POTENTIAL ALTERNATIVE SERUM AND URINE CREATININE REAGENT FROM THE PHENOLIC CONTENT OF THE *Cymbopogon citratus* (LEMONGRASS) LEAVES EXTRACT

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ABSTRACT

Accurate assessment of kidney function is essential for early detection, diagnosis, and management of renal diseases, with serum and urine creatinine levels widely used as biomarkers for evaluating glomerular filtration rate (GFR). The study was conducted to assess the potential of lemongrass phenolic crude extract (LPCE) as an alternative reagent for the determination of creatinine levels in serum and urine. The study employed an experimental-comparative design to evaluate its effectiveness as a natural, sustainable alternative, using the Jaffe Kinetic Method and a commercial Profame reagent kit as controls. Phenolic extraction was achieved using column chromatography, and total phenol content was measured using the Folin-Ciocalteu method. The researchers assessed the percent yield of LPCE and tested four concentrations: 100%, 75%, 50%, and 25% to determine the best formulation. The extract was formulated into a test reagent and applied to serum and urine samples collected from five volunteer donors. This research included a 23.01% phenolic yield from lemongrass leaves, with 6.25% LPCE identified as the most suitable concentration for serum creatinine determination, demonstrating comparable performance to the standard reagent in terms of pH, color intensity, and accuracy. Therefore, the hypothesis asserting no significant differences among phenolic crude extract concentrations in total phenolic content is rejected, whereas the hypothesis proposing no significant difference in performance relative to the standard creatinine reagent is accepted. Overall, the study aimed to develop a lemongrass-based creatinine reagent as a safer, more affordable, and eco-friendly alternative to synthetic reagents, using locally sourced materials to reduce costs and reliance on imports.

Keywords: Column chromatography, jaffe kinetic method, kidney function test, picric acid, and phenolic extract

INTRODUCTION

Background of the Study

The kidneys are crucial to overall health, as they perform several key functions. They help clear the plasma of unnecessary substances, including metabolic waste and excess compounds. One key indicator of kidney function is creatinine, a byproduct of muscle metabolism. It is a critical indicator of renal function, with serum concentrations inversely correlated with the GFR. Creatinine levels can rise due to kidney problems, dehydration, or high muscle mass, and drop with low muscle mass, overhydration, or pregnancy, with "normal" levels varying based on factors like age, sex, and body size (National Kidney Foundation, 2024). Elevated serum creatinine levels are typically indicative of impaired glomerular function (Bishop *et al.,* 2022). The protein-to-creatinine ratio in urine is a widely used metric for estimating daily protein excretion and has been shown to detect kidney disease; however, its accuracy is influenced by urine concentration (Yang *et al.,* 2015).

Creatinine is usually measured using synthetic chemical reagents, which pose environmental and health risks. In contrast, natural plant products offer a promising,

eco-friendly alternative. Herbs and plant-derived substances are rich in bioactive compounds, including antioxidants, which have gained significant attention for their health benefits. The Folin-Ciocalteu reaction, widely used to measure antioxidant capacity, highlights the importance of phenolic compounds as free radical neutralizers (Raventeros, 2017; Noreen, 2017).

Among these natural products, *Cymbopogon citratus* (lemongrass) exemplifies the potential of plant-based solutions. As a widely distributed herb in the Poaceae family, lemongrass contains a variety of bioactive compounds, including phenols, flavonoids, and tannins. These phenolic compounds not only exhibit antioxidant properties but also show therapeutic potential, particularly in the treatment of liver disease (Machraoui *et al.,* 2018; Irfan *et al.,* 2022). The integration of lemongrass and similar plant-based products into clinical diagnostics could replace synthetic reagents, offering a sustainable approach to health management while reducing environmental harm. This underscores the dual role of natural plant products in advancing both healthcare and ecological sustainability.

However, few studies have explored how plant-derived compounds can serve as reliable reagents for this critical diagnostic process, despite the potential environmental and health benefits. This study aims to develop a plant-based creatinine reagent to make laboratory tests safer, more affordable, and more accessible, while reducing reliance on harmful synthetic chemicals. In the study of Cai *et al.* (2019), fabricating a lemongrass membrane using only dried plant material and water—without pricier chemicals—demonstrating high efficacy in dye adsorption and microbial inhibition, while repeatedly highlighting low cost and abundance of the raw material and its simple, scalable fabrication. Similarly, an optimized decoction study achieving approximately 72 mg GAE per 100 mL used solely water, heat, and basic filtration**,** avoiding organic solvents, thus significantly reducing operational costs (Muala *et al.,* 2021).

