Trop J Nat Prod Res, January 2025; 9(1): 176 - 182

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

Tropical Journal of Natural Product Research

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

Original Research Article

Nephroprotective Effect of Ethyl Acetate Fraction of Cogongrass (*Imperata cylindrica*) Root Acute Kidney Injury

Widya M Rahmawati, Okid P Astirin*, Shanti Listyawati

Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta 57126, Central Java, Indonesia.

ARTICLE INFO

Article history:
Received 29 November 2024
Revised 03 December 2024
Accepted 27 December 2024
Published online 01 February 2025

ABSTRACT

Acute kidney injuries have become a worldwide public health issue. Cogongrass's roots have antioxidant, anti-inflammatory, and vasodilatory activities that support nephroprotection. This study aimed to evaluate the active components in the ethyl acetate fraction of cogongrass and their potential protective effects on renal function. Cogongrass root was macerated in 96% ethanol and fractionated in petroleum ether and ethyl acetate. The components of the ethyl acetate fraction were observed using liquid chromatography high-resolution mass spectrometry. The *in vivo* study included three treatment groups with orally administered doses of cogongrass fractions at 300, 350, and 400 mg/kg BW, as well as a normal and negative control. Intraperitoneal injection of 250 mg/kg BW folic acid was used to model acute kidney injury. Measurement of serum BUN and creatinine levels to determine kidney function, while kidney histology is observed for tubular kidney profiling. The active compounds of the cogongrass roots fraction were 5-methoxyflavone, chryptochlorogenic acid, and formononetin. Doses of a cogongrass roots fraction (300-400 mg/kg BW) decreased serum creatinine and BUN levels in acute kidney injury. However, the dose of 350 mg/kg BW had a lower tubular profile damage score, characterised by tubular cell swelling and necrosis, compared to other doses. The ethyl acetate fraction of cogongrass root showed significant therapeutic potential in overcoming folic acid-induced acute kidney injury.

Keywords: Acute Kidney Injury, Blood Urea Nitrogen, Creatinine, *Imperata cylindrica*, Renal.

Introduction

Acute kidney injury (AKI) is a condition of sudden kidney dysfunction confirmed by decreased urine volume and increased creatinine levels for seven days.[1] AKI cases affect more than 13 million people in the world, with 1.7 million deaths each year.[2] Approximately 40% of AKI cases are caused by drug- and toxin-induced renal ischemia.[3] It has the potential for developing to chronic kidney diseases in the long term.[4-5] Therapeutic strategies for AKI are mostly supportive because there are no drugs and therapies that target AKI directly.[3] The exploration of secondary metabolite compounds has significant potential in the management of AKI.

Cogongrass roots are used as a traditional medicine mixture in renal medicine in China.[6] Cogongrass roots contain compounds with antioxidant, anti-inflammatory, and vasodilator activities.[7-8] Ethyl acetate extract from cogongrass roots protects against adriamycin-induced nephrosis, with a focus on glomerular injury.[9] Cyilindrin from cogongrass roots also alleviated folic acid-induced renal fibrosis.[10] The folic acid model demonstrates the progression from AKI to CKD, with renal tubular injury as the primary cause of damage.[11]

The ethyl acetate component of cogongrass root's ability to reduce the consequences of AKI and prevent CKD is not fully known. The objectives of this research are to identify the active compounds in the ethyl acetate fraction of the ethanol extract of cogongrass and to evaluate the nephroprotective effect.

*Corresponding author. E mail: parama_astirin@staff.uns.ac.id,
Tel: +62 (0271) 669376

Citation: Rahmawati WM, Astirin OP, Listyawati S. Nephroprotective Effect of Ethyl Acetate Fraction of Cogongrass (*Imperata cylindrica*) Root Acute Kidney Injury. Trop J Nat Prod Res. 2025; 9(1): 176 – 182.
https://doi.org/10.26538/tjnpr/v9i1.25

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

Materials and Methods

Collection and Identification of plant materials

Cogongrass roots were collected from Bendosari District, Sukoharjo Regency, Central Java (Indonesia) in January 2024. Plants were identified and authenticated by Mr. Suratman at the Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, with voucher 150/UN27.9.6/Lab/2024.

