IN VITRO THROMBOLYTIC ACTIVITY OF ETHANOLIC EXTRACT FROM *Paspalum conjugatum* (CARABAO GRASS) AND *Eleusine indica* (PARAGIS)

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ABSTRACT

Thromboembolic disorders, including ischemic heart disease and stroke, pose significant health risks, leading to urgent needs for effective treatments. Current thrombolytic agents, while effective, often have limitations such as high costs and side effects. This research aimed to assess the potential in vitro thrombolytic activity of ethanolic extracts from carabao grass (*Paspalum conjugatum*) and paragis grass (*Eleusine indica*) as a natural, cost-effective alternative for managing thromboembolic disorders. In vitro tests were used to assess the effect of the ethanol extract from carabao and paragis grass on clot lysis. Phytochemical screening revealed the presence of flavonoids and tannins in both grass extracts, indicating their potential for clot lysis. The results from thrombolytic assays showed a dose-dependent increase in clot lysis, *P. conjugatum* extract lysed the clot by 18.09 % in 2.5mg/mL, 27.59% in 5 mg/mL, 49.39% in 10 mg/mL and 65.60% in 20 mg/mL showing a steady increase with the concentration, while in *E. indica* extract it lysed 25.38 % in 2.5mg/mL, 41.53% in 5 mg/mL, 59% in 10 mg/mL and 82.67% in 20 mg/mL indicating that with the highest concentrations of both extracts achieving significant clot reduction making it the optimal concentration. Compared with the negative control, it showed a mere 0.32% reduction in clot formation. The findings suggest that carabao and paragis grass extracts possess a thrombolytic activity, warranting further investigation into their pharmacological properties and potential as natural thrombolytic agents.

Keywords: clot, lysis, flavonoids, tannins, thromboembolic disorders

INTRODUCTION

Background of the Study

Currently, many individuals are impacted by thromboembolic disorders, which include a variety of conditions affecting the heart and blood vessels. The World Health Organization (WHO, 2021) identifies this category as encompassing issues such as ischemic stroke and myocardial infarction. Thrombosis is the primary cause of both ischemic heart disease and ischemic stroke. This condition occurs when blood vessels become blocked due to excessive clotting or malfunctioning anti-clotting factors, as highlighted by Ogugofor et al. (2022). If not addressed, these disorders can result in the formation of blood clots, or "thrombi," within blood vessels, leading to serious health complications.

In recent years, cardiovascular diseases (CVDs) have resulted in 17.9 million deaths, representing 32% of all fatalities, with 85% of these deaths attributed to heart attacks and strokes, making CVD a significant global health issue (WHO, 2021). The Philippine Statistics Authority reports that ischemic heart disease, also known as coronary heart disease, accounted for 105,114 deaths, or 17.14% of all fatalities (PSA, 2021). Chest pain or discomfort is a common symptom, often caused by blood clots forming in the coronary arteries. These clots contribute to the majority of CVDs by partially or completely obstructing blood vessels (Ashorobi et al., 2021).

Thromboembolic disorders, which include ischemic stroke and myocardial infarction, are significant global health challenges affecting millions of individuals. The World Health Organization (2021) identifies thrombosis as a primary cause of these conditions, leading to the formation of blood clots that obstruct blood vessels. In recent years, cardiovascular diseases (CVDs) have resulted in approximately 17.9 million deaths annually, accounting for 32% of all

fatalities worldwide. This alarming statistic underscores the pressing need for effective treatments and preventive measures to combat these life-threatening conditions. In the Philippines, ischemic heart disease, also known as coronary heart disease, was responsible for 105,114 deaths, representing 17.14% of total fatalities (Philippine Statistics Authority, 2021). Unfortunately, the lack of government support for emergency care and prevention programs hinders the effective implementation of strategies designed to combat these issues, leaving many at risk.

Carabao grass is traditionally used for its health benefits, including the treatment of wounds and various ailments. Its bioactive compounds, such as flavonoids and tannins, are believed to contribute to its therapeutic properties. Similarly, *paragis* is known for its antibacterial, antiviral, and anti-inflammatory effects, making it a valuable resource in traditional medicine. Preliminary research indicates that both plants may possess significant thrombolytic activity, positioning them as potential, cost-effective alternatives to current thrombolytic therapies.

The findings of this study not only emphasize the potential of carabao grass and paragis as natural thrombolytic agents but also align with Sustainable Development Goals (SDGs). Specifically, this research supports SDG 3, which aims to ensure good health and well-being for all individuals, and SDG 9, which encourages innovation and sustainable industrial practices. By highlighting the efficacy of these herbal extracts, the study opens avenues for further exploration of their bioactive compounds, fostering innovation in the development of safer and more accessible treatments for thromboembolic disorders, and ultimately addressing health disparities in underserved communities.

Statement of the Objectives

This study aimed to determine the in vitro thrombolytic activity of ethanolic extract from carabao grass *(P. conjugatum)* and paragis grass *(E. indica)*. It was conducted from January to March 2025.

