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Antihyperuricemic Activity of n-Hexane, Ethyl Acetate, and Aqueous Fractions of Ethanol Extract of *Chromolaena odorata* Leaves in Mice: *In Vivo* Study

Aisyiah*, Adhi Wardhana Amrullah, Rolando Rahardjoputro

Bachelor of Pharmacy Study Program, Faculty of Health Sciences, Universitas Kusuma Husada, Surakarta, Central Java, Indonesia

ABSTRACT: Hyperuricemia is a metabolic disorder characterised by elevated uric acid levels in the blood, which may lead to complications such as gout, arthritis, kidney stones, and renal failure. In recent years, medicinal plants have gained increasing attention as alternative therapies for hyperuricemia. *Chromolaena odorata*, commonly known as kirinyuh, is one such plant suspected to have antihyperuricemic properties. This study aimed to evaluate the antihyperuricemic activity of the n-hexane, ethyl acetate, and aqueous fractions of *C. odorata* leaf ethanol extract in male Swiss Webster mice induced with chicken liver juice. The extract was obtained by maceration using 96% ethanol and subsequently fractionated using solvents with different polarities. The experiment employed a pretest-posttest control group design, involving 12 groups: a normal control, a negative control (0.5% Na-CMC), a positive control (allopurinol at 13 mg/kg body weight), and nine treatment groups that received each fraction at doses of 20, 40, and 80 mg/kg body weight. Uric acid levels were measured at three intervals: baseline (day 0), post-induction (day 7), and after treatment (day 14), using the Easy Touch GCU Multi-Function Monitoring System. The results demonstrated that all three fractions reduced uric acid levels, with the ethyl acetate fraction at 80 mg/kg body weight (BW) showing the most significant effect, achieving a 21.34% reduction. These findings suggest that *Chromolaena odorata* leaves, especially the ethyl acetate fraction, possess promising antihyperuricemic potential and could serve as a natural alternative for managing hyperuricemia.

Keywords: Antihyperuricemic; *Chromolaena odorata*; fractionation; Hyperuricemia;

*Corresponding author:
Name : Aisyiah
Email : aaisyiah01@gmail.com
Address : Faculty of Health Sciences, Universitas Kusuma Husada, Jl. Jaya Wijaya No..11, Kadipiro, Kec. Banjarsari, Kota Surakarta, Jawa Tengah

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INTRODUCTION

The shift toward a modern lifestyle, characterized by speed and convenience, has led to changes in consumption patterns and a decline in physical activity. These lifestyle changes have contributed to the rising prevalence of non-communicable diseases, one of which is hyperuricemia. Hyperuricemia is a condition characterized by elevated levels of uric acid in the blood that exceed normal limits, which may result from increased production, decreased excretion, or a combination of both mechanisms (Rakanita *et al*., 2017). Although hyperuricemia may not always present clinical symptoms, it poses a significant risk of chronic complications such as gout arthritis, an inflammatory joint condition caused by the accumulation of monosodium urate (MSU) crystals (Mahmudah *et al*., 2023). One of the main risk factors for hyperuricemia is a purine-rich diet. Consumption of foods such as red meat, organ meats, seafood, legumes, and certain vegetables, including long beans and spinach, can elevate uric acid levels in the body (Nurcahyani *et al*., 2022). According to data from the World Health Organization (WHO) in 2018, the global prevalence of gout arthritis was 34.2%, and it had increased by 33.3% (Mahmudah *et al*., 2023). In Indonesia, the Basic Health Research (RISKESDAS) in 2018 reported a prevalence of joint diseases of 7.30%, with the highest incidence (18.95%) among individuals aged 75 years and above.

Conventional treatment of hyperuricemia typically involves the use of drugs such as allopurinol and colchicine. While effective, long-term use of these medications may lead to adverse effects, including hepatic and renal dysfunction as well as hypersensitivity reactions. Therefore, the development of alternative therapies derived from natural products has become a promising and relevant approach. Various secondary metabolites, including flavonoids, alkaloids, phenolics, tannins, coumarins, and iridoid glycosides, have demonstrated antihyperuricemic potential through mechanisms that involve xanthine oxidase inhibition (Ling & Bochu, 2014). Flavonoids have received particular attention due to their low toxicity and wide-ranging biological activities, including antioxidant, anticancer, anti-inflammatory, and antihyperuricemic properties (Li *et al*., 2024; Xue *et al*., 2023). Their ability to inhibit xanthine oxidase is attributed to the presence of a C-2=C-3 double bond in their molecular structure. In addition to flavonoids, alkaloids such as colchicine also exhibit antihyperuricemic activity by inhibiting xanthine oxidase and exerting anti-inflammatory effects (Pracila *et al*., 2020; Wajdie *et al*., 2018).

