ISSN 2548-6462 (online), ISSN 2088-8740 (print)

DOI: 10.30644/rik.v15i2.1190

Antihyperglycemic activity of the combination of *Pandanus Amaryllifolius* Roxb. and *Cosmos caudatus Kunth.* leaf infusion in male white mice

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Accepted: 28 February 2026; revision: 30 April 2026; published: 31 May 2026

Abstract

Background: *Pandanus amaryllifolius* Roxb. (pandan leaves) is widely cultivated in Sumatra, including Jambi Province, and is commonly used as a traditional food ingredient. Its potential medicinal value, particularly in combination with *Cosmos caudatus Kunth*. (kenikir leaves), for managing hyperglycemia has not been extensively investigated. Evaluating the antihyperglycemic activity of decoctions from these plants may provide scientific evidence for their development as phytotherapeutic agents targeting non-communicable diseases. This study aimed to evaluate the antihyperglycemic activity of combined decoctions of pandan and kenikir leaves in hyperglycemic mice.
Method: A quasi-experimental study with a static-group comparison design was conducted using 30 male Swiss mice divided into five groups. Hyperglycemia was induced with glucose (0.2 mL/20 g BW). The groups consisted of a negative control (tragacanth), a positive control (glibenclamide 0.01 mL/20 g BW), and three treatment groups receiving decoctions of pandan and kenikir in water at ratios of 5:5:90, 10:10:80, and 20:20:60, respectively. Blood glucose levels were measured on days 1, 3, and 7. Two-way ANOVA and Duncan's post hoc test were used to evaluate the data.
Results: The combination of pandan and kenikir leaves demonstrated significant antihyperglycemic activity compared with the negative control ($p < 0.05$). Among the tested ratios, the 20:20:60 formulation showed the closest effect to glibenclamide after 7 days of treatment.
Conclusion: Decoctions of *P. amaryllifolius* combined with *C. caudatus* exhibit promising antihyperglycemic effects, suggesting their potential as a complementary herbal therapy for hyperglycemia management. Further studies on the mechanism of action and clinical applicability are warranted.

Keywords: *Pandanus amaryllifolius*; *Cosmos caudatus*, Antihyperglycemic.

INTRODUCTION

Local communities in Jambi Province, which largely depend on forest resources for their livelihoods, have long used herbs as medicine. Forests are crucial to their survival because they hunt and gather food there, and they have both traditional and commercial value. A community's healing traditions are closely linked to its culture. Through a process of socialization passed down from generation to generation and accepted as truth, the diversity of plant species used in traditional medicine and perceptions of health are shaped (1,2). *Pandanus amaryllifolius* Roxb. is an aromatic plant belonging to the genus Pandanus and the *Pandanaceae* family, which has benefits as an antioxidant, anti-inflammatory, and helps prevent diabetes (3). Kenikir leaves (*Cosmos caudatus* Kunth.) are also used as antioxidants, antidiabetics, anti-inflammatories, and antimicrobials (4). These two leaves are rich in antioxidant compounds such as flavonoids, alkaloids, and terpenoids, which can lower the incidence of degenerative disorders and shield the body from harm brought on by free radicals (5). Research shows that extracts of pandan leaves and kenikir leaves have an effect in lowering blood sugar levels, so they have the potential to help in managing diabetes (6). Based on the results of phytochemical screening, the solvent fraction of the methanol extract of

fragrant pandan stems contains compounds that are positively concentrated in the n-hexane fraction (alkaloids, saponins, steroids, triterpenoids, and tannins), the ethyl acetate fraction (alkaloids, flavonoids, saponins, and tannins), the methanol fraction (alkaloids, flavonoids, and saponins), and the water fraction (alkaloids) (7). Giving boiled pandan leaf water to diabetes patients has good benefits, namely, it can be used as a substitute to lower blood sugar levels in diabetes patients (8). However, the combination of these two plants still requires further research to prove this claim scientifically.

