Trop J Nat Prod Res, September 2025; 9(9): 4279 - 4284 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Antioxidant Activity of *Muntingia calabura* L. Leaf Extract and its Effect in Acute Gout Arthritis Rat Model: Reactive Oxygen Species Modulation

Nita Parisa[1]*, Lazahra Salsabiltha[2], Ayeshah A. Rosdah[1], Syarinta Adenina[1], Veny Larasati[3], Bintang A. Prananjaya[4] Theodorus[1]

[1]*Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, 30139, South Sumatra Province, Indonesia*
[2]*Undergraduate of Medical Doctor, Faculty of Medicine, Universitas Sriwijaya, Palembang, 30139, South Sumatra Province, Indonesia*
[3]*Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, 30139, South Sumatra Province, Indonesia*
[4]*Department of Psychiatry, Faculty of Medicine, Universitas Sriwijaya, Palembang, 30139, South Sumatra Province, Indonesia*

ARTICLE INFO

Article history:
Received 13 June 2025
Revised 06 July 2025
Accepted 30 July 2025
Published online 01 October 2025

ABSTRACT

Gout arthritis is an inflammatory joint condition caused by uric acid crystal accumulation. The inflammatory mechanism involves complex pathways that increase reactive oxygen species (ROS) levels. *Muntingia calabura L.* leaves contain bioactive compounds, such as phenolics, including flavonoids, which are known to reduce ROS production by mitigating pro-inflammatory signaling pathways. This study aimed to evaluate the phenolic content and effectiveness of *Muntingia calabura L.* leaves extract as an anti-inflammation and antioxidant agent in regulating ROS levels in Wistar rats with acute gout arthritis. Five groups of rats were used, which consisted of positive control, negative control, and treatment groups receiving the extract at doses of 50, 100, and 200 mg/kg BW. Folin-Ciocalteu assay revealed a high phenolic content of 26.12 ± 0.18%, with strong antioxidant activity (IC_{50} = 23.85 ± 0.16 µg/mL). In line with this, LC-MS-MS showed that flavonoids were the most predominant compounds in the extract. Meanwhile, the extract administration slightly reduced pain-induced monosodium urate (MSU) crystal injection. Interestingly, 50 mg/kg BW of the extract was identified as the most effective dose in reducing MSU-induced ROS production—even stronger than that of colchicine (p = 0.0049). This study highlighted the significant antioxidant activity of the extract due to its phenolic content, which markedly lowered ROS levels in the animal model.

Keywords: Antioxidant, Gout arthritis, *Muntingia calabura*, Phenolics, Reactive oxygen species

Introduction

Gout arthritis is an inflammatory condition caused by monosodium urate (MSU) crystal deposition due to hyperuricemia.[1] This disease has an incidence of 0.1–0.3%, and it is recorded to affect 1–4% of the global population. Its prevalence rises with age, which reaches 11–13% in individuals over 80 years. Men are more frequently affected, with a male-to-female ratio of 3:1 to 10:1.[2] Inflammation is initiated by macrophage phagocytosis of MSU crystals, activating NADPH oxidase and generating reactive oxygen species (ROS). ROS trigger the activation of the NLRP3 inflammasome, leading to IL-1β release, which amplifies inflammation through TNFα, IL-6, and chemokines, promoting neutrophil infiltration and joint damage.[1,3] Colchicine is a drug of choice for acute gout treatment. However, its use is limited by its gastrointestinal toxicity, hematologic disturbances, and nephrotoxicity. This drug also has a narrow therapeutic index.[4,5] Thus, safer and more effective alternatives for acute gout treatment are required. Cherry tea (*Muntingia calabura L.*) leaves contain phenolics, in which flavonoids are members of this group, and they exhibit anti-inflammatory and antioxidant properties.

*Corresponding author. Email: nitaparisa@unsri.ac.id
Tel: +62711373438

Citation: Parisa N, Salsabiltha L, Rosdah AA, Adenina S, Larasati V, Prananjaya BA, Theodorus. The antioxidant activity of *Muntingia calabura* L. leaf extract and its effect in acute gout arthritis rat model: Reactive oxygen species modulation. Trop J Nat Prod Res. 2025; 9(9): 4279 – 4284 https://doi.org/10.26538/tjnpr/v9i9.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

These compounds reduce ROS production, suppress inflammatory pathways, and mitigate oxidative stress, preventing excessive immune activation and tissue damage.[6–9] The compounds regulate ROS and inflammation through multiple mechanisms. They act as antioxidants, directly scavenging ROS and inhibiting ROS-producing enzymes like NADPH oxidase and COX. Phenolics and flavonoids suppress NF-κB and MAPK signaling, reducing pro-inflammatory cytokines and enzymes such as COX-2 and iNOS. They also enhance endogenous antioxidants like superoxide dismutase (SOD) and catalase, maintaining redox balance. These actions help control oxidative stress and inflammation, making them potential therapeutic agents for inflammatory diseases.[10,11]

Despite the established role of phenolics in inflammation, the potential of cherry tree leaf extract (CLE) in modulating ROS levels in acute gout arthritis remains unexplored. This study was aimed to evaluate the phenolic content and efficacy of CLE as an anti-inflammatory and antioxidant agent in regulating ROS levels in Wistar rats with acute gout arthritis. This study provides valuable insight into CLE's potential as an alternative therapeutic approach for acute gout arthritis.