Chromolaena odorata (kirinyuh) is a tropical plant species widely considered a weed, yet it contains various secondary metabolites, including flavonoids, alkaloids, tannins, saponins, glycosides, and triterpenoids. These compounds are known to possess pharmacological activities, including antibacterial, anti-inflammatory, and diuretic effects (Fadia *et al*., 2020; Supriningrum *et al*., 2020). Previous research has shown that ethanolic extracts and fractions of *C. odorata* leaves can inhibit the growth of *Propionibacterium acnes*, indicating the presence of bioactive compounds (Komala *et al*., 2021). However, most studies on *C. odorata* have been limited to crude extracts, with few efforts made to isolate and identify more purified and specific active compounds through fractionation. Given the promising secondary metabolite content of *C. odorata* leaves, particularly flavonoids, this study aims to evaluate the antihyperuricemic activity of the n-hexane, ethyl acetate, and aqueous fractions of *Chromolaena odorata* leaves as a potential natural alternative therapy for hyperuricemia.

METHODS

Material and Equipments

The materials used in this study included kirinyuh (*Chromolaena odorata*) originating from Girilayu, Matesih, Karanganyar, Central Java, 96% ethanol, n-hexane, ethyl acetate, distilled water, 0.5% sodium carboxymethyl cellulose (Na-CMC), allopurinol tablets (Errita Pharma®), and chicken liver. The experimental animals used were 36 male Swiss Webster white mice, obtained from the Experimental Animal Development Unit, Gadjah Mada

University, Yogyakarta. The equipment used in this study included a separatory funnel (Pyrex®), laboratory glassware (Pyrex®), water bath (Faithul®), and a uric acid strip test device (Easy Touch GCU Multi Function Monitoring System).

Experimental Prosedure

Plant Identification

The taxonomic identification of *Chromolaena odorata* was carried out at the UPF Yankestrad in Tawangmangu, Karanganyar. The plant was identified and authenticated by a botanist, and a voucher specimen (No. TL.02.04/D.XI.6/24934.1177/2024) was collected and deposited in the herbarium for future reference.

Extraction

The *leaves of Chromolaena odorata* were extracted using the maceration method with 96% ethanol as the solvent. The maceration was carried out in a glass container at a sample-to-solvent ratio of 1:10 (w/v), and the mixture was left to stand in the dark for 72 hours with occasional stirring. After this time, the mixture was filtered to separate the filtration from the residue. The residue was then re-macerated using a fresh batch of ethanol. This process was repeated three times. The combined filtrates were then concentrated using a rotary evaporator to produce a concentrated extract of *C. odorata* leaves (Efendi *et al*., 2023; Syifa *et al*., 2022).

Fractionation

The fractionation process was conducted using three solvents of varying polarity: n-hexane, ethyl acetate, and water. A total of 20 grams of concentrated ethanolic extract of *Chromolaena odorata* leaves was dissolved in 100 mL of distilled water and transferred into a separatory funnel. An equal volume of n-hexane was then added, and the mixture was vigorously shaken. The funnel was allowed to stand until two distinct phases formed: the upper n-hexane layer and the lower aqueous layer. The n-hexane layer was separated, while the aqueous phase was subjected to further partitioning using ethyl acetate in a 1:1 ratio. After shaking and settling, the upper ethyl acetate layer and the lower aqueous layer were separated. The fractionation was repeated several times until the solvent layers became clear. Each collected fraction was then concentrated by evaporating the solvent to obtain a thick extract (Maqfirah *et al*., 2023; Nirwana, 2021; Sugiarti *et al*., 2020; Syifa *et al*., 2022). Phytochemical screening was performed on each concentrated fraction to identify the presence of secondary metabolites, including alkaloids, saponins, tannins, flavonoids, and polyphenols (Amalia *et al*., 2022; Nurlila *et al*., 2024).

