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Antihyperuricemic Activity of *Peperomia pellucida* (L.) Kunth Ethanol Extract and Its Effects on Relative Kidney Weight in Rats

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Abstract: This study aimed to evaluate the effectiveness of ethanolic extract of *Peperomia pellucida* in reducing blood uric acid levels while assessing its subchronic effects on relative kidney weight. An *in vivo* experiment was conducted using 24 male white rats (*Rattus norvegicus*), which were randomly allocated into six treatment groups (4 rats per group): a normal control (P0), a negative control receiving 0.1% Na-CMC (P1), a positive control receiving allopurinol at 1.8 mg/200 g body weight (BW) (P2), and three groups receiving *P. pellucida* extract at doses of 100 mg/kg BW (P3), 150 mg/kg BW (P4), and 200 mg/kg BW (P5). Hyperuricemia was induced by intraperitoneal administration of potassium oxonate at 250 mg/kg BW. Treatments were administered daily for 28 days, followed by measurement of final blood uric acid levels using Point-of-Care Testing and determination of the relative kidney organ index. Welch's ANOVA showed a significant effect of extract administration on the reduction in blood uric acid levels ($F(5, 7.739) = 14.630$; $p = 0.001$). ANCOVA further confirmed significant differences in treatment effectiveness among the groups ($F(5, 17) = 3.897$; $p = 0.015$). The 200 mg/kg BW dose (P5) produced the greatest reduction in uric acid levels, reaching 46.06 ± 10.80%, and was statistically comparable to the positive allopurinol control (50.95 ± 13.19%; $p > 0.05$). In contrast, the Kruskal–Wallis test showed no significant differences in the relative kidney organ index among groups ($H(5) = 4.262$; $p = 0.512$), with mean values ranging from 0.82 ± 0.06% to 0.96 ± 0.54%. These findings indicate that *P. pellucida* extract at 200 mg/kg BW effectively reduces blood uric acid levels without causing significant changes in relative kidney weight during 28 days of administration.
Keywords: *Peperomia pellucida*; uric acid; hyperuricemia; subchronic toxicity; kidney

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INTRODUCTION

Hyperuricemia is a metabolic condition characterized by elevated serum uric acid concentrations above the physiological range (Rosdiana et al., 2018). Uric acid is the final product of purine metabolism in humans, and its circulating concentration is determined by the balance between urate production and excretion. Persistent hyperuricemia promotes urate supersaturation and is the principal prerequisite for the formation and deposition of monosodium urate (MSU) crystals in joints and periarticular tissues. These crystals can initiate an intense inflammatory response, resulting in gout, which commonly manifests as acute joint swelling, erythema, severe pain, and stiffness, particularly in the first metatarsophalangeal joint (Nur, 2022; Dalbeth et al., 2021). Thus, hyperuricemia represents a major modifiable risk factor in the pathogenesis of gout, although not all individuals with hyperuricemia develop clinically apparent gout.

The global burden of gout has increased substantially over recent decades. According to the Global Burden of Disease Study 2021, approximately 55.8 million people were living with gout worldwide in 2020, representing a 150.6% increase in the number of prevalent cases compared with 1990. The global age-standardized prevalence was estimated at 659.3 cases per 100,000 population, with a substantially

higher prevalence among men than women (GBD 2021 Gout Collaborators, 2024). In Indonesia, joint diseases remain an important health problem, with Riskesdas (2018) reporting an overall prevalence of 7.30%, although this figure encompasses joint disorders broadly and should not be interpreted as the prevalence of gout specifically. Dietary purine intake, endogenous purine turnover, renal urate handling, genetic factors, and metabolic conditions collectively influence serum urate concentrations. In most individuals with sustained hyperuricemia, impaired renal urate excretion is considered a major underlying mechanism (Chen et al., 2016; Dalbeth et al., 2021).