Materials and Methods

Plant collection and identification

Cherry leaves were collected on June 19, 2024, in Prabumulih (-3°25'34.79"S, 104°14'4.80"E), South Sumatra, Indonesia. The leaves were botanically determined by Joko Santosa, a taxonomist at Lansida, and deposited (voucher ID: 16/P1-3/19062024/J) in Lansida, Yogyakarta, Indonesia (No. 2406/LHT/166). A total of 1.22 kg of fresh leaves were sorted and washed. The leaves were initially air-dried and moved into an oven with a temperature of 40°C for 24 hours until they were completely dried. The dried leaves were pulverized into powders using a blender and sieved through a no. 40 mesh, and weighed.[12]

Extract preparation

The powdered leaves were macerated in 70% ethanol, ensuring full

immersion in a sealed container. The mixture was stored in a dark environment for at least three days with occasional stirring to enhance solvent penetration and compound dissolution. After maceration, the extract was filtered to separate the leaf residue, and the solvent was removed using a rotary vacuum evaporator set at 50°C.[13] To calculate the amount of extract obtained relative to the powdered leaves used, the concentrated extract was weighed.

Total phenolic content determination

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method.[14] A calibration curve was generated using a series of gallic acid dilutions. The extract sample was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of distilled water, followed by incubation at room temperature for 10 minutes. Next, 1.5 mL of 20% Na_2CO_3 solution was added, and the mixture was heated for 20 minutes at 40°C in a water bath. The mixture's container was placed in an ice bath to stop the reaction, and the absorbance was recorded at 760 nm (Shimadzu, UV Spectrophotometer UV-1800, Japan). The TPC was determined using the standard calibration curve.

Evaluation of antioxidant activity using DPPH

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity of the extract.[15] A 0.4 mM DPPH solution in ethanol was prepared, and its absorbance at 516.5 nm was measured as the control. The extract was mixed with 1 ml of 0.4 mM DPPH solution, adjusted to a final volume of 5 ml, and incubated for 30 minutes in the dark. The absorbance was then recorded, and the percentage of DPPH inhibition was calculated. A standard curve of inhibition percentage vs. concentration was used to determine the IC_{50} value.

Phytochemical identification using liquid chromatography tandem-mass spectrometry (LC-MS-MS)

LC-MS-MS (Waters Xevo TQ-S Micro, USA) was utilized to screen for compounds contained in the CLE as adapted from an earlier report.[16] First, the sample was dissolved in methanol and kept for 24 hours at room temperature. The solution was filtered with a 0.22 µm membrane syringe filter, and 5 µL of the filtered sample was injected into the Acquity UPLC H-Class (Waters Corporation, USA) equipped with Acquity HSS T3 (100 x 2.1 mm, 1.8 µm; Waters Corporation, USA). The column temperature was set at 35 °C, and a mixture of 0.1 % formic acid in water (A) and acetonitrile (B) was used as the mobile phase. Mobile phases A and B with a ratio of 95:5 were used for the first 3 minutes of the elution time. Then, the ratio was changed to a 3:7 ratio for 12 minutes. In the last 3 minutes of the elution, the initial ratio was used again. The mobile phase was kept at a constant flow rate of 0.3 mL/min. The compartment was connected to a Xevo TQ-S Micro MS (Waters Corporation, USA) to determine the MS/MS transitions of the compounds. The compounds were identified by comparing their m/z and MS/MS transitions to those in the UNIFI Library software. The analysis was performed in the 100–800 m/z range and positive ionization mode. To calculate the relative composition of the identified compounds, the area under the peak was determined.

Animals and experimental design

Thirty male Wistar rats (*Rattus norvegicus*), weighing 200–300 g, were used and housed in the Animal House, Faculty of Medicine, Universitas Sriwijaya. Rats were acclimatized for 7 days under controlled conditions (humidity range of 40–70% and temperature range of 20–26°C) with ad libitum access to standard chow and water. Ethical clearance for animal protocols was granted by the Medical Research Ethics Committee, Faculty of Medicine, Sriwijaya University, Indonesia (ethical approval No. 349-2024), which aligns with all international guidelines and regulations.

Rats were randomly divided into negative control, colchicine, and three cherry leaf extract (CLE) treatment groups. Six rats were assigned to each group (Table 1). CLE was administered orally at 50, 100, or 200 mg/kg BW for 7 days based on previous studies indicating 100 mg/kg BW as the effective dose. The dose selection followed a 1/2n, n, 2n pattern (n = 100 mg/kg BW).[17] Colchicine (0.3 mg/kg BW) was given as a positive control for 7 days via oral gavage.[18] Gouty arthritis was induced using monosodium urate (MSU) crystals, prepared from dissolving uric acid in NaOH as previously reported.[19] One hour after the final treatment dose, 50 µL of 25 mg/mL MSU solution was injected intraarticularly into the right knee under anesthesia. After 72 hours, the rats were culled.