Experimental Animal Preparation

The experimental animals used in this study were male Swiss Webster mice aged 2–3 months and weighing approximately 20–30 grams. Prior to the experiment, the mice were acclimatized for 7 days in animal cages at the Laboratory of Pharmacy, Kusuma Husada University, Surakarta. During the acclimatization period, the mice were provided with standard feed and drinking water, and cage hygiene was maintained by replacing the bedding every three days.

Preparation of Chicken Liver Juice

Fresh chicken liver juice was prepared daily for six consecutive days by weighing 60 grams of fresh liver, blending it, and diluting it with distilled water to a final volume of 100 mL (Wikantyasning *et al*., 2024). The resulting solution was administered orally to mice at a dose of 0.5 mL per 20 g of body weight once daily to induce hyperuricemia (Hasan *et al*., 2022; Roseno, 2022).

Preparation of 0,5% Na-CMC Suspension

A total of 0.5 grams of Na-CMC powder was added to a preheated mortar (60°C), followed by 50 mL of hot distilled water. After allowing the powder to swell, the mixture

was stirred until homogeneous. The remaining 50 mL of hot distilled water was gradually added while stirring to obtain a uniform suspension (Lubis *et al*., 2023).

Preparation of Allopurinol Suspension

A total of 65 mg of allopurinol was added to a mortar containing 10 mL of hot distilled water (approximately 60 °C). The mortar was covered and left to stand for approximately 15 minutes, until a transparent mass formed; then, it was triturated until smooth. The Na-CMC gel was added gradually to help dissolve any precipitate that may have formed. Finally, distilled water was added to a total volume of 50 mL, and the mixture was stirred rapidly until a uniform suspension was obtained (Lubis *et al*., 2023).

Preparation for Fraction Suspensions

Each *Chromolaena odorata* leaf fraction was formulated into a suspension by first weighing 0.5 grams of Na-CMC, which was dispersed in 20 mL of hot distilled water and triturated until a uniform gel was formed. Subsequently, 0.2, 0.4, and 0.8 grams of the concentrated fraction were added and mixed thoroughly. The mixture was then transferred into a 100 mL volumetric flask and diluted to the mark with distilled water, followed by stirring until a homogeneous suspension was obtained.

Evaluation of Antihyperuricemic Activity

The test animals were divided into 12 groups, each consisting of 3 male Swiss Webster mice. The groups included a normal control group, a negative control group, a reference group (allopurinol), and six treatment groups that received oral doses of each fraction at 20, 40, and 80 mg/kg body weight. Prior to treatment, the mice were fasted for 18 hours and had free access to water. Baseline serum uric acid levels were measured to determine the normal values. All groups except the normal control group were induced with hyperuricemia by receiving 0.5 mL/20 g body weight (BW) of freshly prepared chicken liver juice orally once daily for six consecutive days. On day 7, serum uric acid levels were measured again to confirm the elevation. Subsequently, all groups except the standard control received their respective treatments orally once daily for six days. Final serum uric acid levels were measured on day 14. Blood samples were collected from the tail vein by cutting 0.1–0.2 cm off the tip of the tail with sterilized scissors. A drop of blood was applied directly to a uric acid test strip inserted into the Easy Touch GCU Multi Function Monitoring System, which displayed the serum uric acid concentration in mg/dL (Nurcahyani *et al*., 2022; Nurlila *et al*., 2024). The percentage reduction in serum uric acid levels was calculated using the following formula (Hasan *et al*., 2022).

$$\% \text{ Reduction} = \frac{\text{Post Induction} - \text{Post Treatment}}{\text{Post Induction}} \times 100\%$$

Data Analysis

The data obtained from this study were analyzed using SPSS (Statistical Product and Service Solutions) version 26.0. A paired-sample *t*-test (pre-test/post-test) was conducted to determine if there were significant differences before and after treatment, under the assumption that the data followed a normal distribution. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant (Nurlila *et al*., 2024).

RESULT AND DISCUSSION

Secondary metabolites were extracted from *Chromolaena odorata* leaves using the maceration method with 96% ethanol, accompanied by two remacerations to enhance efficiency. Maceration was chosen because it is straightforward, does not require

specialized equipment and avoids the heating that could degrade bioactive compounds (Badaring *et al*., 2020; Muchson Arrosyid *et al*., 2023). The process was conducted for 72 hours with intermittent stirring to maximize contact between the sample and the solvent until equilibrium was reached. Ethanol at 96% was used due to its universal polarity, volatility, inertness, selectivity towards active compounds and resistance to microbial growth (Widyawati, 2023). Compared to lower concentrations, 96% ethanol yields higher concentrations and can dissolve non-polar, semi-polar, and polar compounds (Nahor *et al*., 2020; Wendersteyt *et al*., 2021). The extraction process yielded a thick, dark brownish-black extract with a yield of 16.24%, meeting the quality standards outlined in the Indonesian Herbal Pharmacopoeia (2017). The result of the extraction can be seen in Table 1.