Pharmacological management of gout and clinically relevant hyperuricemia commonly involves urate-lowering therapy. Allopurinol, a xanthine oxidase inhibitor, remains the preferred first-line urate-lowering therapy in current gout-management guidelines because it inhibits the conversion of hypoxanthine to xanthine and subsequently xanthine to uric acid (FitzGerald et al., 2020). Nevertheless, long-term pharmacotherapy may be associated with adverse drug reactions, including gastrointestinal disturbances, cutaneous reactions, and, in susceptible individuals, severe allopurinol hypersensitivity reactions (Imbar et al., 2019). These limitations, together with increasing interest in complementary therapies, have encouraged investigation of plant-derived compounds with urate-lowering potential. One medicinal plant of interest is *Peperomia pellucida* (L.) Kunth, commonly known in Indonesia as sirih cina.

Peperomia pellucida is a herbaceous species belonging to the family Piperaceae and has been traditionally used for various inflammatory and musculoskeletal complaints, including rheumatic pain (Koswara, 2025; Yuliana & Ami, 2020). Phytochemical investigations have identified several classes of secondary metabolites in this species, including flavonoids, tannins, alkaloids, saponins, terpenoids, and other phenolic constituents, which may contribute to its reported antioxidant and anti-inflammatory activities (de Fátima Arrigoni-Blank et al., 2004; Alves et al., 2019; Ho et al., 2022). Experimental evidence has also indicated antihyperuricemic activity of *P. pellucida* preparations in animal models. For example, previous studies reported reductions in serum uric acid following administration of *P. pellucida* extracts to hyperuricemic rats, although the extract type, dose, induction method, and treatment duration varied considerably among studies (Alvero et al., 1992; Sio et al., 2001; Imbar et al., 2019).

One plausible mechanism underlying the antihyperuricemic effect of plant-derived flavonoids is inhibition of xanthine oxidase, the enzyme responsible for the terminal reactions of purine catabolism. Xanthine oxidoreductase catalyzes the oxidation of hypoxanthine to xanthine and subsequently xanthine to uric acid; therefore, inhibition of this pathway can reduce uric acid production (Chen et al., 2016). Flavonoids such as quercetin and related compounds have been reported to interact with xanthine oxidase and may contribute to urate-lowering activity (Himawan, 2017). However, the pharmacological activity of *P. pellucida* should not be attributed to a single constituent without direct mechanistic confirmation, because its extracts contain multiple phytochemicals whose concentrations and biological activities may vary according to plant origin, extraction solvent, and processing conditions (Alves et al., 2019; Ho et al., 2022).

Despite its therapeutic potential, the efficacy of a medicinal plant must be evaluated together with its safety profile, particularly when repeated administration is intended. Toxicity is influenced by dose, duration of exposure, route of administration, and biological susceptibility. Previous investigations have generally suggested relatively low toxicity of *P. pellucida* extracts in experimental models; however,

differences in extract preparation, administered dose, and study design limit direct extrapolation between studies (de Fátima Arrigoni-Blank et al., 2004; Ho et al., 2022). Recent experimental evidence has also demonstrated the presence of antioxidant constituents in *P. pellucida* and reported no overt acute or chronic toxicity under specific experimental conditions, although these findings do not eliminate the need for organ-specific safety evaluation (Teodhora et al., 2024). Consequently, toxicity assessment remains important for characterizing dose–response relationships and identifying potential adverse effects associated with repeated exposure to *P. pellucida* extract (Irmayanti et al., 2025).

The kidney is particularly relevant to such an evaluation because it plays a central role in urate homeostasis and is also an important target organ for xenobiotic exposure. Renal urate transport contributes substantially to the regulation of circulating uric acid concentrations, and disturbances in renal urate handling are strongly associated with hyperuricemia (Dalbeth et al., 2021; Otani et al., 2023). In toxicological studies, relative organ weight is frequently used as an initial macroscopic indicator of treatment-related organ changes. However, changes in relative kidney weight alone cannot establish the presence or absence of nephrotoxicity and should ideally be interpreted together with biochemical markers such as serum creatinine and urea and with histopathological findings. Therefore, in the present study, relative kidney weight was used specifically as a preliminary macroscopic indicator of kidney safety rather than as a definitive measure of renal function.