Therefore, using the alloxan induction method, the researchers aimed to determine the effect of a combination of pandan wangi (*Pandanus amaryllifolius* Roxb.) and kenikir (*Cosmos caudatus* Kunth.) leaf infusion on the hypoglycemic potential of the infusion in male white mice. Therefore, the findings of this study can be used as a guide in the development of new drug molecules for the treatment of diabetes mellitus.

METHOD

This type of research is a quasi-experimental study with a static-group comparison design. The sample of this study was freshly picked pandan leaves and kenikir leaves. Sampling of pandan leaves and kenikir leaves was carried out in Jambi City, then the samples were processed until the antihyperglycemic activity test of pandan leaves and kenikir leaves was carried out at the Poltekkes Kemenkes Jambi. The study on the activity of pandan leaves and kenikir leaves on reducing blood sugar levels in mice was conducted in February 2025. Primary data from laboratory research on reducing blood sugar levels in male white mice at various concentrations using a glucose meter was used as a data collection method in this study, in addition, the *One Way ANOVA* method with the *Statistical Product Services Solution* (*SPSS*) program was used to statistically analyze the test data on the activity of pandan leaves and kenikir leaves in reducing blood sugar levels in mice with a 95% confidence level or $\alpha = 0.05$ (9).

Sampling of fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) and kenikir leaves (*Cosmos caudatus* Kunth.) was obtained from Jambi City. The leaves to be prepared are fresh leaves that are not too young and not too old. This is because if the leaves are too young, then the chemical compounds contained are still not perfect; likewise, in old leaves, the chemical compounds contained are reduced. Preparing fresh fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) and kenikir leaves (*Cosmos caudatus* Kunth.) with the time of collection in the morning, the leaves are separated from dirt, then washed clean, drained, and weighed (10).

Take a combination of parts of fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) and kenikir leaves (*Cosmos caudatus* Kunth.) that have been cleaned and weighed according to the planned content. Then put them into a mug with 100 ml of water added. Heat the water in a water bath until it reaches a temperature of 90^0C. Next, add the fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) and kenikir leaves (*Cosmos caudatus* Kunth.) that are in the mug and have been added to the water bath and steamed for 15 minutes. Because they have essential oils, stirring is done once, and filtering is done when it has cooled. This is done so that the essential oils contained in the fragrant pandan leaf infusion (*Pandanus amaryllifolius* Roxb.) and kenikir leaves (*Cosmos caudatus* Kunth.) do not evaporate (11).

The Phytochemistry and Pharmacology Laboratory, Department of Pharmacy, Health Polytechnic, Ministry of Health, Jambi, will conduct this study over roughly five months in 2025 with a certificate number suitable for research ethics, LB.02.06/2/123/2025, issued by KEPK Poltekkes Kemenkes Jambi.

The research procedure was conducted. The research tools consisted of a mouse cage, a place for mice to eat and drink, an animal scale, an analytical scale, an infusion pan, a blender, a beaker, a funnel, filter paper, an oral probe (cannula), a 1 ml syringe, a water bath, and a Gluco test.

Infusions

Take 5:5:90 (Infusion 1), 10:10:80 (Infusion 2), and 20:20:60 (Infusion 3) of cleaned and weighed *Pandanus amaryllifolius* Roxb. and *Cosmos caudatus* Kunth. leaves. Place them in a mug and add 100ml of water. Heat the water in a water bath to 90 ^{0}C. Next, add the *Pandanus amaryllifolius* Roxb. and *Cosmos caudatus Kunth*. leaves, which have been added to the mug and water, are placed in the water bath and steamed for 15 minutes. Because they contain essential oils, stir once and strain once cooled. This is to prevent the essential oils in the bengkal leaf infusion from evaporating (11).

