Trop J Nat Prod Res, February 2025; 9(2): 664 - 669 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

Effect of *Kaempferia galanga* L. on Xanthine Oxidase Activity and Arterial Stiffness in Hyperuricemia

Patonah Hasimun*, Yoga A. R. Muzaki, Jajang J. Sodik, Ika K. Sukmawati, Herni Kusriani

Pharmacology and Clinical Pharmacy Research Group, Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia.

ARTICLE INFO

Article history:
Received 22 December 2024
Revised 31 December 2024
Accepted 01 February 2025
Published online 01 March 2025

ABSTRACT

Hyperuricemia, characterized by excess uric acid production or reduced uric acid excretion, is a significant risk factor for kidney and cardiovascular diseases. The enzyme xanthine oxidase (XOin) plays a crucial role in uric acid production, and contributes to oxidative stress and inflammation. This study aimed to evaluate the XO inhibitory potential of *Kaempferia galanga* L. ethanol extract (KGEE) and its effects on arterial stiffness in hyperuricemic rats. Hyperuricemia was induced by placing the animals on 20% fructose in drinking water for 28 days, followed by potassium oxonate injection (4.5 mg/kg, stat). Twenty-five rats were divided into five groups: normal control, positive control (hyperuricemic rats), allopurinol (1.8 mg/kg), and two KGEE groups (50 and 100 mg/kg). After 28 days of treatment, XO inhibitory activity of liver homogenate and serum uric acid concentration were measured. Arterial stiffness was measured on days 0, 14, and 28 using the pulse wave velocity method. XO inhibitory activity of kaempferol a flavonoid in KGEE was assessed *in silico* by molecular docking simulation. KGEE demonstrated XO inhibitory activity *in vitro* with IC_{50} of 31.47 µg/mL. KGEE stimulated XO inhibition in rats' liver, resulting in percentage inhibition of 79.38% at 100 mg/kg. The extract significantly reduced uric acid levels and improved arterial stiffness, compared to the positive control. *In silico* molecular docking simulations indicated that kaempferol may act as XO inhibitor. Therefore, KGEE shows promise as an effective XO modulator and a potential biomarker for endothelial dysfunction in hyperuricemia, contributing to the amelioration of arterial stiffness. .

Keywords: *Kaempferia galanga* L., Xanthine oxidase, Hyperuricemia, Arterial stiffness, Pulse wave velocity.

Introduction

Hyperuricemia is characterized by elevated uric acid levels, and is associated with cardiovascular diseases and is often observed in patients with metabolic syndrome. Hyperuricemia increases the risk of hypertension, diabetes, and other health issues.[1,2] In 2017, approximately 7.44 million cases of hyperuricemia were associated with metabolic syndrome globally, with a higher prevalence in men (10%) compared to women (6%).[3-5] In Indonesia, the prevalence of hyperuricemia is 18%, with the highest rate (54.8%) found in individuals aged 75 years and older.[6]

Arterial stiffness, a hallmark of vascular aging, is associated with hyperuricemia due to oxidative stress and systemic inflammation caused by elevated uric acid levels. Even though uric acid is typically an antioxidant, it can become harmful at high levels, leading to vascular damage by reducing nitric oxide (NO) availability, promoting endothelial dysfunction, and increasing vascular tone. The renin-angiotensin-aldosterone system contributes to vascular fibrosis, and hyperuricemia-induced oxidative stress, which further increases arterial stiffness through the production of endothelin-1, a potent vasoconstrictor.[7]

*Corresponding author. E mail: patonah@bku.ac.id
Tel: +628562201473

Citation: Hasimun P, Muzaki A.R.Y, Sodik J.J, Sukmawati K.I, Kusriani H. Effect of *Kaempferia galanga* L. on Xanthine Oxidase Activity and Arterial Stiffness in Hyperuricemia. Trop J Nat Prod Res. 2025; 9(2): 664 – 669 https://doi.org/10.26538/tjnpr/v9i2.31

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

Kaempferia galanga L., a plant from the Zingiberaceae family, is believed to have anti-hyperuricemic properties, mainly through the inhibition of xanthine oxidase (XO).[8] The bioactive compound kaempferol found in the plant's rhizome exhibits anti-inflammatory and vasorelaxant properties.[9,10] Kaempferol has demonstrated a variety of pharmacological benefits, with several studies confirming its ability to inhibit XO. Studies have suggested that *Kaempferia galanga* L. extract reduced inflammatory markers such as TNF-α and IL-1β.[11] Other investigations further supported its role in lowering uric acid levels in hyperuricemic animal models.[12] Therefore, this study aimed to investigate the anti-hyperuricemic effects of *Kaempferia galanga* L., its potential to inhibit XO activity, and its impact on arterial stiffness and vascular health in hyperuricemic animal model.