The extract doses of 100, 150, and 200 mg/kg body weight were selected to investigate antihyperuricemic activity within a comparatively lower dose range while simultaneously monitoring an initial indicator of organ safety. Previous experimental studies of *P. pellucida* have employed markedly different dose ranges, including doses of 200 mg/kg body weight and substantially higher doses depending on the type of preparation and experimental model (Sio et al., 2001; Imbar et al., 2019). Therefore, evaluating several lower-dose levels may help clarify whether meaningful antihyperuricemic activity can be achieved without requiring higher extract exposure. Rather than assuming that these doses have never been investigated, the present study addresses the comparatively limited evidence regarding dose-dependent efficacy and repeated-dose safety within this specific dose range and experimental design.

Although previous studies have demonstrated the antihyperuricemic potential of *P. pellucida*, most have primarily evaluated reductions in uric acid levels, while relatively limited attention has been given to antihyperuricemic efficacy and preliminary kidney safety within the same repeated-dose experimental framework. This represents an important research gap because an effective urate-lowering intervention should ideally provide therapeutic benefit without inducing detectable adverse effects in organs involved in urate handling and xenobiotic elimination. Therefore, this study aimed to evaluate the antihyperuricemic activity of ethanolic *P. pellucida* extract at doses of 100, 150, and 200 mg/kg body weight in hyperuricemic male rats and, simultaneously, to assess relative kidney weight as an initial macroscopic indicator of organ safety following 28 days of administration. We hypothesized that *P. pellucida* extract would reduce blood uric acid levels in a dose-dependent manner without significantly altering relative kidney weight. This integrated evaluation is expected to provide additional preclinical evidence regarding both the efficacy and preliminary safety profile of *P. pellucida* as a potential herbal intervention for hyperuricemia.

METHOD

Study Design and Ethical Approval

This study was conducted over a six-month period, including a 28-day treatment period. Animal husbandry, preparation of *Peperomia pellucida* extract, blood uric acid measurements, treatment administration, and organ collection were carried out at the Integrated Laboratory Unit (UPT Laboratorium), Universitas Sebelas Maret (UNS), Indonesia.

The experiment employed a completely randomized design. A total of 24 male rats (*Rattus norvegicus*) were randomly assigned to six experimental groups, with four animals per group. The groups consisted of a normal control group (P0), which was not subjected to hyperuricemia induction; a negative control group receiving 0.1% sodium carboxymethyl cellulose (Na-CMC) (P1); a positive control group receiving allopurinol (P2); and three treatment groups receiving ethanolic extract of *P. pellucida* at doses of 100 mg/kg BW (P3), 150 mg/kg BW (P4), and 200 mg/kg BW (P5).

All experimental procedures involving animals were reviewed and approved by the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta, Indonesia, under Ethical Approval No. 453/III/HREC/2026.

Experimental Animals and Acclimatization

Healthy male *R. norvegicus* aged 2–3 months and weighing 150–200 g were used in this study. Animals were included if they were physically active and free from visible physical abnormalities. Rats that experienced >10% body-weight loss during the 7-day acclimatization period or became ill or died before or during the experimental period were excluded.

The minimum sample size was determined using the Federer formula: [(n-1)(t-1) ≥15], where *n* represents the number of animals per group and *t* represents the number of treatment groups. Based on six experimental groups, a minimum of four animals per group was required.

Animals were randomly allocated to the six treatment groups using simple random sampling based on a random-number generator. Before the experimental procedures, all rats were acclimatized to laboratory conditions for one week to allow adaptation to the environment and minimize stress that could affect experimental outcomes (Azhar, 2022). During acclimatization and the treatment period, general health status was monitored based on locomotor activity, fur appearance, body weight, food intake, and water intake.

Body-Weight Measurement

Rat body weight was measured using a digital balance (OHAUS) from week 0 to week 4. Serial body-weight measurements were used as part of the monitoring of potential subchronic toxic effects during the treatment period (Magfirah, 2020).