The application of tools and materials

Getting the Test Animals Prepared

The formula can be used to determine the experimental test animals employed (9):
(t-1) (r-1) ≥ 15,
(t-1) (r-1) ≥ 15
(5-1) (r-1) ≥ 15
4 (r-1) ≥ 15
4r = 15 + 4
r = 19/4 = 4,75 = 5+1 = 6
Information:
t: Group
r: Number of test animals
Thus, six mice are used in one treatment group. Thus, thirty mice were employed for each of the five treatment groups.

Drug solution, carrier, and diabetes inducer preparation

a. 5 mg solution of glibenclamide
five milligrams x 0.0026 = 0.013 milligrams (0.65 mg per kilogram of body weight). In a mortar, grind one Glibenclamide tablet until it is uniform. Then, add ten milliliters of 0.5 percent tragacantha suspension, take one milliliter of the solution, dilute it with 0.5 percent tragacantha suspension until you have a 7.7 milliliter solution, and make sure the glibenclamide suspension solution is consistent by stirring it (12,13).
b. Preparing a 0.5% Tragacanth suspension
0.5 g of Tragacanth should be weighed, dispersed in hot water 20 times, allowed to sit for 15 minutes, ground, and then the remaining water added till 100 ml is obtained (14).
c. 175 mg/kgBW preparation Alloxan solution
3.5 milligrams per 0.2 milliliters x 10 milliliters = 175 mg; 175 milligrams per kilogram of body weight x 0.02 kilogram = 3.5 milligrams. After weighing 175 mg of Aloxan into a glass beaker, add ten milliliters of Aqua Pro injection and mix until everything is uniform (13,15,16).

Treatment of Test Animals

Thirty male white mice (*Mus musculus*) weighing twenty to thirty grams each, aged two to three months, were employed as test subjects in this investigation. They were split into six test groups. Initially, male white mice were housed in a cage for about a week, during which time they were provided regular food and enough water to drink. Except for the negative control group, all mouse groups were given intraperitoneal injections of alloxan at a dose of 175 mg/kgBW and a volume of 0.2 milliliter every 20 grams to induce diabetes. The following day, the blood sugar level is measured. Given the statistically substantial rise, the treatment can be prolonged by delivering a 10% sugar solution along with food and drink, as needed. The five groups of mice were then given a negative control (tragacanth), a positive control (glibenclamide 0.01 mL/20 g BW), and three treatment groups receiving decoctions of pandan and kenikir in water at ratios of 5:5:90 (Infusion 1), 10:10:80 (Infusion 2), and 20:20:60 (Infusion 3). Next, measure the mice's blood sugar levels on days 1, 3, and 7.

Techniques for gathering and analyzing data

The test animals' blood glucose levels are measured and shown in a table following treatment. ANOVA analysis was then used for statistical analysis.

RESULTS

Following the research, the following data were acquired:

Table 1. The percentage of test animals' blood glucose levels that decreased

No.	Treatment Group	Blood Glucose Levels of Test Animals (mg/dL)										
		Fast	Diabetic	Days to-								
				1	SD	%	3	SD	%	7	SD	%
1.	Control negative	128,4	208,4	178	7,582	14,58	123,8	7,049	40,59	109,4	1,140	47,50
2.	Control positive	111,2	216,5	131,2	7,293	39,39	113,6	7,924	47,52	108,4	7,700	49,93
3.	Infusion 1	121,7	212,6	192,6	5,941	9,40	186,8	6,723	12,13	180,8	7,328	14,95
4.	Infusion 2	123,1	211,5	182	6,324	13,94	174,2	6,300	17,63	167,2	6,379	20,94
5.	Infusion 3	110,3	201,5	133,6	7,635	33,69	115,8	8,318	42,53	107,8	2,408	46,50

Table 2. Normality Test

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual for Levels_Sugar_Blood	,073	75	,200*	,973	75	,106

The results of the study showed that the combination of fragrant pandan leaf (*Pandanus amaryllifolius* Roxb.) and kenikir leaf (*Cosmos caudatus* Kunth.) infusions had significant antihyperglycemic activity in male white mice. The normality test (Shapiro-Wilk, p = 0.106) and homogeneity (Levene, p > 0.05) ensured that the data were normally distributed and homogeneous, so that the ANOVA analysis could be used with good validity.