Table 1 Extraction Yield of *Chromolaena odorata* Leaves

Simplicia Weight (g)	Solvent Volume (mL)	Extract Yield (g)	% Yield (w/w)
200	2000	32,47	16,24

In this study, fractionation was performed using the liquid–liquid partition method, which utilizes the polarity difference between two immiscible solvents. This method is based on the principle of 'like dissolves like', whereby compounds dissolve preferentially in solvents with similar polarity (Sogandi *et al*., 2019). The process was performed in steps according to solvent polarity, starting with non-polar solvents, then semi-polar solvents, and finally polar solvents. Three solvents with different polarities were used: n-hexane (non-polar), ethyl acetate (semi-polar), and water (polar). First, the ethanolic extract of *Chromolaena odorata* leaves was dissolved in water. Then, it was partitioned with n-hexane in a 1:1 ratio using a separatory funnel. The mixture was shaken until two distinct phases formed: a non-polar brownish-black phase and a polar reddish-brown phase. Shaking was performed to improve the efficiency of metabolite transfer into the respective solvents. Phase separation occurred due to differences in polarity and density, with the heavier solvent forming the lower layer and the lighter solvent remaining on top (Luntungan *et al*., 2021).

The densities of n-hexane, ethyl acetate and water are 0.655 g/ml, 0.902 g/ml and 0.997 g/ml respectively (Taufik *et al*., 2023). As shown in Table 2, the aqueous fraction (polar solvent) produced the highest yield at 47.06%, followed by the ethyl acetate fraction (semi-polar) at 21.72%, and the n-hexane fraction (non-polar) at 16.78%. These findings indicate that the ethanolic extract of *Chromolaena odorata* contains a higher proportion of polar bioactive compounds compared with semi-polar and non-polar constituents.

Table 2 Fraction Yield of *Chromolaena odorata* Leaves

Fraction	Extract Weight (g)	Fraction Yield (g)	% Yield (w/w)
N-Hexane	32	5,37	16,78
Ethyl Acetate	32	6,95	21,72
Aqueous	32	15,06	47,06

In this study, phytochemical screening tests were performed on each fraction of *Chromolaena odorata* leaves. Phytochemical screening is a preliminary analytical method

used to identify the presence of secondary metabolite compounds in a sample, either in its entirety or in specific parts of plant or animal materials (Jonathan *et al*., 2024). This test is crucial as an initial step in identifying bioactive compounds with potential pharmacological properties, such as antibacterial, antiviral, and particularly antihyperuricemic activity. The results of the phytochemical screening of the *Chromolaena odorata* leaf fractions are presented in Table 3. Based on the findings, the n-hexane and ethyl acetate fractions of the ethanolic extract of Kirinyuh leaves were found to contain secondary metabolites, including tannins, flavonoids, and polyphenols.

Table 3 Phytochemical Screening Results of *Chromolaena odorata* Leaf Fractions

Parameters	Results			
	Extract	**F.N.H**	**F.E.A**	**F.A**
Alkaloid	+	-	-	+
Saponin	-	-	-	-
Tanin	+	+	+	+
Flavonoid	+	+	+	+
Polyphenol	+	+	+	+

F.N.H : n-Hexane Fraction
F.E.A : Ethyl Acetate Fraction
F.A : Aqueous Fraction
(+) : Compound group present
(–) : Compound group absent

In contrast, the water fraction of the extract exhibited a more diverse composition, including alkaloids, tannins, flavonoids, and polyphenols. Although tannins, polyphenols, and flavonoids are generally polar, certain derivatives, such as aglycones or methylated forms, exhibit reduced polarity. This enables them to partition into the non-polar (n-hexane fraction) (Palaiogiannis *et al*., 2023).