Materials and Methods

Plant collection and identification

The rhizomes of *Kaempferia galanga* L. were sourced from the Indonesian Spices Medicinal and Aromatic Plants Instrument Standard Testing Institute in Bogor, West Java, Indonesia in December 2023. The plant material was identified at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, West Java, Indonesia. Voucher number: 31/HB/02/2024 was assigned.

Phytochemical analysis

Phytochemical screening was performed to identify the presence of secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, and steroids-triterpenoids.[13]

Preparation of extract

Kaempferia galanga rhizomes (2000 g) were cleaned, sliced, and dried at 37°C. The dried material was ground and macerated in 20 L of 96% ethanol for 3 days in a dark place. The resulting extract was filtered and concentrated using a rotary evaporator at 40°C.

Determination of in vitro xanthine oxidase inhibitory activity

The *in vitro* xanthine oxidase (XO) inhibitory activity of KGEE was determined using the spectrophotometric method. In a 96-well microplate was added 35 µL phosphate buffer (pH 7.5), followed by 30 µL of XO (0.2 U/mL) and 50 µL of KGEE (15.625; 31.25; 62.5; 125; 250; 500 µg/mL). the buffer (115 µL) was used as blank, while a mixture of 85 µL of buffer and 30 µL of XO was used as the control. Allopurinol at the sample concentrations as the test extract was used as the standard (positive control). The plate was incubated at 25°C for 15 minutes. Xanthine substrate (60 µg/mL) was then added and incubated for another 30 minutes, followed by 25 µg/mL I M HCl. The absorbance of the resulting mixture was measured at 290 nm using a microplate reader. The determination was done in triplicate. The percentage XO inhibition was calculated using the following formula:
XO inhibition (%) = [(A - B) / A] × 100.
Where;
A is the absorbance of control minus absorbance of blank,
B is the absorbance of sample minus absorbance of blank.

Animals

Twenty-five male Wistar rats, 2-3 months old and weighing 200-250 g, were acclimatized to the laboratory condition (24–26°C, 70–75% humidity, and a 12-hour light-dark cycle). The animals were fed with standard rodent pellets and had unrestricted access to drinking water. All procedures adhered to ethical guidelines for the use and handling of experimental animals. Ethical approval was given by the Faculty of Medicine, Padjadjaran University, Bandung (registration number 115/UN6.KEP/EC/2024).

Induction of hyperuricemia and animal grouping

Hyperuricemia was induced by administering 20% fructose in drinking water for 28 days, followed by a potassium oxonate injection on the final day. The rats were divided into five groups of 5 animals each, namely; normal control group (not hyperuricemic), positive control group (hyperuricemic rats but not treated), allopurinol group (hyperuricemic rats, received allopurinol 1.8 mg/kg), and *Kaempferia galanga* L. ethanol extract (KGEE) groups (hyperuricemic rats, received KGEE at doses of 50 and 100 mg/kg) for 28 days. All groups, except the normal control group had hyperuricemia induction.

Determination of xanthine oxidase inhibitory activity using rat liver homogenate

A 20% liver homogenate from all treatment groups was reacted with xanthine substrate at concentrations of 0.13, 0.26, 0.39, and 0.53 mM in phosphate buffer (pH 7.5). The reaction mixture was incubated for 15 min, after which 0.2 U/mL of xanthine oxidase was added, then incubated for 30 min, and the absorbance of the resulting mixture was measured using a spectrophotometer at 576 nm. The buffer solution was used as blank, while the buffer + enzyme without the test sample was used as the control.

Measurement of blood uric acid level

The blood uric acid level was measured on days 0 and 28. Blood samples were collected from the orbital sinus. The blood samples were centrifuged at 3000 rpm for 5 minutes to separate plasma. A 5 µL plasma sample was mixed with 500 µL of uric acid Reagent Kit (ProLine®) and incubated at 20-25°C for 10 minutes. Uric acid levels were measured using a Microlab 300 UV spectrophotometer at 239 nm.

Measurement of arterial stiffness

Arterial stiffness was measured on days 0, 14, and 28 using the pulse wave velocity (PWV) method as described by Zakaria and Hasimun (2017).[14] To perform PWV measurements, the rats were first anaesthesized by placing them in an anaesthesia chamber, after the animals became unconscious, they were transferred to the PWV compartment. An electrocardiogram sensor was used to record the heart electrical activity by placing the electrodes on the right, left, and hind paw. In contrast, a photoplethysmography sensor was placed at the tip of the tail to monitor blood flow changes. Increased PWV values indicated greater arterial stiffness.