Statement of the Problem

The study was conducted to assess the potential of lemongrass phenolic crude extract (LPCE) as an alternative reagent for the determination of creatinine levels in serum and urine. Carried out from January to April 2025, it specifically aimed to answer the following research problems:

1. What is the percent yield of the LPCE of the lemongrass leaves?
2. What is the optimum concentration of LPCE as a potential serum and urine creatinine reagent under the following concentrations:
    a. 100%
    b. 75%
    c. 50%
    d. 25%?
3. Is there a significant difference in the performance of the LPCE against the standard creatinine reagent in terms of pH, color intensity, and accuracy?

Statement of the Null Hypothesis

There is no significant difference in the performance of the lemongrass phenolic crude extract against the standard creatinine reagent in terms of pH, color intensity, and accuracy.

METHODOLOGY

Research Design

The study used a comparative-experimental design. Creatinine levels were measured using the Jaffe kinetic method as the control to test the potential of Lemongrass Phenolic Crude Extract (LPCE) as an alternative reagent for serum and urine samples. Different LPCE concentrations were tested to determine the most effective concentration, and the results were compared with the standard Profame reagent kit to assess accuracy.

Study Site and Sample Collection

The research was conducted at Saint Mary's University, Bayombong, Nueva Vizcaya, in a research laboratory equipped to analyze lemongrass's phenolic properties and its potential as a creatinine testing reagent. Lemongrass samples were collected from Brgy. Pogonsino, Bagabag, Nueva Vizcaya.

Plant Certification

The lemongrass samples were submitted to the Bureau of Plant Industry in Nueva Vizcaya for identification and certification, confirming them as *Cymbopogon citratus*.

Blood and Urine Donors

Blood and urine samples were collected from five volunteer students at Saint Mary's University who gave their consent to join the study. The donors met the required criteria and were informed about the study's purpose and procedures. Using these samples, the researchers aimed to obtain enough data for accurate correlation and analysis.

Data Gathering Procedure

Preparation of Lemongrass Leaves

The leaves were washed with running water, air-dried, then oven-dried at 50°C. They were powdered using a blender, weighed on a digital balance, and prepared for maceration. Samples were placed in a 500mL Erlenmeyer flask, soaked in 80% ethyl alcohol for 24 hours, then decanted and filtered to obtain the crude ethanol extract. The extract was finally concentrated using a rotary evaporator at 50°C.

Phenol Extraction Through Column Chromatography

Fifty grams of silica gel was mixed with hexane to form a slurry, then packed into a chromatography column, avoiding air bubbles. The column was tapped to settle the gel evenly, and excess hexane was drained. Using the wet method, the sample was applied, and elution was carried out with hexane, chloroform, and methanol while keeping the column moist. As the solvents passed through, colored bands from amber yellow to light yellow appeared and were collected in test tubes. Each fraction was tested with Ferric chloride, where a blue color indicated the presence of phenolic compounds.

Total Phenol Determination Through Folin-Ciocalteu Method

The phenolic content of lemongrass leaves was measured using the Folin-Ciocalteu Method. A calibration curve was prepared by mixing 1 mL of Gallic acid solutions (40–200 µg/mL) with 1 mL of diluted Folin-Ciocalteu reagent and 3 mL of 20% sodium carbonate. After two hours of incubation, absorbance was read at 765 nm using a spectrophotometer. The lemongrass extract was treated with the same reagents, incubated at 37°C for three hours, and its absorbance was also measured at 765 nm.

Phenolic Reagent Preparation for Creatinine

The preparation of the phenolic reagent involved dissolving 200 µL of total phenol concentration from lemongrass leaves in 1000 µL of dimethyl sulfoxide (DMSO) to improve yield and reduce contamination. Then, 4000 µL of 63% nitric acid was added drop by drop while cooling the mixture in an ice bath to maintain stability.