Table 1: Experimental design of the animal groups

Group	Treatment
Positive control	Rats were administered colchicine (0.3 mg/kg BW) orally for 7 days, followed by MSU injection intraarticularly.
Negative control	Rats were administered 0.5% of Na-CMC orally for 7 days, followed by MSU injection intraarticularly.
CLE50	Rats were administered **CLE at 50 mg/kg BW orally for 7 days**, followed by **MSU injection intraarticularly**.
CLE100	Rats were administered **CLE at 100 mg/kg BW orally for 7 days**, followed by **MSU injection intraarticularly**.
CLE200	Rats were administered **CLE at 200 mg/kg BW orally for 7 days**, followed by **MSU injection intraarticularly**.

Animal evaluation

Pain evaluation

Pain around the MSU injection site was assessed at 6, 24, 48, and 72 hours to monitor pain response. The pain scores were determined as follows: 0 (no response) if there was no reaction to pressure; 1 (mild response) if there was avoidance of strong pressure; 2 (moderate response) if there was avoidance of light pressure; and 3 (severe response) if there was even avoidance even before pressure was applied, including reacting to the researcher's approach.

Reactive oxidative stress measurement

After the animals were culled using an intraperitoneal injection of ketamine, knee joint tissue was isolated and homogenized. The homogenate was centrifuged at 5,000 rpm for 5 minutes. The supernatant was then taken and utilized for ROS measurement using ROS ELISA kit (ABclonal, Massachusetts, USA) as instructed by the manufacturers (FineTest, Wuhan, China). The ROS levels were measured at 450 nm.

Statistical analysis

Data was analyzed using GraphPad Prism version 8.0.1 and presented as mean ± standard deviation. Normality was assessed with the Shapiro-Wilk test, followed by a homogeneity test with Levene's test. One-way ANOVA with Tukey's post-hoc was used for homogenous and normally distributed data, while the Kruskal-Wallis test was applied if the data was not homogenous and not normally distributed. A p-value < 0.05 showed statistical significance.

Results and Discussion

Extract relative percentage, total phenolic content, and antioxidant activity

The extraction process produced a concentrated ethanolic extract of 16.16% (w/w) relative to the powdered leaves used. In this study, the TPC of the extract was 26.12 ± 0.18%, suggesting that the CLE had a high phenolic content (Table 2). Previous studies reported that the TPCs of cherry leaves extracted using 70% and 96% ethanol were in a range of 58.50–361.22 mg/g, where CLE extracted using 70% ethanol had higher TPC compared to CLE extracted using 96% ethanol.[21–23] Differences in the content and composition of secondary metabolites in plant extract are not only influenced by solvents but also by the geographical location of where the plant grows and the environmental conditions.[24,25]

Table 2: Total phenolic content and IC_{50} of cherry leaf extract

Sample	Total phenolic content (% w/w)	IC_{50} (µg/mL)
Chery leaves extract	26.12 ± 0.18	23.85 ± 0.16

Phenolic compounds are abundant, second only to carbohydrates, and exhibit a wide range of structures. The content of phenolic compounds is correlated to the anti-inflammatory and antioxidant of medicinal plants.[26,27] In line with this, the CLE showed high free radical scavenging potential with an IC_{50} value of 23.85 ± 0.16 µg/mL (Table 2). Similarly, previous studies revealed strong antioxidant activity of the extract via the superoxide anion, ferric-reducing antioxidant power, and DPPH assays.[23,28]

Phytochemical identification using liquid chromatography tandem-mass spectrometry (LC-MS-MS)

Further compound identification using LS-MS-MS showed that the majority of the predicted compounds belong to the flavonoids, except for methyl gallate and D-fructopyranose (Table 3). Since flavonoids are a subclass of phenolic compounds, it is not surprising that the TPC of CLE was high, which is likely attributed by the high flavonoid content. In line with this finding, several studies have also identified various flavonoids and phenolics in cherry leaves, including (2S)-5,7-dihydroxyflavanone (pinocembrin), gnaphaliin, 40 -hydroxy-7-methoxyflavanone, quercetin, 6,7-dimethoxy-5-hydroxyflavone, and methyl gallate.[29–31]

Table 3: Analysis result of liquid chromatography-tandem mass spectrometry (LC-MS-MS)