Botanical Identification of *Peperomia pellucida* (L.) Kunth

The plant used in this study was identified as *Peperomia pellucida* (L.) Kunth, a member of the family Piperaceae. The taxonomic classification was described according to Permadani (2024) as follows:

Kingdom	: Plantae	Genus	: *Peperomia*
Divisio	: Magnoliophyta	Species	: *Peperomia pellucida* (L.) Kunth
Classis	: Magnoliopsida		
Ordo	: Piperales		
Familia	: Piperaceae		

Figure 1. *Peperomia pellucida* (L.) Kunth used in the study

The botanical identity of *P. pellucida* was described with reference to published botanical information. A voucher specimen of *P. pellucida* documented in the scientific literature from the Icoaraci district of Belém, Brazil, is deposited under voucher number 190136 IAN.

Preparation of *Peperomia pellucida* Dried Plant Material

Peperomia pellucida samples were collected from areas surrounding the Universitas Muhammadiyah Surakarta (UMS) campus. The stems, leaves, and flowers were collected and washed thoroughly.

The plant material was dried under direct morning sunlight between 08:00 and 11:00 Western Indonesian Time (WIB). This procedure was used to facilitate moisture reduction, minimize fungal growth and undesirable enzymatic reactions, and limit prolonged exposure to ultraviolet radiation and excessive heat that could affect the stability of bioactive compounds. The dried plant material was subsequently ground using a blender to obtain powdered crude plant material (simplisia) (Andini, 2019).

Preparation of *Peperomia pellucida* Ethanolic Extract

A total of 400 g of powdered dried *P. pellucida* material was macerated with 96% ethanol for 3 × 24 h and maintained in a shaded area. Every 24 h, the macerate was filtered to obtain the filtrate, after which fresh solvent was added to the remaining plant material and the maceration process was repeated.

The filtrates obtained from successive extraction cycles were pooled and concentrated using a rotary evaporator at 60–70°C and 40 rpm for approximately 45–120 min until a concentrated extract was obtained. This evaporation process was intended to remove residual organic solvent from the extract (Purba, 2026).

Basis for Selection of Ethanol Concentration

The use of 96% ethanol was supported by previous findings on the extraction of flavonoids from *P. pellucida*. Pratiwi (2023) reported that qualitative analysis using magnesium powder and concentrated HCl produced a positive flavonoid reaction, indicated by reddish-brown coloration.

Quantitative analysis using UV–Vis spectrophotometry at 430 nm showed total flavonoid contents of 2.78576 g/100 g quercetin equivalent (QE) in the 70% ethanol extract, 5.132503 g/100 g QE in the 80% ethanol extract, and 6.77682 g/100 g QE in the 96% ethanol extract. The highest reported flavonoid content was therefore obtained using 96% ethanol (Pratiwi, 2023).

Extract Yield

The percentage extract yield was calculated by comparing the weight of the concentrated extract obtained after evaporation with the initial weight of dried plant material, according to the following equation:

$$\text{Extract yield (\%)} = \left(\frac{\text{Weight of Concentrated Extract (g)}}{\text{Weight of Dried Crude Plant Material (g)}}\right) \times 100\%$$

The initial amount of dried powdered *P. pellucida* used for extraction was 400 g.

Phytochemical Screening

Qualitative phytochemical screening was performed according to Permadani (2024) to detect alkaloids, steroids, triterpenoids, flavonoids, tannins, phenolic compounds, and saponins in *P. pellucida*.

1. Alkaloid test

The sample was crushed and mixed with 1 mL of ammonia solution and 10 mL of chloroform. The mixture was filtered, and the filtrate was extracted with 10 mL of 2 N sulfuric acid until two distinct layers formed. The acidic phase was separated into two test tubes. Mayer's reagent was added to the first tube, with the formation of a white precipitate indicating a positive reaction. Wagner's reagent was added to the second tube, with the formation of a yellow-to-brown precipitate indicating the presence of alkaloids.

2. Steroid and triterpenoid tests

A 10-g sample was ground and extracted using hot methanol, followed by partitioning with *n*-hexane. The *n*-hexane fraction was treated with Liebermann–Burchard reagent. A green or blue coloration indicated the presence of steroids, whereas red, pink, or brown coloration accompanied by precipitate formation indicated the presence of triterpenoids.