The solution was gently stirred and heated in a boiling water bath for 4 hours. During this process, brown gas formed, and after 1.5–2 hours, the solution's color changed from deep to amber yellow. The mixture was then cooled, and 100 µL of the phenol picric acid solution was diluted with 6400 µL of distilled water to produce a 6.25% concentration.

Blood and Urine Collection

Serum samples were collected through venipuncture in the antecubital vein and placed in 4 mL yellow-top tubes for serum separation under the supervision of a Registered Medical Technologist. Tubes were labeled with patient details, then centrifuged at 3500 rpm for five minutes to separate serum from cells. The serum was pipetted into a clean container and stored properly to prevent contamination.

Urine samples were provided by five random donors during lab hours. Each was collected midstream in a sterile container, stored at room temperature, and tested within one hour. Collection details such as date and time were recorded. The urine creatinine test followed the protocol of the commercial creatinine kit.

Determination of Creatinine Level

Serum and urine creatinine were measured using the Jaffe method. Samples were mixed with Jaffe's reagent, and in a separate set, a phenolic reagent was used instead of picrate. Absorbance was read at 505 nm with a UV-VIS BK1000 spectrophotometer, using distilled water as the blank.

Tests were done in triplicate and compared with a pathologic control. Results were recorded and checked against the manufacturer's reference values to ensure accuracy.

Treatment of Data

The creatinine results from serum and urine were analyzed using a two-sample T-Test to compare different reagent concentrations. Data were processed using tables, reference parameters, pH readings, color grading, and spectrophotometer absorbance values, which are presented in the appendices.

Ethical Consideration

This study was approved by the Saint Mary's University Research Ethics Board (SMUREB) of Saint Mary's University, 2nd floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, Nueva Vizcaya, Philippines under the approval code of SMUREB 2025-0892. SMUREB can be reached via email at reb@smu.edu.ph or by phone at 0917-705-3041.

RESULTS AND DISCUSSIONS

Section 1. Percent Yield

Section 1 presented the percent yield of the lemongrass phenolic extract from 323.35 grams of dried leaves.

Figure 1

Percent Yield of the Phenolic Content of Lemongrass Leaves

$$\text{Percent yield (\%)} = \frac{74.42\ grams}{323.35\ grams} \text{ x } 100 = 23.01\%$$

Figure 1 indicates that the percentage yield of lemongrass phenolic extract was 23.01%. Using methanol as a solvent, the extraction produced an amber-yellow mass weighing 74.42 g.

Based on existing studies, a phenolic yield of 23.01% can be considered relatively high, particularly when compared to yields obtained through efficient extraction methods such as sonication, which have been reported to reach up to 26.7% (Md Saad et al., 2022; Tapas et al., 2022). Although this yield is not directly linked to creatinine reagent production, it aligns with literature benchmarks and indicates strong potential for application in reagent formulation.

In conclusion, the 23.01% yield of phenolic extract from lemongrass using methanol shows high extraction efficiency, similar to those reported with advanced methods. Although not directly linked to creatinine reagent production, this yield supports the potential of lemongrass as a natural source of phenolic compounds for reagent use.

Section 2. Different Concentrations of the Phenolic Extract

Section 2 emphasized the different concentrations of the phenolic extract used to determine the appropriate dilution for the alternative creatinine reagent.

Table 1

Specific Concentration of Phenolic Extract in Serum Creatinine Determination

		Results (6.25%)	
Sample	Trials	Phenolic Reagent	Jaffe Reagent (Control)
1	Trial 1	6.48	2.0
	Trial 2	14.48	
	Trial 3	23.17	
2	Trial 1	0.69	1.16
	Trial 2	0.41	
	Trial 3	0.34	
3	Trial 1	1.59	2.33
	Trial 2	0.62	
	Trial 3	0.34	
4	Trial 1	0.69	1.834
	Trial 2	2.55	
	Trial 3	0.83	
5	Trial 1	2.69	2.0
	Trial 2	2.0	
	Trial 3	1.38	

Table 1 shows the absorbance values in serum creatinine levels with the 6.25% concentration of the LPCE. It was observed that a 6.25% concentration with a 15:1 dilution makes the LPCE a more reliable method for estimating serum creatinine levels because it attained results that are closer and more aligned with the established Jaffe Reagent (Control) method. However, the Picric Acid of Jaffe Reagent (Control) method generally yielded lower serum creatinine level, while the LPCE showed higher creatinine level.