No	Retention time	MS molecular weight	Molecular weight	Molecular formula	Predicted Compound	% Composition	Classification
1	6.4	185.15	184,15	$C_8H_8O_5$	Methyl gallate	0.17	Phenolic
2	7.59	373.37	372.37	$C_20H_{20}O_7$	Isosinensetin	0.84	Flavone
3	7.81	333.35	332.35	$C_{18}H_{20}O_6$	(2s)-2-(3-hydroxy-4,5-dimethoxyphenyl)-7-methoxy-3,4-dihydro-2h-1-benzopyran-8-ol	1.44	Dihydroflavone
4	7.95	595.52	594.52	$C_{30}H_{26}O_{13}$	Tiliroside	0.28	Flavon
5	8.03	303.24	302.24	$C_{15}H_{10}O_7$	Quercetin	1.26	Flavonol
6	8.19	181.16	180.16	$C_6H_{12}O_6$	D-fructopyranose	1.09	Carbohydrate
7	8.43	317.31	316.35	$C_{17}H_{16}O_6$	3,5-dihydroxy-6,7-dimethoxy-2-phenyl-2,3-dihydro-1-benzopyran-4-one	5.18	Isoflavon
8	8.96	285.27	284.26	$C_{16}H_{12}O_5$	Izalpinin	2.47	Flavonol
9	9.66	301.28	300.26	$C_{16}H_{12}O_6$	Isokaempferide	7.78	Flavone
10	9.99	315.29	314.29	$C_{17}H_{14}O_6$	Kumatakenin	5.72	Flavone
11	10.18	241.27	240.25	$C_{15}H_{12}O_3$	7-hydroxyflavanone	1.19	Dihydroflavone
12	10.37	299.29	298.29	$C_{17}H_{14}O_5$	Galangin 3,7-dimethyl ether	10.42	Flavonol
13	10.86	315.29	314.29	$C_{17}H_{14}O_6$	Gnaphaliin	12.13	Flavonol
14	12.2	329.32	328.32	$C_{18}H_{16}O_6$	Alnustin	10.39	Flavone
15	12.88	269.28	268.26	$C_{16}H_{12}O_4$	Tectochrysin	9.75	Flavone
16	13.13	359.35	358.34	$C_{19}H_{18}O_7$	Flindulatin	3.37	Flavonol
17	14.34	299.30	298.29	$C_{17}H_{14}O_5$	Mosloflavone	26.52	Flavonol

Mosloflavone, gnaphaliin, and galangin 3,7-dimethyl ether are the most abundant compounds detected in the LC-MS-MS (Table 3). The anti-inflammation and antioxidant activities of mosloflavone have been documented earlier.[32] Mosloflavone can reduce iNOS, IL-1*β*, and TNF-α levels while its antioxidant activity is evident in human monocyte cell line.[33,34] Gnaphaliin, the second compound with the highest composition on CLE, demonstrates radical-scavengig activity on plasma oxidation and low-density lipoprotein (LDL).[35] Gnaphaliin exerts its anti-inflammatory activity by influencing the arachidonic acid metabolism and inhibiting leukocyte infiltration and phosphodiesterase.[36] Lastly, galangin improves antioxidant levels, reduces oxidative stress, and demonstrates its anti-inflammatory properties by modulating TNF-α, IL-6, IL-1*β*, PGE2, and COX-2.[37,38]

Reduction of pain and reactive oxidative stress levels following administration of cherry leaf extract

Pain-induced MSU was assessed using a scoring system conducted by two independent observers. In the negative control group, pain scores peaked at 24 hours post-induction with a score of 2.8 ± 0.45, then gradually decreased at 48 and 72 hours post-induction (Figure 1). This trend was also noticed in the other groups. The MSU injection successfully triggered an inflammatory response resembling acute gout symptoms in humans, which was reflected in increased pain response. Other inflammatory symptoms usually observed in animals after MSU injection include joint swelling and redness.[19,39] Inflammatory markers peaked at 24 hours post-induction before gradually declining, consistent with previous studies that reported a self-limiting inflammatory response within 72 hours.[34,40,41] The inflammatory cascade was driven by immune cell activation, leading to the production of (ROS), NLRP3 inflammasome activation, and inflammatory mediators release.[42]

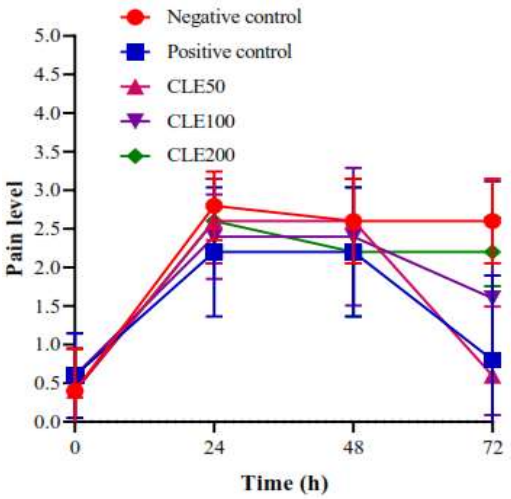

Figure 1: Pain assessment result of MSU-injected rats. The data is presented as mean ± SD (n = 6) and analyzed using one-way ANOVA followed by Tukey post-hoc test. CLE50 = cherry tree leaf extract group receiving a dose of 50 mg/kg BW; CLE100 = cherry tree leaf extract receiving a dose of 100 mg/kg BW; CLE200 = cherry tree leaf extract group receiving a dose of 200 mg/kg BW.