3. Flavonoid test

The methanolic extract was first partitioned with *n*-hexane to separate nonpolar compounds. The resulting residue was then partitioned with 10 mL of 80% ethanol. Approximately 0.5 mg of magnesium powder and several drops of 0.5 M HCl were added to the ethanolic filtrate. The formation of pink, dark-red, or purple coloration indicated the presence of flavonoids.

4. Tannin test

A 0.5-g portion of methanolic extract was heated in 10 mL of hot distilled water. The mixture was filtered, and several drops of 0.1% iron(III) chloride solution were added to the filtrate. The development of a brownish-green or blue-black color indicated the presence of tannins.

5. Phenolic Compound Test

Phenolic compounds were evaluated according to the procedure described by Permadani (2024). The development of a blackish-green or bluish-black coloration was considered indicative of phenolic compounds.

6. Saponin Test

The extract or sample residue was mixed with distilled water and vigorously shaken for 1 min. Several drops of 1 N HCl were subsequently added, and the mixture was allowed to stand for 30 min. Stable foam measuring approximately 1–10 cm in height and persisting for at least 30 min was considered a positive indication of saponins.

Preparation of 0.1% Na-CMC Solution

A total of 0.1 g sodium carboxymethyl cellulose (Na-CMC) was weighed using an analytical balance and dissolved in 100 mL of distilled water. The preparation was stirred using a hotplate magnetic stirrer and allowed to stand for approximately 15 min until homogeneous. The resulting preparation was transferred to a labeled bottle for subsequent use (Manengkey, 2020).

Induction of Experimental Hyperuricemia

Baseline and final blood uric acid levels were measured using an Easy Touch GCU meter. Experimental hyperuricemia was induced using potassium oxonate. Potassium oxonate was dissolved in 0.9% saline or 0.1% Na-CMC to obtain a concentration of 25 mg/mL.

Potassium oxonate was administered intraperitoneally at a dose of 250 mg/kg BW and an administration volume of 1 mL/100 g BW. The inducing agent was administered 1 h before administration of the test preparation for seven consecutive days to maintain the hyperuricemic condition.

Blood uric acid concentrations were monitored until they reached approximately 6–10 mg/dL, which was used as the criterion for experimental hyperuricemia (Subagja, 2021).

Allopurinol Administration

Allopurinol was administered orally according to the method described by Tayeb *et al.* (2016). A human allopurinol dose of 100 mg was converted to the equivalent dose for a 200-g rat using a conversion factor of 0.018, resulting in a dose of 1.8 mg per rat. Accordingly, the positive control group received allopurinol at a dose of 1.8 mg/200 g body weight (BW).

***Peperomia pellucida* Extract Treatment**

The experimental treatment groups received ethanolic extract of *P. pellucida* at doses of 100 mg/kg BW (P3), 150 mg/kg BW (P4), and 200 mg/kg BW (P5). Treatments were administered daily during the 28-day experimental period according to the assigned dose.

Measurement of Blood Uric Acid Levels

Blood uric acid levels were determined using the Point-of-Care Testing (POCT) method with an Easy Touch GCU meter (Ermiyanti, 2022). Measurements were performed at the initial and final experimental time points. Before blood collection, the rats were fasted for 12 h. Blood samples were obtained from the tail vein and immediately applied to uric acid test strips. Blood uric acid concentrations were subsequently recorded using the Easy Touch GCU meter.

Kidney Collection and Weighing

Kidneys were collected and weighed on day 28 as part of the assessment of possible subchronic effects associated with *P. pellucida* extract administration (Alipin, 2021). Following the final blood uric acid measurement, the rats were euthanized by cervical dislocation. The kidneys were subsequently excised and weighed using an analytical balance (Whidyastuti *et al*., 2019).

Relative Kidney Organ Index

The relative kidney organ index was determined by comparing kidney weight with the corresponding body weight of each animal, according to the following equation:

$$\text{Relative kidney organ index (\%)} = \frac{\text{Kidney Weight (g)}}{\text{Body Weight (g)}} \times 100$$

Relative kidney weight was used as an initial macroscopic indicator of potential treatment-related changes in the kidney. Because organ weight alone does not directly assess renal function or microscopic tissue damage, the parameter was interpreted as an initial indicator of kidney safety. A more comprehensive renal safety assessment would require complementary measurements such as serum creatinine, urea, and histopathological examination.