Molecular docking validation of xanthine oxidase inhibitory activity

Molecular docking validation was performed using AutoDock version 4.2.3. The protein (XOR) was pre-prepared, and the native ligand was separated from the protein using Discovery Studio, after which it was re-docked into the target protein. The grid centre was positioned nearly at the centre of the ligand, encompassing all residues in the binding site. The grid box was determined by setting the central region of the native ligand and performing docking with a maximum number of genetic algorithms (GA).[15] The number of GAs run was set to 100, a medium number of evaluations, and the algorithm used was the Lamarckian Genetic Algorithm (LGA). The docking process was considered valid when the Root Mean Square Deviation (RMSD) was ≤ 2Å.[16]

Molecular docking simulation

The molecular docking simulation of test ligand with the target protein was performed using AutoDock version 4.2.3. The grid box settings were adjusted according to the size and area determined from the validation results. The docking results were then interpreted by examining the ΔG (binding free energy) and KI (inhibition constant).

Molecular docking visualization

The Discovery Studio Visualizer 2016 and Visual Molecular Dynamics applications were used to examine the binding interactions between the test ligand and the target protein. Docking results were visualized by showing the interactions between the ligand and the protein, as well as the intermolecular bonds with amino acid residues of the target protein.[16]

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of five replicates. Differences between means were determined using one-way Analysis of Variance (ANOVA) with SPSS 25.0 software. P-value < 0.05 was considered statistically significant.

Results and Discussion

Extract yield

The extract yield from *Kaempferia galanga* L. was 11.79%, which met the Indonesian Herbal Pharmacopoeia's standard of over 10% for a good yield. This high yield suggested a greater concentration of bioactive compounds in the extract.

Phytochemical constituents of Kaempferia galanga

The phytochemical screening of *Kaempferia galanga* rhizomes identified alkaloids, flavonoids, tannins, and triterpenoids in the ethanol extract. This result is consistent with previous studies which showed that *Kaempferia galanga* contained flavonoids with XO inhibitory activity.[17,13]

Xanthine oxidase inhibitory activity

The *in vitro* and *in vivo* xanthine oxidase inhibitory activity of *Kaempferia galanga* extract are presented in Table 1 and Figure 1. Results were presented in terms of IC_{50} values (Table 1) and percentage inhibition (Figure 1). The *in vitro* result showed that *Kaempferia galanga* extract exhibited a dose-dependent xanthine oxidase inhibitory activity. Higher concentrations of KGEE resulted in higher percentage xanthine oxidase inhibition. At the lowest concentration of 15.265 µg/mL, KGEE showed an average percentage xanthine oxidase inhibition of 34.5%, and this value increased significantly as the concentration of KGEE increased. KGEE had an IC_{50} value of 31.47 µg/mL, while allopurinol, the standard xanthine oxidase inhibitor used

in this study had an IC_{50} value of 1.54 µg/mL, which is much lower compared to that of KGEE. A lower IC_{50} value, indicate a higher xanthine oxidase inhibitory activity.

Table 1: *In vitro* xanthine oxidase inhibitory activity of *Kaempferia galanga* extract

Replicate	IC_{50} (µg/mL)	
	Allopurinol	**KGEE**
1	1.01	30.98
2	1.70	30.08
3	1.90	33.34
Mean ± SD	1.54 ± 1.47	31.47 ± 1.67

KGEE = *Kaempferia galanga* Ethanol Extract

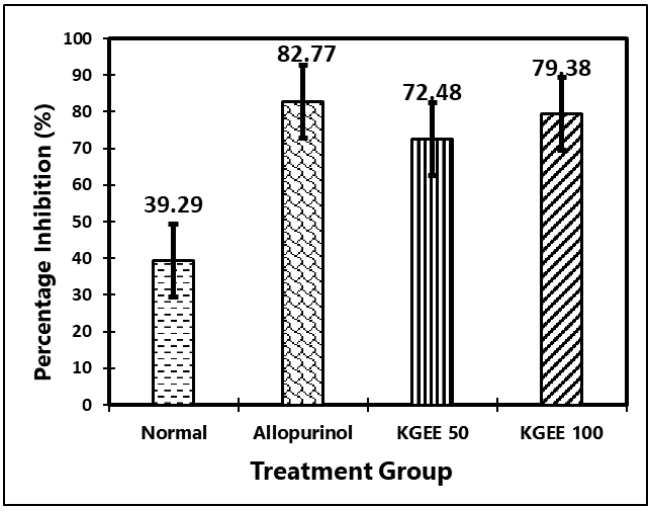

Figure 1: *In vivo* **x**anthine oxidase inhibitory activity of *Kaempferia galanga* extract

Similarly, Figure 1 shows that KGEE stimulated xanthine oxidase inhibition in the rat liver, resulting in percentage inhibition of 72.48% and 79.38% at 50 and 100 mg/kg doses, respectively. These values were significantly ($P < 0.05$) higher than that of the normal control group, which showed a percentage inhibition of 39.29%. However, the standard drug allopurinol had a significantly ($P<0.05$) higher inhibition of 82.77% than the KGEE groups.