The administration of colchicine reduced the pain level to 2.2 ± 0.84, although it was not significant compared to the negative control group (p = 0.07). Colchicine, which works as an anti-inflammatory, can alleviate pain that accompanies gout flares. However, the drug is not usually used as an analgesic.[43,44] Therefore, the pain reduction response noticed in this study was only moderate.

Following the administration of CLE, there was a slight decrease in pain response in all CLE groups, with CLE100 reaching the lowest pain level at 24 hours post-induction. Further comparison between the CLE groups revealed that at 48 and 72 hours post-induction, CLE200 and CLE50 had the lowest pain levels, respectively. These results indicate that it may be possible to use CLE to lower pain associated with gout arthritis. On the other hand, these findings do not indicate that CLE is void of any anti-inflammatory activity. Given that Parisa et al. (2025) has shown the anti-inflammatory activity of CLE by modulating TNF-α level, further investigation should be conducted using more specific parameters against inflammation. When this paper was written, our research group was evaluating CLE's anti-inflammatory activity by observing changes in knee swelling, redness, and other inflammatory cytokines.

The Shapiro-Wilk and Levene's tests showed that ROS data had p-values of more than 0.05, indicating normal distribution and variance homogeneity. Subsequently, the data was analyzed using one-way ANOVA. As shown in Table 4, the highest ROS level post-induction was observed in the negative control group (11.242 ± 0.795 U/ml), indicating that MSU triggers ROS production. Oxidative stress plays a central role in joint damage, characterized by elevated ROS, nitric oxide (NO), and malondialdehyde (MDA) levels, alongside reduced activity of endogenous antioxidant enzymes such as glutathione (GSH) and SOD. This imbalance exacerbated tissue damage and inflammation.[45,46]

Table 4: Reactive oxidative species levels of all treatment groups

Group	ROS Level (U/ml)
Negative control	11.242 ± 0.795
m	4.875 ± 1.026
CLE 50 mg/kg BW	3.409 ± 0.345
CLE 100 mg/kg BW	6.472 ± 0.318
CLE 200 mg/kg BW	8.860 ± 0.419

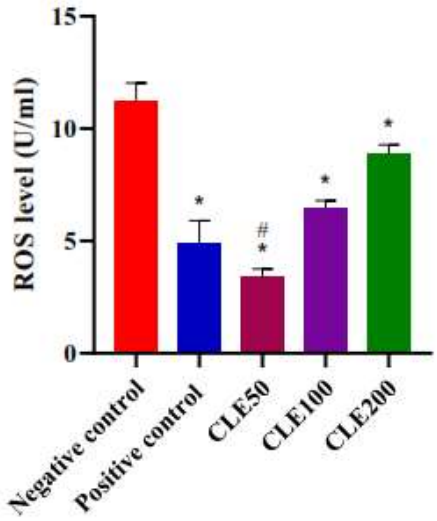

Figure 2: ROS measurement result of MSU-injected rats. The data is presented as mean ± SD (n = 6) and analyzed using one-way ANOVA followed by Tukey post-hoc test. *p < 0.0001 vs. negative control group; # p < 0.005 vs. positive control group; CLE50 = cherry tree leaf extract group receiving a dose of 50 mg/kg BW; CLE100 = cherry tree leaf extract receiving a dose of 100 mg/kg BW; CLE200 = cherry tree leaf extract group receiving a dose of 200 mg/kg BW.

Colchicine was able to dampen ROS production (p < 0.0001), confirming the antioxidant activity of colchicine and the protective effect against oxidative stress reported in earlier studies.[47,48] A marked reduction in ROS levels was also observed in CLE50 (p < 0.0001), CLE100 (p < 0.0001), and CLE200 (p < 0.0001) groups, where CLE50 had the lowest ROS level among the CLE groups (Figure 2). These results indicate that CLE at 50 mg/kg BW is the most effective CLE dose. Surprisingly, the ROS reduction activity of CLE50 was even significantly higher than that of colchicine (p = 0.0049), suggesting superior activity of CLE50. The *in vivo* antioxidant activity of CLE in the acute gout arthritis model further corroborates the *in vitro* findings in this study. Although CLE50 activity was better than colchicine, CLE exhibited a dose-independent response where the administration of higher CLE doses resulted in increased ROS levels. This dose-independent response has also been identified in previous reports, where the lowest dose of CLE was found to be more effective than higher CLE doses.[19,28] The pharmacology activity of an active compound may decline if it is present in high concentrations outside the therapeutic window.[31]

Overall, the biological activities of CLE are most likely owing to the phenolic and flavonoid contents. These two groups are known for their strong antioxidant properties and have been widely studied for their ability to counteract oxidative stress through direct ROS scavenging, inhibition of ROS-generating enzymes, and upregulation of endogenous antioxidant defenses. Flavonoids such as quercetin and rutin have been shown to attenuate oxidative stress by reducing lipid peroxidation, leukocyte infiltration, and inflammatory cytokine release while enhancing SOD and GSH activity.[6,10,49] Additionally, CLE's protective ability may contribute to restoring oxidative balance in inflamed tissues. The observed effects of CLE in modulating pain and oxidative stress further solidified its role as a promising therapeutic agent.