RESULTS AND DISCUSSION

Administration of *Peperomia pellucida* extract for 28 days affected blood uric acid levels in the experimental rats (Table 1). The greatest reduction in uric acid levels was observed in the P5 group receiving *P. pellucida* extract at 200 mg/kg BW, whereas the P1 negative-control group receiving 0.1% Na-CMC showed an increase rather than a reduction in uric acid levels. Based on the percentage changes presented in Table 1, the P5 group showed a 46.06% reduction, while the P1 group showed a −9.36% change.

Table 1. Mean blood uric acid levels and percentage changes in male rats administered *Peperomia pellucida* extract

Group	Initial uric acid level (mg/dL)	Final uric acid level (mg/dL)	Mean change (%) ± SD
P0 (control) + 2 mL distilled water	3.6 ± 0.4	3.35 ± 0.45	7.00 ± 3.85[a]
P1 (negative control + 0.1% Na-CMC)	8.7 ± 2.0	9.58 ± 3.32	−9.36 ± 27.92[ab]
P2 (positive control + allopurinol)	8.0 ± 2.0	3.75 ± 0.42	50.95 ± 13.19[b]
P3 (*P. pellucida* extract, 100 mg/kg BW)	6.7 ± 2.5	3.88 ± 1.27	39.70 ± 18.10[ab]
P4 (*P. pellucida* extract, 150 mg/kg BW)	5.7 ± 0.6	4.00 ± 0.92	30.01 ± 10.40[ab]
P5 (*P. pellucida* extract, 200 mg/kg BW)	6.2 ± 0.3	3.32 ± 0.26	46.06 ± 10.80[ab]

To evaluate the effect of treatment on blood uric acid levels, statistical analysis was performed after assessing the assumptions of normality and homogeneity of variance (Tables 2 and 3).

Table 2. Normality tests for blood uric acid levels in rats

Variable	Kolmogorov–Smirnov statistic	df	p-value	Shapiro–Wilk statistic	df	p-value	Interpretation
Uric acid level (mg/dL)	0.145	24	0.200	0.954	24	0.337	Normally distributed (p > 0.05)

As shown in Table 2, both the Kolmogorov–Smirnov and Shapiro–Wilk tests yielded p-values greater than 0.05. Therefore, the blood uric acid data were considered normally distributed, and the analysis proceeded to the homogeneity-of-variance test (Table 3).

Table 3. Homogeneity-of-variance test for blood uric acid levels

Variable	F	df1	df2	p-value	Interpretation
Uric acid level (mg/dL)	1.843	5	18	0.155	Homogeneous variance (p > 0.05)

The Levene test showed a p-value of 0.155 (p > 0.05), indicating that the assumption of homogeneity of variance was satisfied. The data were therefore further analyzed using analysis of covariance (ANCOVA) (Table 4).

Table 4. ANCOVA of blood uric acid levels in rats

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Corrected Model	80.030[a]	6	13.338	6.626	0.001
Intercept	25.446	1	25.446	12.641	0.002
AUakhir	14.413	1	14.413	7.160	0.016
Dose	39.225	5	7.845	3.897	0.015
Error	34.220	17	2.013	—	—

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Total	1119.170	24	—	—	—
Corrected Total	114.250	23	—	—	—

Dependent variable: Uric acid level (mg/dL)

The ANCOVA results indicate that the covariate was significantly associated with the outcome (F = 7.160; p = 0.016). As described in the original analysis, this finding indicates that baseline (*pretest*) uric acid levels significantly influenced final uric acid levels, supporting the methodological rationale for controlling baseline values using ANCOVA. After adjustment for baseline uric acid levels, a significant difference among treatment groups (P0–P5) remained (F = 3.897; p = 0.015). These findings indicate that treatment with *P. pellucida* extract was associated with significant differences in blood uric acid levels among the experimental groups.