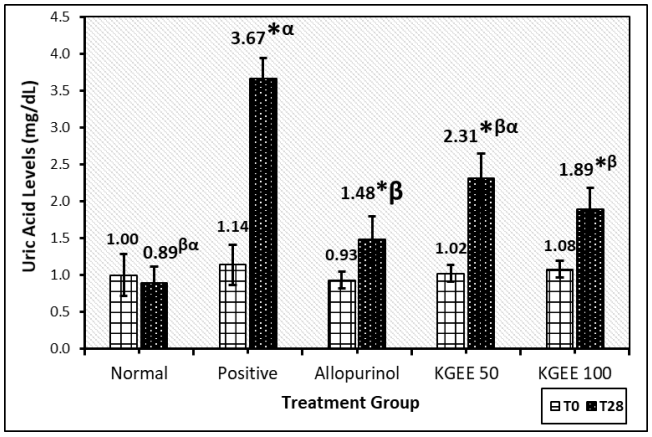

Figure 2: Uric acid levels in the different groups. KGEE: *Kaempferia galanga* L. ethanol extract 50 mg/kgBW, 100 mg/kgBW; (*) Statistically significant difference from the normal control group ($p<0.05$); (β) Statistically significant difference from the positive control (untreated hyperuricemic rats) ($p<0.05$); (α) Statistically significant difference from the Allopurinol group ($p<0.05$).

Xanthine oxidase (XO) is an enzyme that converts hypoxanthine to uric acid, generating reactive oxygen species (ROS). Dysregulation of XO can lead to hyperuricemia, gout, and oxidative tissue damage.[12] KGEE effectively inhibited XO likely through antioxidant and anti-inflammatory mechanism. Also, flavonoids in KGEE might help lower proinflammatory cytokines and increase the activity of antioxidant enzymes like superoxide dismutase and catalase.

Inhibition of XO is an effective way of reducing uric acid levels. Flavonoids in KGEE, such as quercetin and kaempferol bound to the XO active site, reducing their interaction with substrates and decreasing uric acid production.[13] It has also been shown that ethyl-p-methoxycinnamate isolated from *Kaempferia galanga* L. extract inhibited cyclooxygenase-1 and -2, contributing to the anti-inflammatory effects and supporting its role in reducing uric acid levels.[18]

Effect of Kaempferia galanga extract on serum uric acid levels

Figure 2 shows the serum uric acid levels in all groups on day zero (T0) and day 28 (T28). The result showed that the uric acid levels were not significantly different among all the groups at T0. However, at T28, significant differences ($p < 0.05$) were observed in the serum uric acid levels between the extract-treated groups and the positive control group, confirming that the induction of hyperuricemia was successful. There was a significant reduction in uric acid levels in allopurinol group compared to the positive control ($p<0.05$). Similarly, both KGEE 50 mg/kg and 100 mg/kg significantly ($p<0.05$) reduced uric acid levels, with values of 2.31 mg/dL and 1.89 mg/dL, respectively compared to the positive control group, which had uric acid concentration of 3. 67 mg/dL. This indicated that KGEE had the potential to lower uric acid in condition of hyperuricemia.

Potassium oxonate functions as a competitive inhibitor of uricase. By inhibiting uricase activity in rodents, potassium oxonate mimics the human metabolic condition of uric acid accumulation. This leads to an artificial elevation of serum uric acid levels, creating a reproducible and reliable model of hyperuricemia. Potassium oxonate inhibit uricase, which leads to hyperuricemia by preventing the conversion of uric acid to allantoin.[19] Hyperuricemia induces arterial stiffness through inflammation and oxidative stress, increasing collagen density and reducing elasticity.[20] Furthermore, uric acid activates the NLRP3 inflammasome, leading to the release of proinflammatory cytokines IL-1β and IL-18.[21] It has been reported that *Kaempferia galanga* extract inhibited the NF-κB pathway, thereby reducing inflammation and cytokines like TNF-α and IL-6.[22] Higher doses of *Kaempferia galanga* extract were more effective due to their XO inhibition, antioxidant, and anti-inflammatory properties.[22] Allopurinol reduces uric acid levels and free radical production, thereby reducing oxidative stress and inflammation.[23] Recent studies have shown that allopurinol improved endothelial function and reduced arterial stiffness, as evidenced by decreased PWV value in hyperuricemia.[24,25]

Effect of Kaempferia galanga extract on arterial stiffness

This study evaluated the anti-hyperuricemic effect of KGEE in hyperuricemic rats using PWV as the primary indicator of arterial stiffness. PWV is a reflection of arterial elasticity, with higher values indicating reduced elasticity and increased cardiovascular risk. Figure 3 shows the PWV values in hyperuricemic rats treated with KGEE and allopurinol compared to the controls. The result showed that in the normal control group, PWV remained stable from T0 to T28, which suggested that arterial elasticity remained unchanged under normal condition (that is without hyperuricemia). In contrast, in the positive control group (potassium oxonate-induced hyperuricemia), PWV values significantly increased from T0 to T28, which indicated that 20% fructose-induced hyperuricemia led to arterial stiffness. Conversely, allopurinol-treated group showed a significantly reduced PWV from T14 to T28. This therefore emphasized the protective effect of allopurinol against arterial stiffness. KGEE at 50 and 100 mg/kg effectively prevented PWV increase, with the 100 mg/kg dose being more effective, making it the optimal dose for reducing arterial stiffness and potentially lowering cardiovascular disease risk.