The determination of antihyperuricemic activity of the three fractions of *Chromolaena odorata* leaves ethanol extract was conducted by observing changes in blood uric acid levels in experimental animals. The test animals used were male Swiss Webster mice, aged 2–3 months and weighing between 20 and 30 grams, to minimize biological variation and control for variables. Male mice were chosen due to their hormonal stability, as female mice undergo hormonal fluctuations during specific physiological conditions, such as oestrous cycles, pregnancy, and lactation, which may affect their psychological state (Yusuf *et al*., 2022). Before treatment, all mice underwent an adaptation period and were subsequently fasted for 18 hours, with no food but free access to water. This procedure aimed to stabilize blood uric acid levels and eliminate the influence of food intake on them. Uric acid levels were measured three times: on day 0, considered the baseline (normal) uric acid level of the test animals; on day 7, after the induction of hyperuricemia; and on day 14, following the administration of each treatment. Based on Figure 1, it can be seen that each fraction at different doses produced varying data. These differences are considered reasonable, as they may result from physiological variations among the test animals, such as differences in enzyme activity and metabolic processes during the treatment period, which can influence the measured uric acid levels (Himawan *et al*., 2020).

Statistical analysis of blood uric acid levels in mice was performed using SPSS version 26.0 (Statistical Package for the Social Sciences). Data normality was first tested using the Shapiro–Wilk test to determine the distribution of a small sample (Suardi, 2019).