Extraction and fractionation of cogongrass roots

Cogongrass root powder (600 gram) was macerated with 96% ethanol (1:10, y/v) for 24 hours and then remacerated for 24 hours.[12] The ethanol extract was fractionated with petroleum ether and ethyl acetate.[10] The ethanol extract was dissolved in distilled water (1:15, y/v) and then partitioned with petroleum ether (1:1, v/v). The water phase was partitioned again with ethyl acetate (1:7, v/v) two times. The ethyl acetate fraction was concentrated using a rotary evaporator and water bath to form a paste.

Treatment of experimental animals

The experimental procedure was approved by Dr. Moewardi's Health Research Ethics (No. 158/I/HREC/2024). Twenty male BALB/C mice (age 8–10 weeks, weight 20–28 g) were randomly allocated into five different groups (n = 4 for each group): 1) control (K); 2) folic acid-induced AKI group (K-); 3) AKI treated with cogongrass fraction (300 mg/kg/day) (P1); 4) AKI treated with cogongrass fraction (350 mg/kg/day) (P2); and 5) AKI treated with cogongrass fraction (400 mg/kg/day) (P3). The AKI group was induced with 250 mg/kg BW folic acid diluted in 0.3 mol/L sodium bicarbonate ($NaHCO_3$), while the control group was induced with 0.3 mol/L $NaHCO_3$ intraperitoneally at day 0.[10] The treatment group received an oral cogongrass fraction diluted in a 0.5% CMC solution, while groups K and K- received a 0.5% CMC solution orally for 14 days. Blood was collected from the orbital veins, and mice were sacrificed on day 14 to harvest the kidney.[10]

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

Measurement of BUN and serum creatinine
Blood samples were centrifuged at a speed of 2000×g in 10 minutes at 4°C.[13] Creatinine levels were measured using the Jaffe method, while BUN levels were measured enzymatically (Glory Diagnostics kit).

Evaluation of kidney histology tissue
The paraffin method with hematoxylin-eosin staining was used to create kidney histology. This is done by fixing the kidney in a 10% formalin solution, then immersing it in paraffin, cutting it with a microtome 7 µm thick, and staining it with H&E.[14] Three preparations were repeated for each organ on the same part.
A light microscope was used to observe 100 randomly selected tubules at magnifications ranging from 400×.[15] The tubule profiles were assessed using the following criteria: The tubule profiles received a score of 0 for normal condition, a score of 1 for regions characterized by tubular epithelial cell hypertrophy, necrosis, vacuolar degeneration, and/or desquamation impacting approximately 25% of the tubular profile, a score of 2 for similar changes affecting approximately 25% to 50% of the tubular profile, a score of 3 for similar changes affecting approximately 50% to 75% of the tubular profile, and a score of 4 for similar changes affecting approximately 75% of the tubule profile.