Ekpenyong (2015) investigated how varying concentrations and durations of lemongrass leaf infusions affect renal function markers, specifically serum and urinary creatinine, CCr, and eGFR. The study demonstrated that different doses affect creatinine levels and kidney function, supporting the concentration- and dilution-dependent effects observed in LPCE. It found that a 6.25% LPCE concentration provided the most consistent and reliable results compared to the control.

To conclude, a 6.25% LPCE concentration at a 15:1 dilution shows promise as a reliable alternative to the standard Jaffe method for serum creatinine testing. Although it produced slightly higher values, its consistency with the control supports its potential as a natural reagent. These findings also show that different lemongrass concentrations can affect creatinine levels, highlighting LPCE's suitability for clinical use.

Table 2

Specific Concentration of Phenolic Extract in Urine Creatinine Determination

		Results (6.25%)	
Sample	Trials	Phenolic Reagent	Jaffe Reagent (Control)
1	Trial 1	6.48	2.0
	Trial 2	14.48	
	Trial 3	23.17	
2	Trial 1	0.69	1.16
	Trial 2	0.41	
	Trial 3	0.34	
3	Trial 1	1.59	2.33
	Trial 2	0.62	
	Trial 3	0.34	
4	Trial 1	0.69	1.834
	Trial 2	2.55	
	Trial 3	0.83	
5	Trial 1	2.69	2.0
	Trial 2	2.0	
	Trial 3	1.38	

Table 2 shows the results of urine creatinine determination using different concentrations of the alternative creatinine reagent. The phenolic reagent yielded inconsistent values across the five samples. For instance, in Sample 2 at 50% concentration, an unusually high result of 242.0 was recorded, while in sample 3 at 100%, a negative value of -13.63 was observed which can also be seen in the results of the other samples in different concentrations like sample 1 at 100%, Sample 4 at 50%, and sample 5 both at 100% and 50% concentrations.

Previous studies have identified challenges in measuring creatinine in urine due to nonspecific interactions with common urinary compounds such as urea and uric acid. These substances can interfere with chromophore formation in colorimetric assays. Lewinska *et al*. (2019) reported that such interference affects the traditional Jaffe method, leading to decreased absorbance and reduced accuracy in spectrophotometric analysis. This can result in variability and unreliable outcomes when urine is used for creatinine determination.

These findings suggest that using urine samples with LPCE may lead to less consistent and reliable creatinine results compared to the more stable outcomes seen with serum samples. While LPCE shows promise for serum testing, its use in urine still needs further improvement or alternative methods to ensure accuracy and reliability.

Section 3. Performance of LPCE in terms of pH and color intensity

Section 3 assessed the performance of LPCE in terms of pH and color intensity and when tested spectrophotometrically.

Table 3
Phenolic Extract for Serum Creatinine Determination

	pH	Color Intensity
6.25% Concentration of Phenol Extract	1	Y02
Picric acid of Jaffe	3	Y11

Table 3 shows that a 6.25% concentration of LPCE exhibits an acidic pH of 1 and a color intensity of Y02, which is comparable to the Picric acid used in the Jaffe method, having an acidic pH of 3 and a color intensity of Y11. The notable outcome here is that the LPCE managed to achieve similar color intensity under more acidic conditions, suggesting its capability to function efficiently outside the pH range typical for the Jaffé reaction. This is particularly significant because both pH and color intensity are essential parameters in colorimetric analysis.

These results align with phytochemical research showing that *Cymbopogon citratus* is rich in active phenolic compounds—including flavonoids, tannins, terpenoids, and coumarins—which are known for strong chromogenic properties (Shendurse *et al.,* 2021; Anupam Kumar, 2020). Similarly, Asaolu *et al.* (2023) confirmed the presence of bioactive phenolic compounds in lemongrass, which are known to produce strong color reactions in biochemical tests. Taken together, the strong chromogenic behavior of LPCE at acidic pH, combined with its biological activity, demonstrates its potential as an effective, natural alternative reagent for creatinine detection.