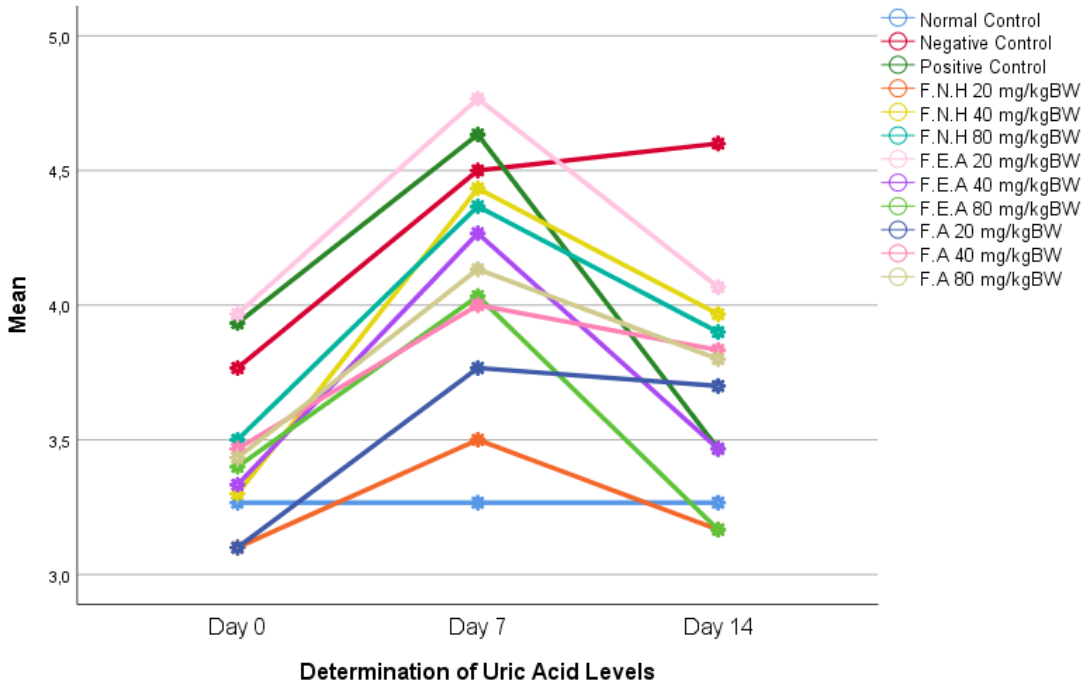

Figure 1. Mean Serum Uric Acid Levels in Mice for Each Treatment Group

This test was chosen because the sample size was less than 50 ($n < 50$). In this test, data are considered normally distributed if the significance value is greater than 0.05 ($p > 0.05$) (Sativa & Annisa, 2020; Suardi, 2019). Based on the results of the normality test, the significance value for uric acid levels before and after treatment was $p > 0.05$, indicating that the data were normally distributed. As a result, further analysis was conducted using the paired sample t-test. This test is used to evaluate whether there is a statistically significant difference between two related groups, to assess the effect of a treatment on a particular variable (Montolalu & Langi, 2018). The purpose of the paired sample t-test is to determine whether there is a significant difference in mean uric acid levels before and after treatment (Sativa & Annisa, 2020). The decision criteria are based on the p-value, where $p < 0.05$ indicates a statistically significant difference, and $p > 0.05$ indicates no significant difference (Nurhamidah *et al*., 2022). The results of the paired sample t-test are presented in Figure 2., significant differences ($p < 0.05$) in uric acid levels were observed in the positive control group; the n-hexane fraction at doses of 40 mg/kgBW and 80 mg/kgBW; the ethyl acetate fraction at all tested doses; and the water fraction at a dose of 80 mg/kgBW. In contrast, the negative control group showed an increase in uric acid levels after treatment, while the normal control group exhibited no significant changes, indicating that neither group showed a therapeutic effect. The significant reduction in uric acid levels in the treatment groups suggests that the *Chromolaena odorata* (kirinyuh) leaf fractions, particularly the ethyl acetate fraction, possess potential antihyperuricemic activity.

The antihyperuricemic activity of the n-hexane, ethyl acetate, and water fractions of *Chromolaena odorata* leaves is presented in Table 4. Based on the observations, each fraction at different doses exhibited varying percentages of uric acid level reduction. The normal control group showed no reduction (0%) in uric acid levels, while the negative control group showed a 2.22% increase. These results indicate that there is no therapeutic

effect in either group. In contrast, the positive control group (allopurinol) demonstrated a significant reduction of 25.05%. Administration of the kirinyuh leaf fractions at various doses resulted in dose-dependent reductions in uric acid levels. The n-hexane fraction yielded reductions of 9.43% (20 mg/kg body weight), 10.38% (40 mg/kg body weight), and 10.76% (80 mg/kg body weight). The ethyl acetate fraction exhibited higher activity, resulting in reductions of 14.68% (20 mg/kg BW), 18.74% (40 mg/kg BW), and 21.34% (80 mg/kg BW). Meanwhile, the water fraction exhibited the lowest activity, with reductions of 1.86% (20 mg/kg BW), 4.25% (40 mg/kg BW), and 7.99% (80 mg/kg BW).

These results indicate that *Chromolaena odorata* leaf fractions possess antihyperuricemic activity in mice. Among all treatments, the ethyl acetate fraction at a dose of 80 mg/kg body weight (BW) demonstrated the most effective reduction (21.34%), which was nearly comparable to that of the standard drug, allopurinol. The percentage reduction in uric acid levels in the treatment groups did not surpass that observed in the allopurinol group. This is likely due to the nature of *Chromolaena odorata* (kirinyuh) leaf fractions, which consist of a mixture of compounds rather than a single, pure active compound; some of these compounds may contribute to or interfere with the overall antihyperuricemic activity (Juwita *et al*., 2017).