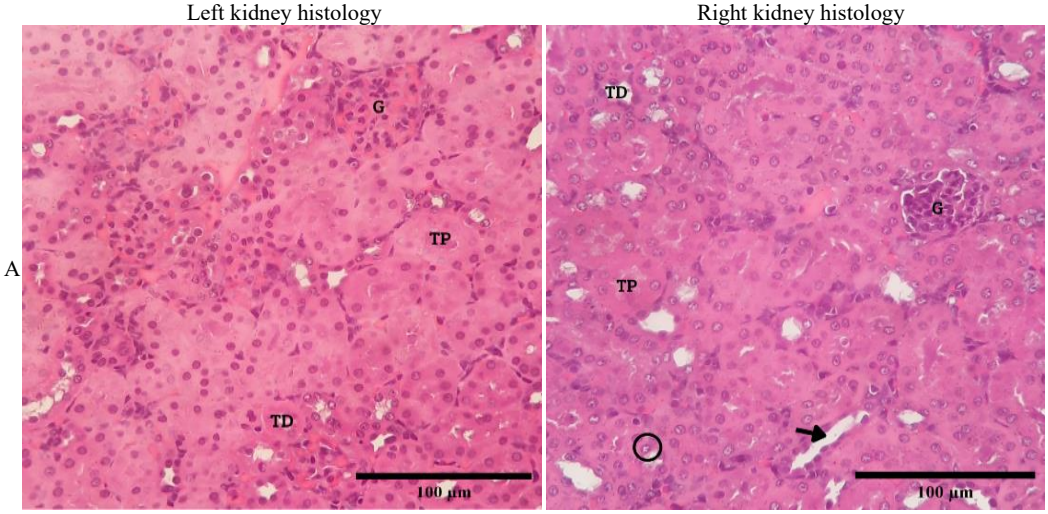
Identification of secondary metabolite compounds resulting from the ethyl acetate fraction of cogongrass roots
Compounds were identified with liquid chromatography and Orbitrap high-resolution mass spectrometry. Untargeted analysis employs complete MS/dd-MS2 collection mode with a positive polarity/ ionization state. The scanning range was 66.7-1000 m/z with a mass accuracy of <5 ppm. The resolution employed was 17500 for dd-MS2 and 70000 for full MS. Compound Discoverer software was used to look at the raw data chromatograms and figure out what chemicals were in the ethyl acetate part of the cogongrass roots.[16]

Interaction of active compounds of cogongrass root with protein in acute kidney injury by molecular docking
The protein structures of AKT1 (PDB ID: 3O96) and EGFR (PDB ID: 1M17) from the Protein Data Bank were prepared using PyMol 3.0.3. Active compounds from cogongrass roots were taken from the PubChem database in SDF format. Molecular docking was carried out using PyRx 0.8 to estimate the binding affinity of compounds with proteins. The coordinates of the docking center for AKT1 are at 8.998 on the X-axis, -7.639 on the Y-axis, and 10.533 on the Z-axis, while EGFR is at 21.813 on the X-axis, -0.209 on the Y-axis, and 52.176 on the Z-axis. The interaction of the compound and protein with the lowest binding affinity is visualized using Discovery Studio v16.

Statistical analysis
Statistical analysis was performed using SPSS software. Differences between groups were evaluated by one-way ANOVA, completed by the post-hoc Tukey test. Quantitative data is provided as a mean ± SD with a significant level of $P < 0.05$.

Results and Discussion

Acute kidney injury has a dire prognosis. Kidney transplantation therapy is an option due to worsening kidney function and progression of AKI to the final stage, or CKD.[17] Therefore, effective treatment for this disease is urgently needed.
The acute kidney injury with folic acid has a central pathology involving tubular obstruction and oxidative stress, which promote necrosis of tubular epithelial cells and the release of cytokines.[18] Folate accumulates significantly in the kidney due to its high affinity for folate receptors in the proximal tubule. The low solubility of folic acid at neutral and acidic pH levels leads to the formation of folate crystals as pH decreases throughout the tubules .[19] Folic acid precipitation causes primary tubular obstruction, resulting in increased intratubular pressure and glomerular filtration pressure.[20] Folic acid causes reversible and sometimes irreversible damage to tubular cells (**Figure 1**). Cell swelling, or hydropic degeneration, is reversible due to an increase in water volume. This condition was caused by transporter disorders, such as Na+/K+-ATPase pump inhibition. Prolonged reversible damage can cause irreversible damage. Necrosis, such as pyknosis, karyolysis, and karyorrhexis, are examples of irreversible forms of damage. Cell regeneration can repair this irreversible damage.[21] The study's results (**Figure 2**) indicate that the histological damage in the negative mice group was more severe than that of the normal and treatment groups. This also demonstrates that administering the ethyl acetate fraction of cogongrass roots reversed the damage caused by folic acid.