Conclusion

This study demonstrates that CLE effectively reduces oxidative stress in an acute gout arthritis model. The 50 mg/kg BW dose shows the highest effectiveness in lowering ROS levels, suggesting an optimal therapeutic dose. Meanwhile, administering higher doses of CLE does not produce better outcomes. The protective activity against oxidative

stress is most likely mediated by its high phenolic and flavonoid compounds. All in all, these findings highlight the potential of CLE for acute gout treatment. Nevertheless, further research is needed to explore its molecular mechanisms and clinical applications.

Conflict of Interest
The author`s declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The study was funded by DIPA of the Public Service Agency of Universitas Sriwijaya, as stated in the letter No. SP DIPA 023.17.2.677515/2024; in line with the Rector's Decree No. 0013/UN9/LP2M.PT/2024.

References

1. Nguyen LK, Tran CV, Pham ND, Tran TV. Phytochemical screening, antioxidant and xanthine oxidase inhibitory activities of *Vitis Heyneana* Schult. Trop J Nat Prod Res. 2023;7(9):3981-3988. http://www.doi.org/10.26538/tjnpr/v7i9.20
2. Singh JA, Gaffo A. Gout epidemiology and comorbidities. Semin Arthritis Rheum. 2020;50(3):S11–S16. https://doi.org/10.1016/j.semarthrit.2020.04.008
3. Kim SK. The mechanism of the NLRP3 inflammasome activation and pathogenic implication in the pathogenesis of gout. J Rheum Dis. 2022;29(3):140–153. https://doi.org/10.4078/jrd.2022.29.3.140
4. Alkadi H, Khubeiz MJ. Colchicine: A Review About Chemical Structure and Clinical Using. Infect Disord Drug Targets. 2017;17(July). https://doi.org/ 10.2174/1871526517666171017114901
5. Stewart S, Yang KCK, Atkins K, Dalbeth N, Robinson PC. Adverse events during oral colchicine use: A systematic review and meta-analysis of randomised controlled trials. Arthritis Res Ther. 2020;22(1). https://doi.org/10.1186/s13075-020-2120-7
6. Al-Khayri JM, Sahana GR, Nagella P, Joseph B V, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Mol. 2022;27(9):2901. https://doi.org/10.3390/molecules27092901
7. Vuolo MM, Lima VS, Junior MRM. Phenolic compounds: Structure, classification, and antioxidant power. In: Bioactive compounds. Elsevier; 2019. p. 33–50. https://doi.org/10.1016/B978-0-12-814774-0.00002-5
8. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol Rep. 2019;24:1–10. https://doi.org/10.1016/j.btre.2019.e00370
9. RosyidulIbad M, Nasution TH, Andarini S. Effect of kersen leaf extract (*Muntingia calabura*) on the erythema degree in inflammatory process of guinea pigs (*Cavia porcellus*) with shallow second degree burns. J Nurs Sci. 2013;1(2):157–161.
10. Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB. Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. Int J Mol Sci. 2016;17(6):921. https://doi.org/10.3390/ijms17060921
11. Choy KW, Murugan D, Leong X fang, Abas R, Alias A, Mustafa MR. Flavonoids as Natural Anti-Inflammatory Agents Targeting Nuclear Factor-Kappa B (NFκB) Signaling in Cardiovascular Diseases: A Mini Review. Front Pharmacol. 2019;10:1–8
12. Syabania M, Pambudi DB, Wirasti W, Rahmatullah S. Characterization and Evaluation of Kersen Leaf Extract (*Muntingia calabura* L.) Granule by Wet Granulation Method. In: Proceedings of the National Seminar on Health. 2021. p. 1737–1746. https://doi.org/10.3389/fphar.2019.01295