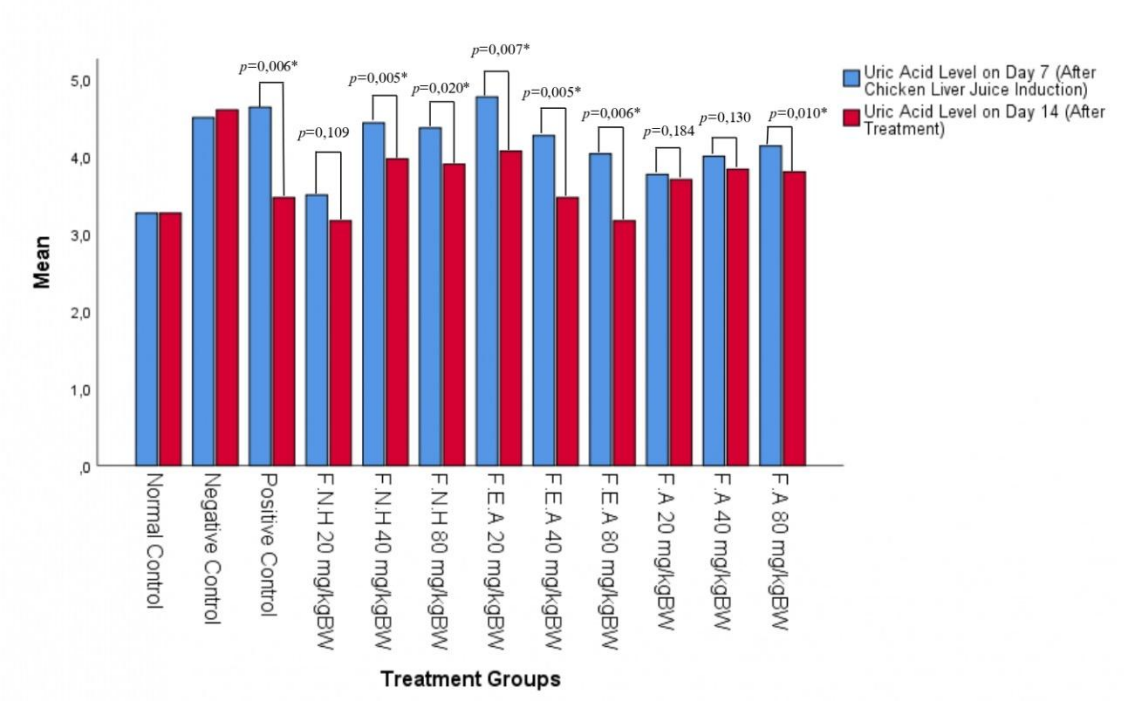

Figure 2. Graph of Uric Acid Level Reduction (* = p<0.05 compared to Uric Acid Levels on Day 7)

Each fraction of *Chromolaena odorata* leaves exhibits antihyperuricemic activity, which is believed to be associated with the presence of secondary metabolites, including flavonoids, tannins, and polyphenols. These compounds have been reported to possess various pharmacological effects, including antihyperuricemic properties. According to previous theories and research findings, the antihyperuricemic activity of these secondary metabolites is primarily attributed to their flavonoid content (Aulena *et al*., 2024; Juwita *et*

al., 2017; Xue *et al*., 2023). This is further supported by studies on other members of the Asteraceae family, such as *Elephantopus scaber*, which has been reported to contain flavonoids that contribute to the reduction of uric acid (Gunarti & Hidayah, 2022). Similar findings were observed in the present study, reinforcing the role of flavonoid compounds in the antihyperuricemic activity of the *C. odorata* leaf fractions.

Table 4 Mean and Percentage Reduction of Uric Acid Levels in Mice

Treatment Groups	Serum Uric Acid Levels in Mice (mg/dL)			
	UA7	UA14	ΔUA (UA7-UA14)	% Reduction
Normal Control	3,27 ± 0,15	3,27 ± 0,15	0,00 ± 0,00	↓0,00
Negative Control	4,50 ± 0,70	4,60 ± 0,70	-0,10 ± 0,00	↑2,22
Positive Control	4,63 ± 0,55	3,47 ± 0,47*	1,16 ± 0,15	↓25,05
F.N.H 20 mg/kgBW	3,50 ± 0,10	3,17 ± 0,21	0,33 ± 0,21	↓9,43
F.N.H 40 mg/kgBW	4,43 ± 0,15	3,97 ± 0,21*	0,46 ± 0,06	↓10,38
F.N.H 80 mg/kgBW	4,37 ± 0,21	3,90 ± 0,30*	0,47 ± 0,12	↓10,76
F.E.A 20 mg/kgBW	4,77 ± 0,57	4,07 ± 0,55*	0,70 ± 0,10	↓14,68
F.E.A 40 mg/kgBW	4,27 ± 0,45	3,47 ± 0,42*	0,80 ± 0,10	↓18,74
F.E.A 80 mg/kgBW	4,03 ± 0,21	3,17 ± 0,21*	0,86 ± 0,12	↓21,34
F.A 20 mg/kgBW	3,77 ± 0,15	3,70 ± 0,17	0,07 ± 0,06	↓1,86
F.A 40 mg/kgBW	4,00 ± 0,26	3,83 ± 0,32	0,17 ± 0,12	↓4,25
F.A 80 mg/kgBW	4,13 ± 0,42	3,80 ± 0,44*	0,33 ± 0,06	↓7,99

UA7 : Uric acid level on Day 7 (after induction with chicken liver juice)
UA14 : Uric acid level on Day 14 (after treatment)
ΔUA : Change in uric acid level between Day 7 and Day 14
F.N.H : *n*-Hexane Fraction
F.E.A : Ethyl Acetate Fraction
F.A : Aqueous Fraction
(*) : Significant difference (p <0,05) compared to uric acid level on Day 7
(↓) : Decrease in uric acid level
(↑) : Increase in uric acid level