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ISSN 2616-0692 (Electronic)

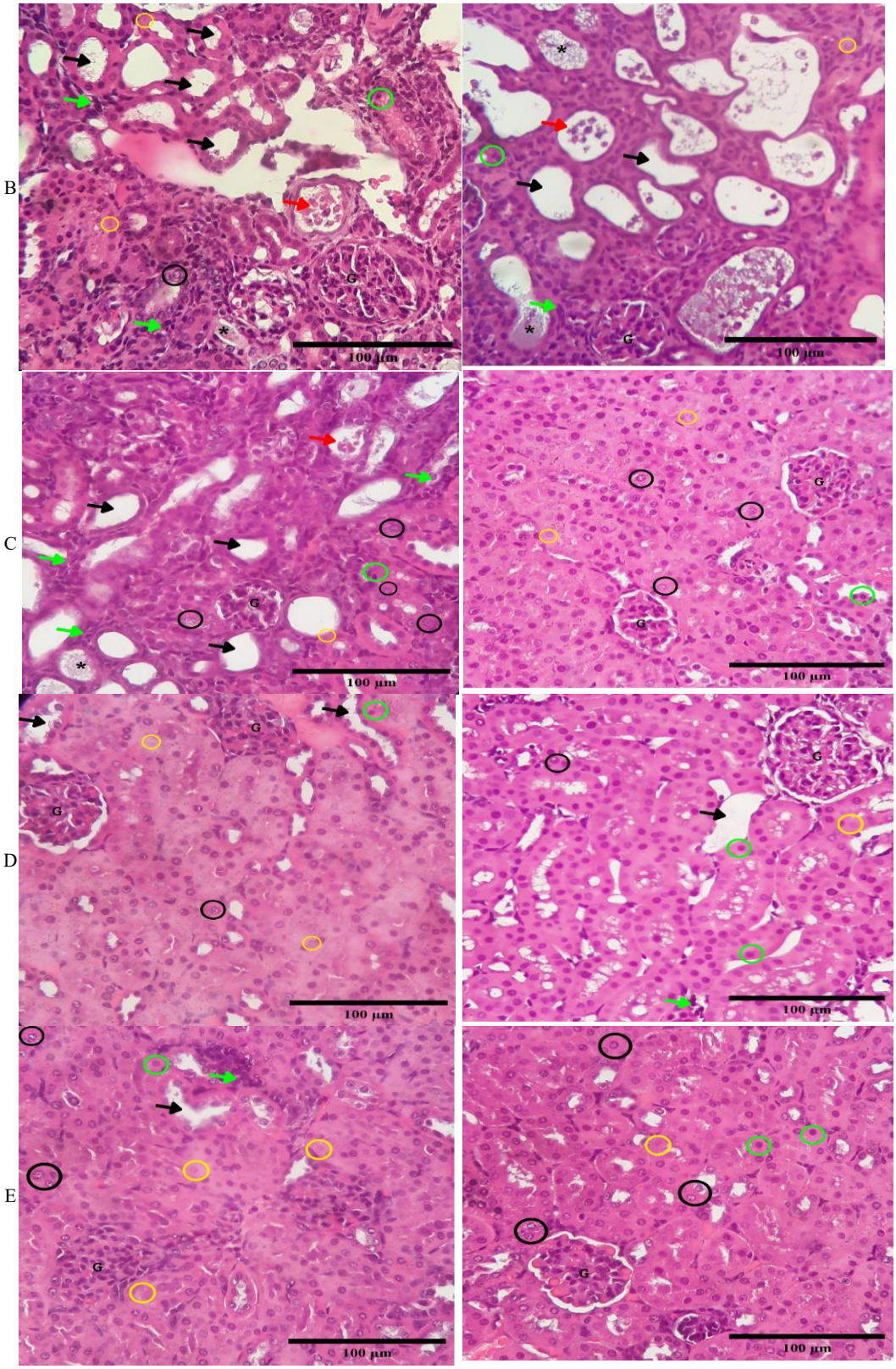

Figure 1: Kidney histology (A) normal control, (B) negative control, (C) 300 mg/kg BW dose group, (D) 350 mg/kg, BW group, and (E) 400 mg/kg BW dose group. 400x magnification. G represents glomerulus, TP represents proximal tubule, and TD represents distal tubule. Black arrows indicate dilatation of the proximal tubule, green arrows indicate interstitial infiltration, red arrows indicate desquamation of tubular cells, and circles indicate necrosis with details in black representing karyorrhexic cells, yellow color karyolysis, and green color representing pyknosis.