Following a normality test, the Shapiro-Wilk value shows that the significance value is more than 0.05, indicating that the research data in Table 1 is normally distributed (Table 2).

Table 3. Homogeneity Test

Levene's Equality of Error Test Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Blood Sugar Levels	Based on Mean	1,559	14	60	,118
	Based on Median	,554	14	60	,889
	Based on the Median and with adjusted df	,554	14	47,319	,886
	Based on trimmed mean	1,482	14	60	,146

The research data is therefore deemed homogeneous as the homogeneity test shows that the significance value is greater than 0.05 (Table 3). Given the aforementioned data, this study satisfies the prerequisites to proceed to the ANOVA test step, which determines whether the variance is homogeneous and the data is normally distributed. The ANOVA test was then performed, and the results are shown in Table 4.

The significance value in Table 2 shows that the variance of data between groups is homogeneous. The fulfillment of the assumptions of normality and homogeneity ensures that the results of the ANOVA analysis obtained are valid and can be interpreted scientifically.

The blood sugar levels of the mice in each research data set differ significantly between the groups, according to the mouse group's ANOVA test findings, where the significance value is less than 0.05. Similarly, the observation day's significance value demonstrates a significant difference, as demonstrated by the p-value less than 0.05, suggesting that the observation day affects the experimental animals' blood sugar levels, the observation day and the treatment group are related, as indicated by the significant value of <0.05 for this association (Table 4).

Also, a different test, the *Duncan post hoc test*, was conducted to determine the efficacy of this infusion and the optimal dosage for reducing the mice's blood sugar levels. The results of the *Duncan post hoc test* are shown in Table 5.

Table 4. *ANOVA* Test

Evaluations of Between-Subjects Effects

Dependent Variable: Blood Sugar Levels

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	78682,987[a]	14	5620,213	125,507	,000
Intercept	1621851,213	1	1621851,213	36218,205	,000
Group_Mice	61540,320	4	15385,080	343,570	,000
Day_Observation	10918,827	2	5459,413	121,916	,000
Group_Mice * Day_Observation	6223,840	8	777,980	17,373	,000
Error	2686,800	60	44,780		
Total	1703221,000	75			
Corrected Total	81369,787	74			

Table 5. *Post hoc Duncan* Test

Group Mice	N	1	2	3	4
Positive control	15	117,73			
Infusa 3	15	118,93			
Negative control	15		137,07		
Infusa 2	15			174,47	
Infusa 1	15				187,07
Sig.		, 625	1,000	1,000	1,000

Duncan's further test showed that the negative control group had the highest blood glucose level (137.07 mg/dL), while the positive control (117.73 mg/dL) and infusion group 3 (118.93 mg/dL) showed a significant decrease. Meanwhile, infusion group 2 (174.47 mg/dL) and infusion group 1 (187.07 mg/dL) had lower effectiveness. These findings indicate that a certain combination dose (infusion 3) provides an antihyperglycemic effect comparable to the positive control drug, which is generally a standard antidiabetic agent. These results support the existence of a dose-response relationship and the importance of the duration of administration on the antihyperglycemic effectiveness of the pandan-kenikir combination.

DISCUSSION

Pharmacologically, *Cosmos caudatus* leaves are rich in polyphenols (including quercetin) and consistently exhibit α-glucosidase inhibition—a mechanism that slows carbohydrate breakdown and lowers postprandial glucose. Metabolomics studies and bioactivity assays confirm variations in kenikir metabolite content across growth stages, which are associated with stronger α-glucosidase inhibitory activity; this explains why standardized ingredients and postharvest processes are crucial for consistent antihyperglycemic effects (17,18).