Flavonoids, one of the most abundant classes of secondary metabolites found in various plant species, are known to possess hydroxyl groups that can interact with the active site of xanthine oxidase through hydrogen bonding and hydrophobic interactions. These interactions result in competitive inhibition of the enzyme's natural substrate, thereby reducing uric acid production (Li *et al*., 2024; Xue *et al*., 2023). Both *in vitro* and *in silico* studies have demonstrated that flavonoids, such as quercetin, kaempferol, and luteolin, exhibit strong binding affinities to the active residues of xanthine oxidase and significantly inhibit its enzymatic activity (Cheng-yuan & Jian-gang, 2023; Zhang *et al*., 2023).

In addition to their role as xanthine oxidase inhibitors, some flavonoids have also been reported to promote uric acid excretion via the kidneys by modulating the expression of uric acid transporters such as URAT1 (Urate Transporter 1) and GLUT9 (Glucose Transporter 9). These transporters are primarily involved in uric acid reabsorption in the proximal renal tubules. A reduction in the expression or activity of URAT1 and GLUT9 can lead to increased urinary excretion of uric acid, thereby contributing to lower serum uric acid levels (Zhao *et al*., 2022). Several flavonoids, such as luteolin, have been reported to reduce the expression of URAT1 and GLUT9 in the kidneys, thereby promoting increased

uric acid excretion and lowering serum uric acid levels. For instance, a study by Cheng-yuan & Jian-gang (2023) demonstrated that luteolin not only inhibits xanthine oxidase activity but also downregulates the mRNA expression of URAT1 and GLUT9, while upregulating the expression of OAT1 (Organic Anion Transporter 1) and OAT3 (Organic Anion Transporter 3), which are involved in uric acid excretion. This enhances the renal clearance of uric acid.

Therefore, flavonoids can lower uric acid levels through two distinct mechanisms: (1) by inhibiting uric acid production via xanthine oxidase inhibition, and (2) by enhancing uric acid excretion through the suppression of reabsorption transporters such as URAT1 and GLUT9. This dual mechanism highlights flavonoids as promising candidates for the development of effective and safe natural therapies for gout, a condition associated with hyperuricemia.

Polyphenol compounds are also known to reduce the risk of gout by lowering blood uric acid levels (Latief *et al*., 2021). Polyphenols may exert their uric acid-lowering effects through three primary mechanisms: inhibition of xanthine oxidase (XO) activity, enhancement of renal uric acid excretion, and antioxidant and anti-inflammatory actions (Behl *et al*., 2021; Li *et al*., 2024). Chronic hyperuricemia induces oxidative stress in the renal cortex, which can contribute to the development of chronic kidney disease (Zhang *et al*., 2023). As potent antioxidants, polyphenols help reduce oxidative stress by neutralizing free radicals generated by the elevated activity of endogenous enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Additionally, certain polyphenols can inhibit inflammatory signalling pathways, including NF-κB and MAPK, by suppressing the expression of pro-inflammatory cytokines such as TNF-α and IL-1β, which are known to exacerbate kidney damage associated with uric acid crystal deposition (Behl *et al*., 2021). Tannins are secondary metabolites believed to possess antihyperuricemic activity. In general, tannins are known for their potent antioxidant properties and their ability to neutralize free radicals involved in oxidative processes, including those in the purine-based uric acid biosynthesis pathway. Tannins are derivatives of saponins, which have also been reported to exhibit antihyperuricemic effects by inhibiting xanthine oxidase activity (Latief *et al*., 2021).

CONCLUSION

The administration of n-hexane, ethyl acetate, and water fractions of the ethanolic extract of *Chromolaena odorata* demonstrated antihyperuricemic activity, which is believed to be associated with the presence of secondary metabolites such as flavonoids, tannins, and polyphenols. Among the tested fractions, the ethyl acetate fraction at a dose of 80 mg/kg body weight exhibited the most effective antihyperuricemic activity, as indicated by the highest percentage reduction in uric acid levels compared to the other fractions.

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AUTHOR CONTRIBUTION

AWA: guidance of academic advisors
RR: guidance of academic advisors

ETHICS APPROVAL

The *in vivo* test had been approved by the Health Research Ethics Committee of Dr. Moewardi Regional Hospital, with ethical clearance number 227/II/HREC/2025.

CONFLICT OF INTEREST (If any)

None to declare

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