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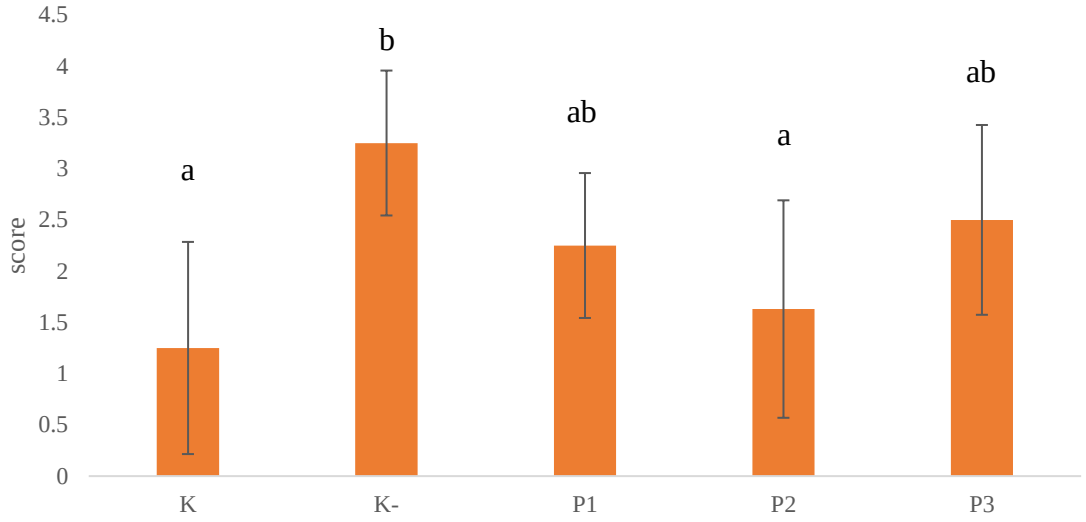

Figure 2: Effect of cogongrass fraction on kidney profile damage score following induction of acute kidney injury. Different letters indicate significant differences ($p \leq 0.05$) between experimental groups (n = 5). K = control group; K- = folic acid-induced AKI group; P1=AKI treated with cogongrass fraction (300 mg/kg/day); P2 = AKI treated with cogongrass fraction (350 mg/kg/day); and P3 = AKI treated with cogongrass fraction (400 mg/kg/day).

The results showed that folic acid administration in mice significantly increased BUN and creatinine levels compared to control animals. This indicates a decrease in kidney function. Disrupting the blocking of sodium ion reabsorption in the renal tubules sets off tubuloglomerular feedback signals, which slow down the glomerular filtration rate to keep the sodium ions in the body.[22] The increase in intratubular pressure causes an increase in urea reabsorption.[20] Renal disorders also disrupt the expression of organic cations and anions in the proximal tubule, causing decreased secretion and potential accumulation of exogenous and endogenous substrates, including creatinine and BUN.[22] Administration of the ethyl acetate fraction of cogongrass roots at a dosage of 300–400 mg/kg significantly reduced BUN and creatinine levels compared to the negative control (**Figure 3**). This shows that cogongrass root secondary metabolites improve kidney function.

