 2016. The cAMP Pathway as Therapeutic Target in Autoimmune and Inflammatory Diseases. *Frontiers in Immunology*, 7, 123.

Ramsay, R. R., Popovic-Nikolic, M. R., Nikolic, K., Uliassi, E., and Bolognesi, M. L. 2018. A Perspective on Multi-target Drug Discovery and Design for Complex Diseases. *Clinical and Translational Medicine*, 7(1), 3.

Singh, B., Bani, S., Gupta, D. K., Chandan, B. K., and Kaul, A. 2003. Anti-inflammatory Activity of "TAF" An Active Fraction from The Plant Barleria prionitis Linn. *Journal of Ethnopharmacology*, 85(2–3), 187–193.

Verawati, V., Aria, M., Dira, D., Maisa, S., and Maharani, A. 2016. Chemical Characterization and Anti-inflammatory Activity of Piladang Leaf (Coleus Atropurpureus) Extract. *Journal of Chemical and Pharmaceutical Sciences*, 9(4), 2496–2499.

Yang, J., Cai, H. Da, Zeng, Y. L., Chen, Z. H., Fang, M. H., Su, Y. P., Huang, H. H., Xu, Y., and Yu, C. X. 2016. Effects of Koumine on Adjuvant- and Collagen-Induced Arthritis in Rats. *Journal of Natural Products*, 79(10), 2635–2643.

Zhang, A., and Lee, Y. C. 2018. Mechanisms for Joint Pain in Rheumatoid Arthritis (RA): from Cytokines to Central Sensitization. *Current Osteoporosis Reports*, 16(5), 603-610.