Post hoc pairwise comparisons of estimated marginal means with Bonferroni adjustment showed that the positive-control group receiving allopurinol (P2) differed significantly from the distilled-water control *group* (P0). In contrast, comparisons between the positive-control group and the groups receiving *P. pellucida* extract at 100 mg/kg BW (P3), 150 mg/kg BW (P4), and 200 mg/kg BW (P5) showed no statistically significant differences. These results suggest that, within the conditions of this experiment, the uric acid-lowering effects of the three extract doses were statistically comparable to that of the allopurinol positive control.

The reduction in uric acid levels may be associated with the secondary metabolites present in *P. pellucida*, particularly flavonoids such as quercetin and luteolin, as well as alkaloids and tannins (Hulkiawar et al., 2022). Flavonoids may act as competitive inhibitors of xanthine oxidase. Their uric acid-lowering activity has been associated with inhibition of the oxidation of hypoxanthine to xanthine and subsequently of xanthine to uric acid (Marliana & Mariska, 2023).

In addition to flavonoids, other secondary metabolites in *P. pellucida*, including alkaloids, tannins, and saponins, have also been proposed to contribute to its uric acid-lowering activity. These compounds may inhibit serum xanthine oxidase activity while promoting uric acid excretion in urine. They may also exert antioxidant effects by scavenging free radicals generated during metabolic processes (Zustika et al., 2025). Furthermore, *P. pellucida* has been reported to exhibit mild diuretic activity, which may facilitate urinary uric acid excretion and contribute to a uricosuric effect (Agista, 2019; Hulkiawar et al., 2022; Marliana & Mariska, 2023).

Quercetin, one of the flavonoids associated with *P. pellucida*, has been reported to exhibit a strong binding affinity for the active site of xanthine oxidase. Molecular docking studies have also suggested favorable binding stability relative to allopurinol. Uric acid metabolism involves adenosine deaminase (ADA, EC 3.5.4.4), which converts adenosine to inosine. Purine nucleoside phosphorylase (PNP, EC 2.4.2.1) subsequently catalyzes the phosphorolysis of inosine to α-D-deoxyribose-1-phosphate and hypoxanthine. Hypoxanthine is then oxidized by xanthine oxidoreductase (XOR), which exists as xanthine oxidase (XO, EC 1.17.3.2) or xanthine dehydrogenase (XD, EC 1.17.1.4), ultimately resulting in uric acid formation. Quercetin-3′-sulfate has been proposed as an important quercetin metabolite involved in uric acid regulation through inhibition of XOR activity (Muharram et al., 2024).

Previous studies by Agista (2019) and Noer (2022) reported a dose-dependent relationship in the antihyperuricemic activity of *P. pellucida*, with 200 mg/kg BW producing a greater effect than 100 and 150 mg/kg BW. The present findings are generally consistent with those studies, as the 200 mg/kg BW group produced the

greatest reduction among the extract-treated groups, with a mean decrease of 46.06 ± 10.80%.

Relative Kidney Index

The relative kidney index was evaluated after 28 days of *P. pellucida* extract administration as an indicator of potential subchronic effects on the kidneys (Table 5). The highest mean relative kidney index was observed in P5, which received 200 mg/kg BW of the extract (0.96%), whereas the lowest mean value was observed in P0 (0.82%).

Table 5. Mean kidney weight and relative kidney index in male *Rattus norvegicus* administered *Peperomia pellucida* extract

Treatment group	Kidney weight (g)	Mean relative kidney index (%) ± SD
P0 (control) + 2 mL distilled water	2.00 ± 0.22	0.82 ± 0.06[a]
P1 (negative control + 0.1% Na-CMC)	1.85 ± 0.26	0.94 ± 0.07[a]
P2 (positive control + allopurinol)	2.05 ± 0.58	0.86 ± 0.16[a]
P3 (*P. pellucida* extract, 100 mg/kg BW)	2.05 ± 0.25	0.84 ± 0.13[a]
P4 (*P. pellucida* extract, 150 mg/kg BW)	2.20 ± 0.37	0.82 ± 0.09[a]
P5 (*P. pellucida* extract, 200 mg/kg BW)	1.90 ± 0.74	0.96 ± 0.54[a]

The distribution of the kidney data was assessed before inferential statistical analysis (Table 6).