The effect of KGEE on arterial stiffness can be mediated through antioxidant and anti-inflammatory mechanisms. It has been shown from

previous studies that KGEE mitigated oxidative stress by inhibiting Inducible Nitric Oxide Synthase (iNOS) involved in inflammation.

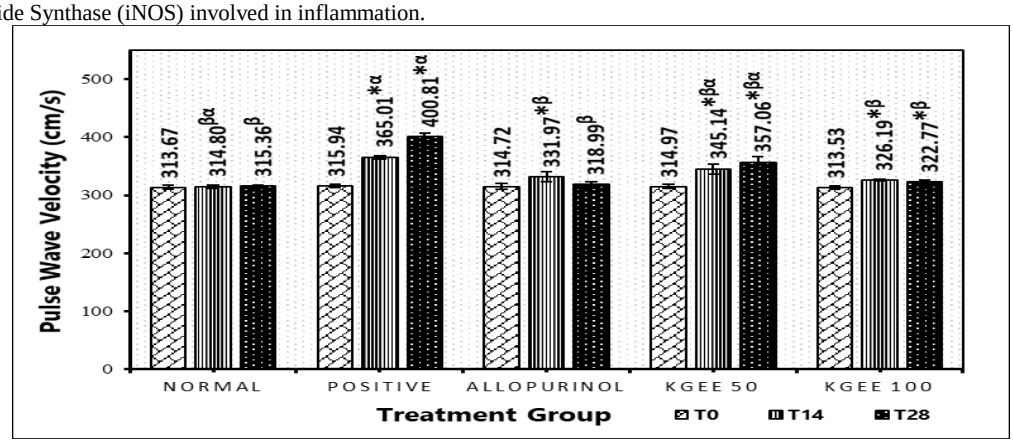

Figure 3: Pulse wave velocity **(**PWV) in all the groups. KGEE: *Kaempferia galanga* L. ethanol extract 50 mg/kgBW, 100 mg/kgBW; (*) Statistically significant difference from the normal control group ($p<0.05$); (β) Statistically significant difference from the positive control group (untreated hyperuricemic rats) ($p<0.05$); (α) Statistically significant difference from the allopurinol group ($p<0.05$).

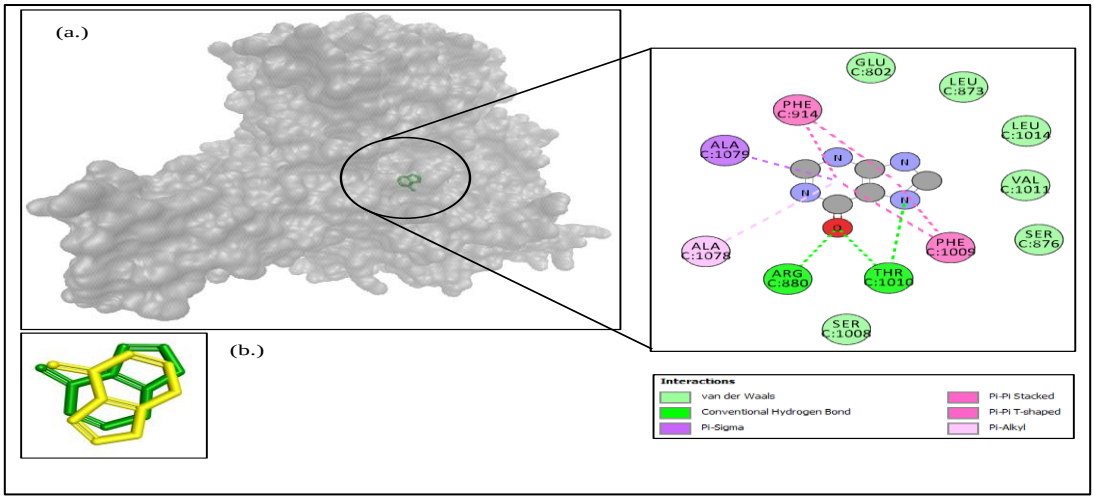

Figure 4: Molecular docking validation **(a)** visualization of native ligand-protein (XOR) complex, **(b)** Visualization overlay. Root Mean Square Deviation (RMSD) was ≤ 2Å.