Overall, the results showed that a 6.25% LPCE produced strong color intensity at a highly acidic pH, similar to the standard Jaffe reagent. This suggested its potential as a natural alternative for creatinine detection. The presence of bioactive phenolic compounds in lemongrass supported its effectiveness in colorimetric analysis, highlighting its suitability for clinical use.

Figure 2
Serum Creatinine Level

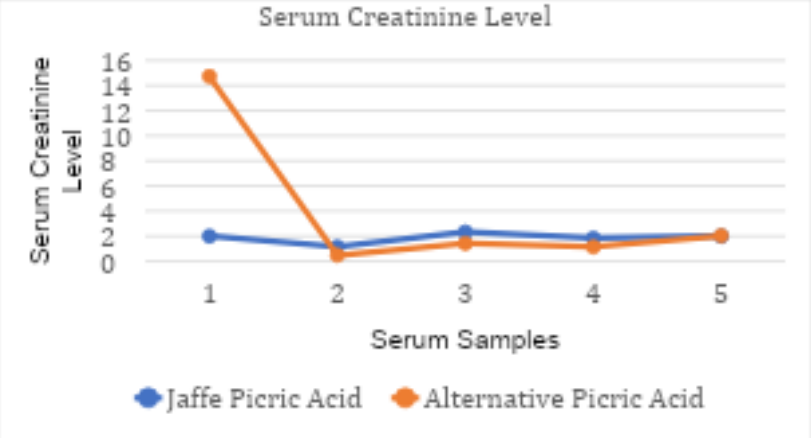

Figure 2 illustrates the comparison of serum creatinine levels determined using the standard reagent Picric Acid of Jaffe and the alternative reagent using 6.25% concentration of phenol extract. The Jaffe method using picric acid generally yielded a lower serum creatinine level, whereas the phenol extract showed a higher creatinine level. Both methods displayed a consistent pattern across the five samples. The Alternative Picric Acid method showed a sharp increase in creatinine level at Sample 1, followed by a gradual decrease from Samples 2 to 5 relative to the initial spike. Similarly, the Jaffe Picric Acid method followed a comparable overall trend, although it showed its peak at Sample 3 rather than Sample 1. This suggests a parallel trend in response between the two methods, indicating that the phenol extract might exhibit a similar detection behavior to the standard reagent, picric acid, at a 6.25% concentration.

The result was supported by the study of Delanghe and Speeckaert (2017), which emphasized the need for alternative methods due to the Jaffe method's susceptibility to interference from substances such as glucose, ketones, and proteins. Moreover, the study of Ou *et al.* (2015), compared LC-MS/MS, enzymatic, and Jaffe methods and found that enzymatic assays demonstrated higher accuracy and lower bias than Jaffe methods, further reinforcing the importance of pursuing more reliable alternatives.

The results showed that the 6.25% phenol extract followed a similar trend to the standard Jaffe method in detecting serum creatinine levels, suggesting its potential as a natural alternative. Although it produced slightly higher values, its consistent pattern supports its reliability. This aligns with previous studies that highlight the limitations of the Jaffe method and the need for more accurate, interference-resistant alternatives.