13. Vonna A, Desiyana LS, Hafsyari R, Illian DN. Phytochemical Analysis and Characterization of Ethanol Extract of Kersen Leaf (*Muntingia calabura* L.). J Bioleuser. 2021;5(1).
14. Ghosal P, Chandra S, Choudhary AN, Saklani S. *Sonchus arvensis*: Antioxidant activity, phenolic profile, and phytochemical screening. Kariri Sci - Cecape Biol Health. 2023;1(1). https://doi.org/10.29327/2256856.2023.1-8
15. Farida Y, Qodriah R, Nilesh S. Quality parameters and determination of total flavonoid levels from the highest antioxidant activity of ethanol 70% extract jackfruit peel (*Artocarpus Heterophyllus* L.) by maceration, reflux, and ultrasonic methods. Int J App Pharm. 2022;100–103. https://dx.doi.org/10.22159/ijap.2022.v14s3
16. Elhady SS, Abdelhameed RFA, Mehanna ET, Wahba AS, Elfaky MA, Koshak AE, Noor AO, Bogari HA, Malatani, RT, Goda MS. Metabolic profiling, chemical composition, antioxidant capacity, and in vivo hepato-and nephroprotective effects of *Sonchus cornutus* in mice exposed to cisplatin. Antioxidants. 2022;11(5):819. doi: 10.3390/antiox11050819
17. Saputra FI. Immunomudulatory effect of kersen (*Muntingia calabura* L.) leaf extract in mice with carbon clearance method [thesis]. Padang: Universitas Andalas; 2021
18. Parisa N, Hidayat R, Maritska Z, Prananjaya BA. Evaluation of the anti-gout effect of *Sonchus Arvensis* on monosodium urate crystal-induced gout arthritis via anti-inflammatory action - an in vivo study. Med Pharm Rep. 2021;94(3):358–365. https://doi.org/10.15386/mpr-1959
19. Parisa N, Kamaluddin MT, Saleh MI, Sinaga E, Partan RU, Irfannuddin, Mangunsong S. The Effectiveness of Tempuyung Leaves' Water Fraction for Inflammation Prevention of Wistar Rats in an Acute Gout Arthritis Model. Indonesian J Pharm. 2025;0(0). https://jurnal.ugm.ac.id/v3/IJP/article/view/14504
20. Mergy MA, Gowrishankar R, Davis GL, Jessen TN, Wright J, Stanwood GD, Hahn MK, Blakely, RD. Genetic targeting of the amphetamine and methylphenidate-sensitive dopamine transporter: on the path to an animal model of attention-deficit hyperactivity disorder. Neurochem Int. 2014;73:56–70. http://dx.doi.org/10.1016/j.neuint.2013.11.009
21. Prayitno SA, Rahim AR. Comparison of Extracts (Ethanol And Aquos Solvents) *Muntingia calabura* Leaves on Total Phenol, Flavonid And Antioxidant (Ic50) Properties. Kontribusia: R D C D. 2020;3(2):319–325. http://dx.doi.org/10.30587/kontribusia.v3i2.1451
22. Upadhye M, Kuchekar M, Pujari R, Kadam S, Gunjal P. *Muntingia calabura*: A comprehensive review. J Pharm Biol Sci. 2021;9(2):81–87. https://doi.org/ 10.18231/j.jpbs.2021.011
23. Sinaga SP, Lumbangaol DA, Iksen RFR, Gurning K. Determination of phenolic, flavonoid content, antioxidant and antibacterial activities of seri (*Muntingia calabura* L.) leaves ethanol extract from North Sumatera, Indonesia. Rasayan J Chem. 2022;15(02):1534–1538. http://doi.org/10.31788/RJC.2022.1526730
24. Akomeng N, Adusei S. Organic solvent extraction and spectrophotometric quantification of total phenolic content of soil. Heliyon. 2021;7(11). https://doi.org/10.1016/j.heliyon.2021.e08388
25. Pant P, Pandey S, Dall'Acqua S. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. Chem Biodivers. 2021;18(11):e2100345. https://doi.org/10.1002/cbdv.202100345
26. Tirado-Kulieva VA, Hernández-Martínez E, Choque-Rivera TJ. Phenolic compounds versus SARS-CoV-2: An update on