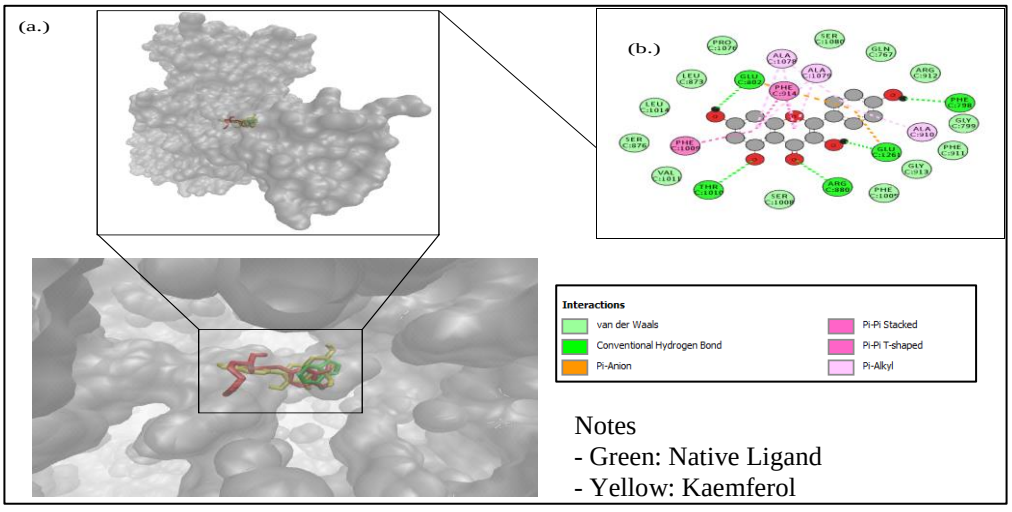

Figure 5: Molecular docking visualization of ligand-protein interactions: **(a)** Native ligand-XOR, and **(b)** Kaempferol-XOR. The native ligand exhibited a ΔG value of -4.87 kcal/mol, while kaempferol demonstrated a ΔG value of -8.97 kcal/mol. Docking analysis revealed that the native ligand, with a ΔG of -4.87 kcal/mol, possesses moderate binding affinity. Moreover, kaempferol, with a ΔG of -8.97 kcal/mol, exhibited a stronger

binding affinity and a more stable interaction with the xanthine oxidase (XOR) active site. The significantly lower ΔG value of kaempferol highlights its potential as a more effective inhibitor compared to the native ligand.

Since iNOS produced large amounts of nitric oxide (NO) during inflammation, its inhibition by KGEE reduced excessive NO production, thereby decreasing necrosis and oxidative stress.[26,27]

Kaempferia galanga bioactive compound such as trans-ethyl p-methoxycinnamate has been shown to exhibit potent antioxidant activity by reducing free radicals and ROS. This decrease in ROS levels minimized oxidative stress and lowered iNOS activation. The compound also inhibited the NF-κB signalling pathway, reducing pro-inflammatory cytokine production. Furthermore, *Kaempferia galanga* has been shown to enhance the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which contributed to lowering ROS levels and reduce the need for iNOS induction. The combination of these effects might have contributed to the ability of *Kaempferia galanga* extract to alleviate hyperuricemia and reduce endothelial dysfunction.[26,27]

Molecular docking data

Molecular docking validation was performed by docking the natural ligand with the target protein and then comparing the binding capacity of the natural ligand before and after docking (Figures 4 and 5), a process known as redocking. This was quantified using RMSD. For the target protein xanthine oxidoreductase (XOR), molecular docking validation produced an RMSD value of 0.248 Å, which indicated that the results met one of the validation criteria which states that molecular docking validation is considered valid when RMSD is ≤ 2 Å. This suggested that the docking method provided minimal deviation and was suitable for further docking simulation of the test ligand.[15]

Interactions between the enzyme and the natural ligand could include hydrogen bonds, hydrophobic interactions, and van der Waals forces, all of which are types of intermolecular bonds (Figure 4). Intermolecular bonds are crucial for the interaction between drugs and their targets.[28] Important amino acid residues in the target XOR binding site include Arg880 and Glu802.[29,30] The results of the redocking interactions showed that the natural ligand interacted with these amino acid residues, forming hydrogen bonds with Arg880. The presence of hydrogen bonds indicated the accuracy of the intermolecular interaction predictions, ensuring that the resulting complex was the most suitable and stable. The interaction results confirmed that molecular docking was valid.[31] The ligand's conformational fit with specific amino acid residues and RMSD value ≤ 2 Å validated the subsequent test ligand (kaempferol) docking parameters.