Figure 3
Urine Creatinine Level

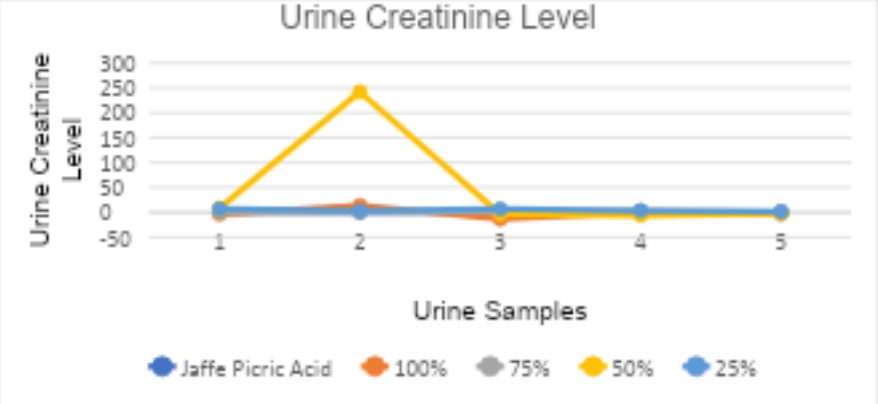

Figure 3 illustrates the comparison of urine creatinine levels determined using the standard reagent Picric Acid of Jaffe and the alternative reagent using 100%, 75%, 50% and 25% concentration of phenol extract. The Picric Acid of the Jaffe method yielded consistently low urine creatinine levels, while the phenol extract showed both high and low creatinine levels, making it unsuitable as a creatinine reagent for urine specimens. Most methods, including 100%, 75%, and 25%, showed both high and low, or negative, creatinine levels throughout, indicating poor detection across the sample points. The 50% concentration displayed a distinct peak at Sample 2, followed by a sharp drop in the succeeding samples, but still indicates an inconsistency when compared to Jaffe Picric Acid. On the other hand, the Jaffe Picric Acid method maintained a relatively steady response, with only minor fluctuations, and showed a strong positive reading.

Due to its inconsistency, urine compounds like urea and uric acid may have interfered with the process. These interfering substances have the potential to react non-specifically with picric acid, thereby disrupting the intended chemical reaction pathway. This interference may either hinder the proper formation of chromophores or trigger competitive side reactions that consume the reagents meant for the target reaction. As a consequence, the expected color development and absorbance increase are diminished, leading to a noticeable decrease in values when measured by spectrophotometry. This reduction in absorbance ultimately affects the accuracy and sensitivity of creatinine detection in the sample (Lewinska *et al.,* 2019).

The phenol extract produced inconsistent, unreliable results for measuring urine creatinine levels, unlike the Jaffe method, which maintained stable readings. This inconsistency may be due to interference from urine compounds such as urea and uric acid, which can affect the chemical reaction and reduce the accuracy of spectrophotometric detection.

Table 4

Descriptive and Inferential Statistics Comparing Jaffe and Alternative Reagents for Serum Creatinine Measurement
ns: not significant

Reagent	n	Mean	SD	t-value	df	p-value
Jaffe Reagent	5	1.86	0.43	0.790^{ns}	4	0.475
Alternative Reagent		3.96	6.04			

Table 4 shows a comparison of serum creatinine levels measured using the standard Jaffe reagent and the alternative lemongrass phenolic crude extract in five serum samples. The analysis showed that the mean creatinine

level for the Jaffe reagent was 1.86 (SD = 0.43), while the alternative reagent yielded a higher and more variable mean of 3.96 (SD = 6.04). However, this difference was not statistically significant, t(4) = 0.79, p = .475, suggesting that the alternative reagent did not significantly differ from the standard method in this small sample.

The study by Selvin *et al.* (2015) investigated the accuracy and precision of different serum creatinine measurement methods—specifically, the Jaffe method, enzymatic assays, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) —with LC-MS/MS serving as the reference standard. The researchers found that the Jaffe method consistently overestimated creatinine levels and showed greater variability than enzymatic methods. This study underscores the importance of evaluating both accuracy and variability when comparing assay methods and highlights how sample size influences the statistical significance of observed differences.

The method comparisons often reveal higher variability in non-reference assays, and lack of significance does not mean no difference especially in small samples. It reinforces that further study is warranted for new assay alternatives like lemongrass extract. These findings indicate that, while the alternative reagent produced more variable results, it may warrant further investigation as a creatinine assay method.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study evaluated Lemongrass Phenolic Crude Extract (LPCE) as an alternative reagent for serum and urine creatinine testing. The extraction yielded 23.01%, and the 6.25% concentration was found to be most suitable. LPCE showed results comparable to those of the standard reagent for pH, color intensity, and accuracy, suggesting its potential as a natural alternative for creatinine detection.

Recommendations

Future researchers may conduct the following:

1. Use HPLC in extracting phenolic compounds from Cymbopogon citratus to achieve higher yield and purity.
2. Assess the stability and shelf-life of the extracts under various storage conditions to confirm long-term effectiveness.
3. Investigate other plant sources rich in phenolic compounds to compare extraction performance and bioactivity.
4. Utilize 24-hour urine collection instead of random sampling to ensure more precise creatinine measurement.

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