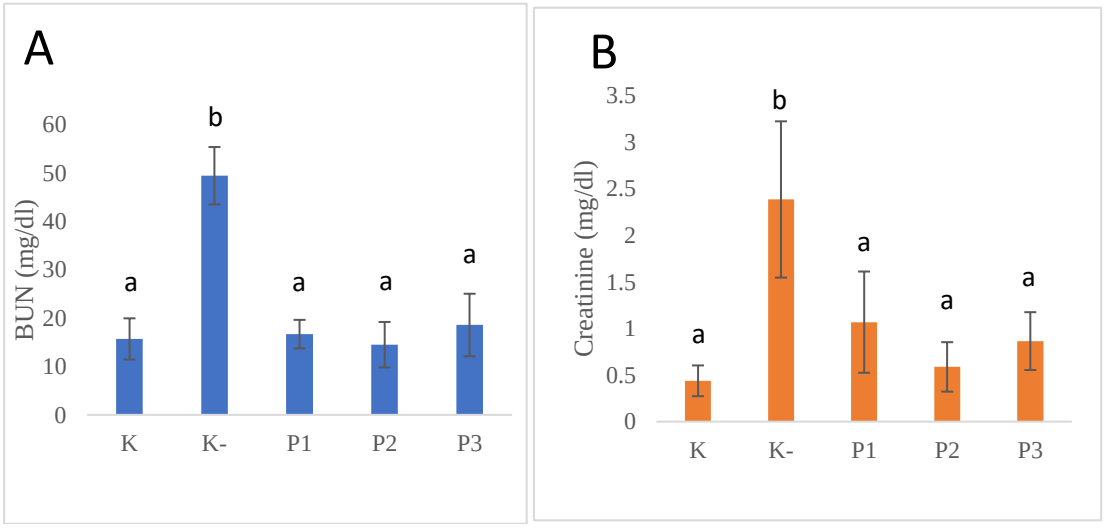

Figure 3: Effect of cogongrass fraction on serum levels of BUN (A) and creatinine (B) in mice with a model of acute renal failure. Different letters represent significant differences ($p < 0.05$) across experimental groups (n = 5). K = control group; K- = folic acid-induced AKI group; P1=AKI treated with cogongrass fraction (300 mg/kg/day); P2 = AKI treated with cogongrass fraction (350 mg/kg/day); and P3 = AKI treated with cogongrass fraction (400 mg/kg/day).

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A total of 414 metabolites were observed, with the 30 compounds shown in **Table 1**. The main secondary metabolites found in the ethyl acetate fraction of cogongrass roots are 5-methoxyflavone, 7-hydroxycoumarine, cryptochlorogenic acid, caffeic acid, ferulic acid, formononetin, and coumarin. These compounds exhibit various bioactivities to improve acute kidney injury. 7-hydroxycoumarin can suppress RIPK1/RIPK3/MLKL-mediated necroptosis.[23] These compounds also act as antioxidants, weaken ROS, and are anti-inflammatory.[24] Ferulic acid is an antioxidant that stimulates the nuclear factor erythroid 2-related factor (Nrf2)/heme oxygenase (HO-1) signaling pathway. It promotes the production of the antioxidant enzyme glutathione S-transferase by boosting Nrf2 and HO-1.

Table 1: List of compounds discovered from the ethyl acetate fraction of cogongrass roots using LC-HRMS

No	*Name*	*Formula*	*Calc. MW*	*RT [min]*	*Presentase area*
1	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.27585	17.453	13.159%
2	Bis(3,5,5-trimethylhexyl) phthalate	$C_{26}H_{42}O_4$	418.30724	17.99	7.965%
3	5-methoxyflavone	$C_{16}H_{12}O_3$	252.07787	9.803	6.574%
4	7-Hydroxycoumarine	$C_9H_6O_3$	162.0313	3.69	6.139%
5	Cryptochlorogenic acid	$C_{16}H_{18}O_9$	354.0941	3.694	4.842%
6	Phthalic acid	$C_8H_6O_4$	166.02624	17.446	4.601%
7	Caffeic acid	$C_9H_8O_4$	180.04186	4.484	2.397%
8	Ferulic acid	$C_{10}H_{10}O_4$	194.05764	3.98	2.383%
9	Formononetin	$C_{16}H_{12}O_4$	268.07298	9.176	2.158%
10	Coumarin	$C_9H_6O_2$	146.03662	5.309	2.102%
11	Hexyl cinnamaldehyde	$C_{15}H_{20}O$	216.15082	11.134	2.012%
12	2,4-Diamino-6-chloropyrimidine	$C_4H_5ClN_4$	144.02086	5.07	1.882%
13	(2Z)-2-(3-Hydroxybenzylidene) heptanoic acid	$C_{14}H_{18}O_3$	234.12511	8.277	1.828%
14	*α*-Lactose	$C_{12}H_{22}O_{11}$	342.11514	0.779	1.478%
15	Hymecromone	$C_{10}H_8O_3$	176.04708	3.812	1.386%
16	(1S,3R,4R,5R)-1,3,4-trihydroxy-5-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}cyclohexane-1-carboxylic acid	$C_{17}H_{20}O_9$	368.11002	5.101	1.188%
17	Vanillin	$C_8H_8O_3$	152.04707	5.546	1.049%
18	IN00458	$C_9H_6O_3$	162.0313	2.168	0.921%
19	Bis(2-ethylhexyl)adipate	$C_{22}H_{42}O_4$	370.30727	17.237	0.905%
20	Citrinin	$C_{13}H_{14}O_5$	250.08365	7.482	0.878%
21	Isovanillic acid	$C_8H_8O_4$	168.04212	4.092	0.737%
22	7-Hydroxy-6-(3-oxobutyl)-2H-chromen-2-one	$C_{13}H_{12}O_4$	232.07311	6.018	0.664%
23	Meglutol	$C_6H_{10}O_5$	162.05244	0.804	0.640%
24	3-(3,4,5-trimethoxyphenyl) propanoic acid	$C_{12}H_{16}O_5$	240.09936	3.403	0.640%
25	NP-000587	$C_{16}H_{18}O_8$	338.09962	4.771	0.632%
26	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278.15121	17.459	0.621%
27	Choline	$C_5H_{13}NO$	103.09972	0.811	0.584%
28	1-Linoleoyl glycerol	$C_{21}H_{38}O_4$	354.27599	14.168	0.559%
29	Flavidin	$C_{15}H_{12}O_3$	240.0783	5.916	0.558%
30	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.03157	0.928	0.544%