Table 6. Normality tests for kidney data

Variable	Kolmogorov–Smirnov statistic	df	p-value	Shapiro–Wilk statistic	df	p-value	Interpretation
Kidney parameter	0.200	24	0.14	0.833	24	0.001	Not normally distributed ($p < 0.05$)

The Shapiro–Wilk test indicated a significant deviation from normality ($p = 0.001$). Therefore, the kidney data were analyzed using the nonparametric Kruskal–Wallis test rather than one-way ANOVA.

Table 7. Kruskal–Wallis test for kidney measurements

Parameter	Kruskal–Wallis H	df	Asymptotic p-value	Interpretation
Kidney weight/index	4.262	5	0.512	No significant difference ($p > 0.05$)

The Kruskal–Wallis test yielded an asymptotic p-value of 0.512, which was substantially greater than the significance threshold of 0.05. Thus, no statistically significant differences in kidney measurements were observed among the treatment groups. These results indicate that administration of *P. pellucida* extract at the tested doses for 28 days did not produce detectable differences in relative kidney weight among the groups.

Changes in organ weight, including marked increases associated with hypertrophy or decreases associated with atrophy, can serve as preliminary indicators of treatment-related organ toxicity (McSweeney et al., 2021). Based on the relative kidney indices presented in Table 5 and the absence of statistically significant differences among groups, the tested doses of *P. pellucida* did not produce detectable alterations in relative kidney weight. This finding is consistent with the report by Razoki et al. (2025), which indicated that active compounds associated with *P. pellucida* at

doses of up to 200 mg/kg BW did not induce inflammatory or necrotic changes associated with organ enlargement at the tested doses. The absence of detectable changes in relative kidney weight may also be related to bioactive constituents such as flavonoids, which possess antioxidant activity and may contribute to protection against oxidative stress (Fadira, 2025).

Physiologically, the absence of significant differences in relative kidney weight indicates that treatment did not produce gross changes consistent with marked renal hypertrophy or atrophy during the 28-day administration period. However, relative organ weight should be interpreted only as a preliminary macroscopic indicator of organ safety. The present findings therefore do not establish the absence of nephrotoxicity or confirm preservation of renal function. A comprehensive assessment of renal safety requires complementary biochemical measurements, including serum creatinine and urea, together with histopathological examination of kidney tissue. Such analyses are particularly important because the kidney is highly susceptible to xenobiotic exposure, and functional or microscopic alterations may occur in the absence of detectable changes in organ weight (Ceriana & Rejeki, 2023).

CONCLUSION

The ethanol extract of *Peperomia pellucida* exhibited antihyperuricemic activity in the potassium oxonate-induced hyperuricemic rat model. Among the tested extract doses, 200 mg/kg BW produced the greatest reduction in blood uric acid levels (46.06 ± 10.80%), with an effect that was statistically comparable to that of the allopurinol-treated positive control. Administration of *P. pellucida* extract for 28 days did not result in significant differences in the relative kidney weight among the treatment groups, suggesting that the tested doses did not induce detectable changes in this macroscopic renal parameter. However, the absence of changes in relative kidney weight alone is insufficient to establish comprehensive renal safety. Further studies incorporating renal biochemical markers, such as serum creatinine and urea, together with histopathological examination, are required to confirm the renal safety profile of *P. pellucida* during repeated administration.

RECOMMENDATION

Based on the findings of this study, the following recommendations are proposed:

1. Development as a Phytopharmaceutical Product: The dose and preparation of *P. pellucida* extract should be further standardized to support its development as a standardized herbal medicine or phytopharmaceutical product that is safe and effective for human use.
2. Clinical Evaluation in Humans: Subsequent studies should include clinical trials in human participants to determine the efficacy and safety of equivalent doses and to establish an appropriate therapeutic dosage range.
3. Long-Term Safety Studies: Further research is required to evaluate the effects of prolonged *P. pellucida* administration on other vital organs, particularly the liver, as well as on overall metabolic function.
4. Public Education: The findings may contribute to public education regarding the potential use of *P. pellucida* as a complementary herbal approach for the management of hyperuricemia. However, its use should remain evidence-based and should consider appropriate dosing and safety precautions.

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