the main findings against COVID-19. Heliyon. 2022;8(9). https://doi.org/10.1016/j.heliyon.2022.e10702
27. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Mol. 2023;28(4):1845. https://doi.org/10.3390/molecules28041845
28. Zakaria ZA. Free radical scavenging activity of some plants available in Malaysia. Iran J Pharmacol Ther. 2007;6:87–91. http://ijpt.iums.ac.ir/article-1-106-en.html
29. Mahmood ND, Nasir NLM, Rofiee MS, Tohid SFM, Ching SM, Teh LK, Salleh MZ, Zakaria ZA. *Muntingia calabura*: A review of its traditional uses, chemical properties, and pharmacological observations. Pharm Biol. 2014;52(12):1598–1623. https://doi.org/10.3109/13880209.2014.908397
30. Jisha N, Vysakh A, Vijeesh V, Latha MS. Anti-inflammatory efficacy of methanolic extract of *Muntingia calabura* L. leaves in Carrageenan induced paw edema model. Pathophysiology. 2019;26(3–4):323–330. https://doi.org/10.1016/j.pathophys.2019.08.002
31. Balan T, Sani MHM, Ahmad SHM, Suppaiah V, Mohtarrudin N, Zakaria ZA. Antioxidant and anti-inflammatory activities contribute to the prophylactic effect of semi-purified fractions obtained from the crude methanol extract of *Muntingia calabura* leaves against gastric ulceration in rats. J Ethnopharmacol. 2015;164:1–15. https://doi.org/10.1016/j.jep.2014.12.017
32. Hnamte S, Parasuraman P, Ranganathan S, Ampasala DR, Reddy D, Kumavath RN, Suchiang K, Mohanty SW, Busi S. Mosloflavone attenuates the quorum sensing controlled virulence phenotypes and biofilm formation in *Pseudomonas aeruginosa* PAO1: In vitro, in vivo and in silico approach. Microb Pathog. 2019;131:128–134. https://doi.org/10.1016/j.micpath.2019.04.005
33. Hájek J. Biological activity of antioxidant compounds in monocytes THP-1 [thesis]. Prague: Charles University; 2015
34. Kim SY, Hassan AH, Chung KS, Kim SY, Han HS, Lee HH, Jung SH, Lee KY, Shin JS, Jang E, Yoon S. Mosloflavone-resveratrol hybrid TMS-HDMF-5z exhibits potent in vitro and in vivo anti-inflammatory effects through NF-κB, AP-1, and JAK/STAT inactivation. Front Pharmacol. 2022;13:857789. https://doi.org/10.3389/fphar.2022.857789
35. Amarowicz R, Pegg RB. Protection of natural antioxidants against low-density lipoprotein oxidation. Adv Food Nutr Res. 2020;93:251-291. https://doi.org/10.1016/bs.afnr.2020.04.002
36. Navarrete A, Balderas-López JL, Rosas-Canales JG, Tapia-Álvarez GR, Alfaro-Romero A, Aviles-Rosas VH, Rodríguez-Ramos F, Avula B, Khan IA. Flavones isolated from *Pseudognaphalium liebmannii* with tracheal smooth muscle relaxant properties. Nat Prod Res. 2025;39(6):1461–1466. https://doi.org/10.1080/14786419.2023.2300402
37. Aloud AA, Veeramani C, Govindasamy C, Alsaif MA, El Newehy AS, Al-Numair KS. Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. Redox Rep. 2017;22(6):290–300. https://doi.org/10.1080/13510002.2016.1273437
38. Lin K, Fu D, Wang Z, Zhang X, Zhu C. Analgesic and anti-inflammatory effects of galangin: A potential pathway to inhibit transient receptor potential vanilloid 1 receptor activation. Korean J Pain. 2024;37(2):151–163. https://doi.org/10.3344/kjp.23363
39. Wang Y, Zhang Y, Yu Z, Bai Y, Zhu M, Lei Y, Dong B, Zhang Q, Gu Q, Xiang Jian. Developing Monosodium Urate Monohydrate Crystals–Induced Gout Model in Rodents and Rabbits. Curr Protoc. 2025;5(3):e70114. https://doi.org/10.1002/cpz1.70114
40. Zhang G, Lin Y, Chen X, Qin J, He Y, Liu T, Zhang L, Zhang L. Cinnamomi cortex extract mitigated monosodium urate-induced acute gouty arthritis in rats through nuclear factor-κB-NOD-like receptor thermal protein domain associated protein 3 signaling pathway. J Vet Med Sci. 2024;86(6):623–630. https://doi.org/10.1292/jvms.23-0085
41. Dai X, Fang X, Xia Y, Li M, Li X, Wang Y, Tao J, Li X. ATP-activated P2X7R promote the attack of acute gouty arthritis in rats through activating NLRP3 inflammasome and inflammatory cytokine production. J Inflamm Res. 2022;1237–1248. https://doi.org/10.2147/JIR.S351660. eCollection 2022
42. Lee YM, Cho SN, Son E, Song CH, Kim DS. Apamin from bee venom suppresses inflammation in a murine model of gouty arthritis. J Ethnopharmacol. 2020;257:112860. https://doi.org/10.1016/j.jep.2020.112860
43. Plotz B, Pillinger M, Samuels J. Colchicine and clinical trials for hand osteoarthritis. Osteoarthr Cartil. 2022;30(1):172-173. https://doi.org/10.1016/j.joca.2020.12.026 1063-4584
44. Latourte A, Pascart T, Flipo RM, Chalès G, Coblentz-Baumann L, Cohen-Solal A, Ea HK, Grichy J, Letavernier E, Lioté F, Ottaviani S, Sigwalt P, Vandecandelaere G, Richette P, Bardin T. 2020 Recommendations from the French Society of Rheumatology for the management of gout: Management of acute flares. J Bone Spine. 2020;87(5):387–393. https://doi.org/10.1016/j.jbspin.2020.05.001
45. Cheng JJ, Ma XD, Ai GX, Yu QX, Chen XY, Yan F, Li YC, Xie JH, Su ZR, Xie QF. Palmatine protects against MSU-induced gouty arthritis via regulating the NF-κB/NLRP3 and Nrf2 pathways. Drug Des Devel Ther. 2023;2119–2132. https://doi.org/10.2147/DDDT.S356307
46. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt Jr PIH. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J. 2016;473(24):4527–4550. https://doi.org/10.1042/BCJ20160503C
47. Zălar DM, Pop C, Buzdugan E, Kiss B, Ştefan MG, Ghibu S, Crişan D, Buruiană-Simic A, Grozav A, Borda IM, Mogoşan CI. Effects of colchicine in a rat model of diet-induced hyperlipidemia. Antioxidants. 2022;11(2):230. https://doi.org/10.3390/antiox11020230
48. El Hasbani G, Jawad A, Uthman I. Colchicine: An ancient drug with multiple benefits. Curr Pharm Des. 2021;27(26):2917-2924. https://doi.org/10.2174/1381612826666201023144320
49. Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem. 2019;299. https://doi.org/10.1016/j.foodchem.2019.125124