Additionally, ferulic acid also suppresses the expression of NFκB and TNFα, thereby reducing the number of lymphocytes infiltrating renal tissue.[25]

The seven active compounds observed interact with protein kinase B (AKT1) and epidermal growth factor receptor (EGFR), which are implicated in renal fibrosis. Binding affinity evaluates the ability of a substance to bind to a target. A lower binding affinity score indicates that the receptor and ligand have a higher affinity.[26] Hydrogen bonds were critical in determining the intensity of the binding energy. The more hydrogen bonds between the ligand and the protein, the stronger the bond affinity.[27] The main active compound and target protein show binding affinity values below -6 kcal/mol, indicating that the compound has a high affinity for the target.

ISSN 2616-0684 (Print)
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Table 2: Results of molecular docking between active compounds and proteins

No	Compounds	*Binding affinity* (kcal/mol)	
		AKT1 (3O96)	EGFR (1M17)
1	*5 - Methoxyflavone*	-9.3	-8.4
2	*7-Hydroxycoumarine*	-7.1	-6.2
3	*Formononetin*	-9.7	-7.7
4	*Cryptochlorogenic acid*	-8.9	-8.2
5	*Caffeic acid*	-6.7	-5.9
6	*Ferulic acid*	-6.6	-6.1
7	*Coumarin*	-7.0	-6.0
8	Native ligan (inhibitor protein)	-14.6*	-7.0**

* 3-[1-[[4-(7-phenyl-3H-imidazo[4,5-g]quinoxalin-6yl)phenyl]methyl] piperidin-4-yl]-1H-benzimidazol-2-one
** 4-anilinoquinazoline

The interaction results presented in **Figure 4**, and the binding affinity values listed in **Table 2**. *In silico* tests, the molecular docking of the seven compounds used was predicted to bind and inhibit the activity of the AKT1 and EGFR proteins. Formononetin and 5-methoxyflavone are the two active molecules that interact most strongly with AKT1 and EGFR. Activation of the EGFR protein facilitates the initiation and progression of renal fibrosis in CKD models.[28] The AKT1 protein play a role in pathological kidney diseases through the proliferation and activity of interstitial fibroblasts, glomerular mesangial cells, and tubular epithelial cells in the progression of renal fibrosis. [29] Consequently, the inhibition of these two proteins can mitigate renal fibrosis and avert the transition of AKI to CKD.