Molecular docking simulations for kaempferol were performed to evaluate the interaction and affinity of kaempferol for the active site of XOR. Test ligands with an inhibition constant value of less than 100 μM are considered potential inhibitors, while those with an inhibition constant greater than 100 μM are regarded as weak inhibitors.[32] Kaempferol exhibited stronger interactions than the native ligand, with an ΔG value of -4.87 kcal/mol for the native ligand and -8.97 kcal/mol for kaempferol. These results showed that kaempferol formed a relatively stronger binding than the native ligand (Figure 5). This suggested that kaempferol has a high potential to be an active compound for drug development. The negative binding energy value indicate that the ligand could interact with the target protein with minimal energy, and a low free binding energy signify that the ligand-protein complex is stable.[33]

The molecular docking visualization results of the kaempferol ligand with XOR showed interactions with crucial amino acid residues, namely Arg880 and Glu802 (Figure 4), forming hydrogen bonds. Hydrogen bonds are interactions between hydrogen atoms and fluorine, oxygen, or nitrogen atoms. They are stronger and more specific than hydrophobic interactions because they can influence the physicochemical properties of a compound, such as boiling point, solubility, melting point, and acidity, which can affect the biological activity of the compound.[34] The ligand's dual function as both a therapeutic agent and a potential biomarker for hyperuricemia is evidenced by its targeted interaction with xanthine oxidase. This interaction mirrors the biochemical condition of hyperuricemia, as the ligand's activity or binding affinity can be linked to uric acid concentrations.[35]

Conclusion

In conclusion, KGEE showed potential as an effective modulator of xanthine oxidase, which may serve as a biomarker for endothelial dysfunction in hyperuricemia and play a role in ameliorating associated arterial stiffness.

Conflicts Of Interest

The authors declare no conflicts 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.

Acknowledgments

The authors thank the Directorate of Research and Community Service at Bhakti Kencana University for financial support through the Internal Excellence Research Grant (number: 038/PEN.DRPM/UBK/VII/2024).

References

1. Copur S, Demiray A, Kanbay M. Uric acid in metabolic syndrome: Does uric acid have a definitive role? Eur J Intern Med. 2022;103:4–12.
2. DiPiro JT, Wells BG, Schwinghammer TL, DiPiro CV. Pharmacotherapy: A Pathophysiologic Approach. 11th ed. United States: McGraw-Hill Education; 2020.
3. Institute for Health Metrics and Evaluation (IHME). Findings from the Global Burden of Disease Study 2017 [Online]. Seattle, Washington: IHME; 2018 [cited 2025 Jan 9]. Available from: https://www.healthdata.org/research-analysis/library/findings-global-burden-disease-study-2017.
4. World Health Organization. World Health Statistics 2016: Monitoring Health for the SDGs. Geneva: WHO; 2016. 1–121p.
5. Li L, Zhang Y, Zeng C. Update on the epidemiology, genetics, and therapeutic options of hyperuricemia. Am J Transl Res. 2020; 12(7):3167–3181.
6. Ministry of Health, Republic of Indonesia. Basic Health Research (RISKESDAS) Report 2018. Jakarta: Ministry of Health, Republic of Indonesia; 2018.
7. Albu A, Para I, Porojan M. Uric acid and arterial stiffness. Ther Clin Risk Manag. 2020; 16:39-54.
8. Kumar A. Phytochemistry, pharmacological activities and uses of traditional medicinal plant Kaempferia galanga L. – An overview. J Ethnopharmacol. 2020; 253:112667.
9. Yao F, Huang Y, Wang Y, He X. Anti-inflammatory diarylheptanoids and phenolics from the rhizomes of kencur (*Kaempferia galanga* L.). Ind Crops Prod. 2018; 125:454-461.
10. Mahobiya A, Singh TU, Rungsung S, Kumar T, Chandrasekaran G, Parida S, Kumar D. Kaempferol induces vasorelaxation via endothelium-independent pathways in the rat-isolated pulmonary artery. Pharmacol Rep. 2018; 70(5):863-874.
11. Lallo S, Hardianti B, Sartini S, Ismail I, Laela D, Hayakawa Y. Ethyl p-methoxycinnamate: An active anti-metastasis agent and chemosensitiser targeting NFκB from

Kaempferia galanga for melanoma cells. Life. 2022; 12(3):337.

12. Chen D, Li H, Li W, Feng S, Deng D. *Kaempferia parviflora* and its methoxyflavones: Chemistry and biological activities. Evid Based Complement Alternat Med. 2018; 2018:4057456.

13. Julianti TB, Bakar MFA, Wikantyasning ER. Phytochemical, Antioxidant Analysis and In Vitro Xanthine Oxidase Inhibitory Activity of Kaempferia parviflora and *Kaempferia galanga*. Trop J Nat Prod Res. 2022; 6(12):1981–1985.

14. Zakaria H and Hasimun P. Non-invasive pulse wave velocity measurement in mice. In: 2017 International Seminar on Sensors, Instrumentation, Measurement and Metrology (ISSIMM). IEEE; 2017. 95–98 p.

15. Morris GM, Huey R, Olson AJ. UNIT using AutoDock for ligand-receptor docking. Curr Protoc Bioinformatics. 2008. 1–40 p.

16. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153(6):1194-1217.