This antihyperglycemic effect may be attributed to the bioactive compounds in pandan wangi leaves, such as flavonoids and alkaloids, which have antioxidant activity and can increase insulin sensitivity. Meanwhile, kenikir leaves are known to contain polyphenols, quercetin, and chlorogenic acid, which play a role in increasing peripheral glucose uptake and inhibiting the enzyme α-glucosidase (19). The combination of these two plants appears to produce a synergistic effect that enhances the ability to lower blood glucose levels (20).

These results align with previous research that reported that kenikir extract has the potential to lower glucose levels through mechanisms that increase insulin sensitivity and inhibit oxidative stress. Similarly, pandan wangi is known to stimulate insulin secretion in animal models of diabetes (21). Therefore, the combination of these two plants could be a candidate phytopharmaceutical for complementary therapy for diabetes mellitus (6).

In humans, 8 weeks of kenikir supplementation in patients with type 2 diabetes mellitus improved glucose levels and insulin sensitivity, lending clinical relevance to

the findings in your animal study. A recent pharmacognosy review also summarized this clinical evidence and positioned kenikir as a candidate for a functional food-based antidiabetic agent (22,23).

For fragrant pandan leaves (*Pandanus amaryllifolius*), human (pilot) evidence indicates a reduction in postprandial glucose after pandan tea consumption, while in animal models, administration of pandan extract increased insulin sensitivity and improved metabolic parameters in rats on a high-fat diet. More recently, studies in gestational diabetes models have also shown glucose reduction and improvements in pancreatic mass index at high doses of pandan extract—suggesting potential protection for pancreatic tissue. Proposed mechanisms include modulation of insulin homeostasis, antioxidant activity, and possible effects on peripheral tissue glucose transport (20,24,25).

Key components such as quercetin—which is abundant in kenikir—have a strong evidence base (both animal and human controlled trials) for lowering fasting glucose and improving insulin sensitivity, as well as protecting pancreatic β-cells via an antioxidative-anti-inflammatory pathway. Recent meta-analyses and reviews reinforce this mechanistic rationale and biologically explain why the kenikir-pandanus combination at the optimal dose (Infusion 3) in your study approximated the effects of the positive control (26,27).

Overall, the significant effects, time-to-treatment interactions, and literature (carbohydrate digestive enzyme inhibition, increased insulin sensitivity, pancreatic protection) support the hypothesis that the pandan–kenikir infusion combination works through a dual mechanism: (1) inhibiting α-glucosidase (suppressing the postprandial glucose spike) common in kenikir; and (2) modulating insulin sensitivity/secretion and relevant oxidative stress in pandan—so that at the correct composition/dose (Infusion 3) the effect is equivalent to the standard agent in a mouse model (17,18).

CONCLUSIONS

This study shows that a combination of *Pandanus amaryllifolius* Roxb. and *Cosmos caudatus* Kunth. Leaf infusions have significant antihyperglycemic activity in male white mice. A certain dose of the combination (3 infusions) produced a blood glucose-lowering effect comparable to that of the positive control. These findings support the potential of this combination as a natural source of ingredients in the development of complementary therapies for the management of diabetes mellitus.

The combination of pandan and kenikir infusions demonstrated significant antihyperglycemic activity in mice, with Infusion 3 producing glucose reductions comparable to the positive control. The most likely mechanisms are α-glucosidase inhibition (primarily in kenikir) and increased insulin sensitivity/secretion and antioxidant activity (primarily in pandan), consistent with recent preclinical and clinical evidence. These findings position the combination as a promising candidate for phytopharmaceutical/complementary therapy for the management of hyperglycemia, and are worthy of further toxicity studies, optimal dose determination, and early clinical trials.

ACKNOWLEDGEMENTS

We are grateful to everyone who has contributed significantly to the completion of this study.

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