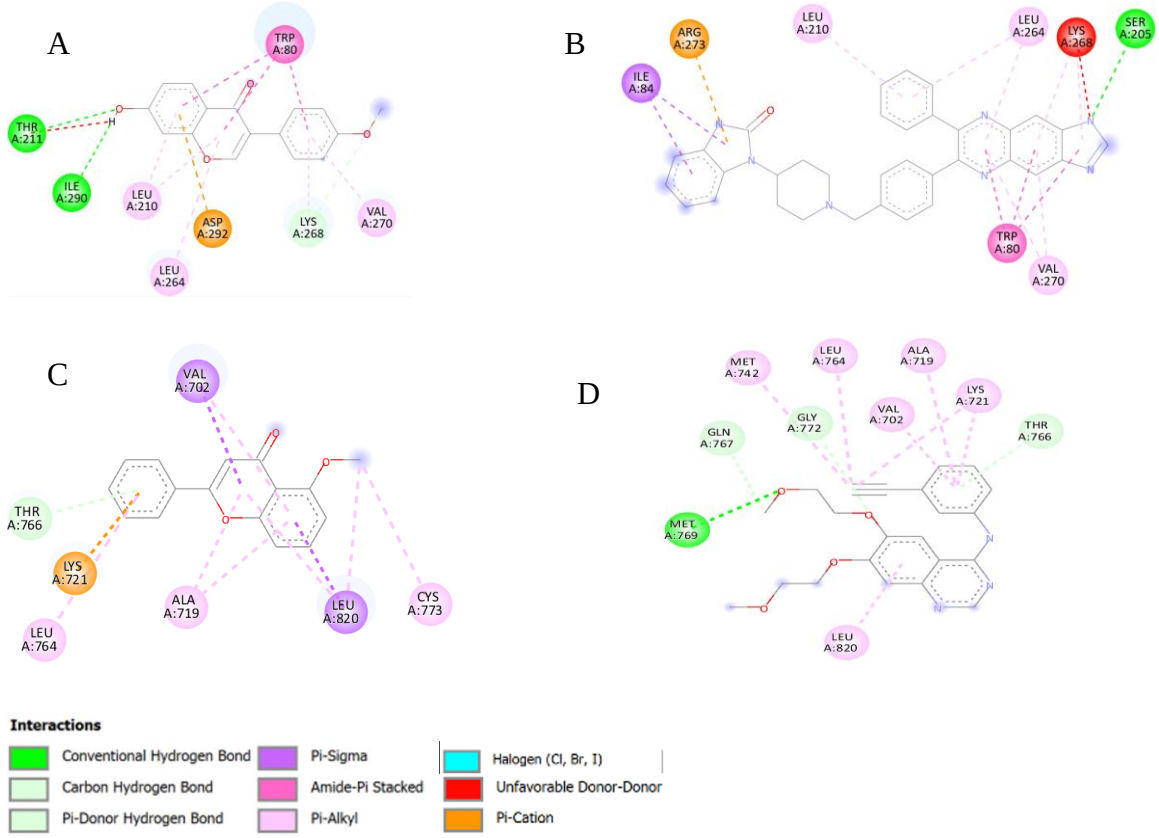

Figure 4: Interaction of active compounds with target proteins (A) interaction of formononetin with AKT1, (B) interaction of inhibitor control with AKT1, (C) interaction of 5-methoxyflavone with EGFR, and (D) interaction of ligand inhibitors with EGFR.

Conclusion

The ethyl acetate fraction of cogongrass roots has secondary metabolite compounds like formononetin, 5-methoxyflavone, and 7-hydroxycoumarin. A 350 mg/kg BW dose of the cogongrass root ethyl acetate fraction reduced both BUN and creatinine levels, as well as the damage to the renal tubular profile. Therefore, the ethyl acetate fraction of cogongrass roots could exert a nephroprotective effect in acute kidney injury. Part of the fraction's mechanism of action is inhibition of AKT1 and EGFR. However, this requires further validation of the fraction's mechanism of action and *in vitro* testing of protein responses in acute kidney injury.

Conflict of Interest

The authors hereby declare that there are no conflicts of interest.

Authors' Declaration

The authors declare that the information provided in this work is original and any responsibility for claims relating to the content of this work will be borne by them.

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

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