17. Ministry of Health of the Republic of Indonesia. Indonesian Herbal Pharmacopoeia Edition II. Jakarta: Ministry of Health of the Republic of Indonesia; 2017.

18. Umar MI, Asmawi MZ, Sadikun A, Atangwho IJ, Yam MF, Altaf R, Ahmed A. Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from *Kaempferia galanga* L. extracts. Molecules. 2012; 17(7):8720–8734.

19. Xu L, Lin G, Yu Q, Li Q, Mai L, Cheng J, Xie J, Liu Y, Su Z and Li Y. Anti-Hyperuricemic and Nephroprotective Effects of Dihydroberberine in Potassium Oxonate- and Hypoxanthine-Induced Hyperuricemic Mice. Front Pharmacol. 2021; 12:1–13.

20. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153(6):1194.

21. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. Int J Mol Sci. 2019; 20(13):1–24.

22. Srivastava N, Ranjana, Singh S, Gupta AC, Shanker K, Bawankule DU, Luqman S. Aromatic ginger (*Kaempferia galanga* L.) extracts with ameliorative and protective potential as a functional food, beyond its flavour and nutritional benefits. Toxicol Rep. 2019; 6:521–528.

23. Zhang D, Zhao M, Li Y, Zhang D, Yang Y, Li L. Natural xanthine oxidase inhibitor 5-o-caffeoylshikimic acid ameliorates kidney injury caused by hyperuricemia in mice. Molecules. 2021; 26(23):1–15.

24. Qazi SU, Qamar U, Maqsood MT. Efficacy of allopurinol in improving endothelial dysfunction: A systematic review and meta-analysis. High Blood Press Cardiovasc Prev. 2023; 30:539-550.

25. Macdonald AS, McConnachie A, Dickie DA, Bath PM, Forbes K, Quinn T, Broomfield NM, Dani K, Doney A, Muir KW, Struthers A, Walters M, Barber M, Bhalla A, Cameron A, Guyler P, Hassan A, Kearney M, Keegan B, Lakshmanan S, Dawson J. Allopurinol and blood pressure variability following ischemic stroke and transient ischemic attack: a secondary analysis of XILO-FIST. J Hum Hypertens. 2024; 38(4):307–13.

26. Wang SY, Zhao H, Xu HT, Han XD, Wu YS, Xu FF, Yang XB, Göransson U, Liu B. *Kaempferia galanga* L.: Progresses in Phytochemistry, Pharmacology, Toxicology and Ethnomedicinal Uses. Front Pharmacol. 2021; 12:1–17.

27. Wang SY, Cai L, Yang N, Xu FF, Wu YS, Liu B. Chemical composition of the *Kaempferia galanga* L. essential oil and its in vitro and in vivo antioxidant activities. Front Nutr. 2023; 10:1–10.

28. Nogrady T and Weaver DF. Medicinal Chemistry: A Molecular and Biochemical Approach. 3rd ed. New York: Oxford University Press; 2005.

29. Bayanati M, Ismail Mahboubi Rabbani M, Sirous Kabiri S, Mir B, Rezaee E. Dipeptidyl Peptidase-4 Inhibitors: A Systematic Review of Structure-Activity Relationship Studies. Iran J Pharm Res. 2024; 23(1):e151581.

30. Roig-Zamboni V, Cobucci-Ponzano B, Iacono R, Ferrara MC, Germany S, Bourne Y, Parenti G, Moracci M, Sulzenbacher G. Structure of human lysosomal acid α-glucosidase-A guide for the treatment of Pompe disease. Nat Commun. 2017; 8(1):1111.

31. Terefe EM and Ghosh A. Molecular Docking, Validation, Dynamics Simulations, and Pharmacokinetic Prediction of Phytochemicals Isolated From Croton dichogamus Against the HIV-1 Reverse Transcriptase. Bioinform Biol Insights. 2022; 16: 11779322221125605.

32. Jha P, Rajoria P, Poonia P, Chopra M. Identification of novel PAD2 inhibitors using pharmacophore-based virtual screening, molecular docking, and MD simulation studies. Sci Rep. 2024; 14(1):1–19.

33. Ross, G. A., Lu, C., Scarabelli, G., Albanese, S. K., Houang, E., Abel, R., Harder, E. D., & Wang, L. The maximal and current accuracy of rigorous protein-ligand binding free energy calculations. Commun Chem. 2023; 6(1):1–12.

34. Kontogeorgis GM, Holster A, Kottaki N, Tsochantaris E, Topsøe F, Poulsen J et al. Water structure, properties and some applications – A review. Chem Thermodyn Therm Anal. 2022; 6:100053.

35. Du L, Zong Y, Li H, Wang Q, Xie L, Yang B, Pang Y, Zhang C, Zhong Z, & Gao J. Hyperuricemia and its related diseases: mechanisms and advances in therapy. Signal Transduct Target Ther. 2024; 9(1).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)