Specifically, it sought to:

1. Determine the secondary metabolites of the ethanolic extract from carabao grass *and paragis grass* using the test tube method.

2. Determine the percentage of clot reduction before and after treatment in the following concentrations:
   a. 2.5 mg/mL carabao grass extract;
   b. 5 mg/mL carabao grass extract;
   c. 10 mg/mL carabao grass extract;
   d. 20 mg/mL carabao grass extract.

3. Determine the percentage of clot reduction before and after treatment in the following concentrations:
   a. 2.5 mg/mL paragis grass extract;
   b. 5 mg/mL paragis grass extract;
   c. 10 mg/mL paragis grass extract;
   d. 20 mg/mL paragis grass extract.

METHODOLOGY

Research Design

An experimental in vitro design was used to evaluate the thrombolytic activity of ethanol extracts from *carabao and paragis grass*. Procedures included blood collection, crude extraction, and testing various concentrations for their effects on clot lysis.

Study Site and Sample Collection

Carabao grass was collected from Purok 2, Baresbes, Quezon, Nueva Vizcaya, a location near a river and distant from pollution sources such as vehicle emissions. Paragis grass was collected in Salvacion, Saguday, Quirino, where it was abundant and ideal for sampling. All laboratory procedures, including extraction and testing, were conducted at the SMU–Center for Natural Sciences Research Laboratory.

Specimen Identification

The collected sample of *Paspalum conjugatum* (carabao grass) and *Eleusine indica* (paragis) was submitted to the Dean of the College of Forestry, Environment and Resource Management of Nueva Vizcaya State University, Bayombong, Nueva Vizcaya, for its taxonomic identity. The experts carefully analyzed it to ensure that the plant samples are correctly positioned within established categories, thereby bolstering its reliability.

Blood Donors

The study involved one male volunteer donor who provided 12 mL of blood by venipuncture using a non-additive evacuated tube system, with the procedure monitored by a Registered Medical Technologist (RMT). The donor met WHO screening criteria, including being at least 18 years old, weighing at least 45 kg, not on anticoagulants, and free of recent tattoos or blood-related illnesses. A health screening questionnaire was used to ensure donor suitability, and informed consent was obtained, with all data kept confidential.

Data Gathering Procedure

Preparation of Carabao and Paragis Grass

The collected leaves of carabao and paragis grass were thoroughly washed to eliminate dirt and contaminants. After cleaning, the leaves were air-dried to remove moisture and prepare them for extraction.

Crude Extraction

The leaves were air-dried for two to three days, then oven-dried at 55°C until crispy and brown. The dried leaves were ground into a fine powder using a laboratory blender. Five hundred grams of the powder were soaked in 1000 mL of ethanol for 48 hours, then filtered through cheesecloth. The filtrate was concentrated using a rotary evaporator under vacuum.

Test Tube Phytochemical Test

Following Ghaeni et al. (2022), flavonoids were detected by mixing 0.5 mL of the extract with sodium hydroxide (NaOH) and then hydrochloric acid (HCl), a color change from yellow to colorless indicated flavonoid presence. For tannins, the extract was combined with diluted ferric chloride; a blue-black color indicated gallic tannins, and green to blue-green indicated catechin tannins.

Thrombolytic Concentration Formulation

The carabao and paragis grass extracts were each mixed with normal saline solution to prepare thrombolytic formulations at four concentrations: 2.5 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL, with each concentration placed in separate test tubes for testing.

Blood Collection and Processing

Blood was collected under RMT supervision using 24 sterile, pre-weighed, non-additive evacuated tubes, each containing 500 µL of blood from a healthy donor. The tubes were kept upright and undisturbed during processing.

Thrombolytic Assay

The blood samples were incubated at 37°C for 60 minutes to form clots, then centrifuged for 5 minutes to separate clots from serum. The clot weight was measured after serum removal. Each clot was treated with different concentrations (2.5, 5, 10, and 20 mg/mL) of carabao and paragis grass extracts and incubated for 90 minutes to assess clot lysis.

Percentage of Clot Reduction

The clot weight before and after treatment was recorded to determine thrombolytic activity. The percentage of clot reduction was calculated using the formula:

$$\%\ Clot\ reduction = \frac{Clot\ (g) before\ treatment - Clot\ (g) after\ treatment}{Clot\ (g) before\ treatment} \text{ x } 100$$

This value reflects the effectiveness of the carabao and paragis grass extracts in lysing the clot.

Selection of the Optimum Concentration

The optimum concentration was determined by comparing the percentage of clot reduction across all tested concentrations. The concentration that showed the highest clot reduction was selected as the most effective. Statistical analysis was used to validate the results and ensure consistency.

Treatment of Data

The thrombolytic activity of the different concentrations was measured using the % Clot reduction method. The mean of the results was computed to select the optimal concentration.

Ethical Consideration

The study received ethical approval from the Saint Mary's University Research Ethics Board (SMUREB), located on the 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Bayombong, Nueva Vizcaya, Philippines. Contact details are email: reb@smu.edu.ph and cellphone: 09177053041.

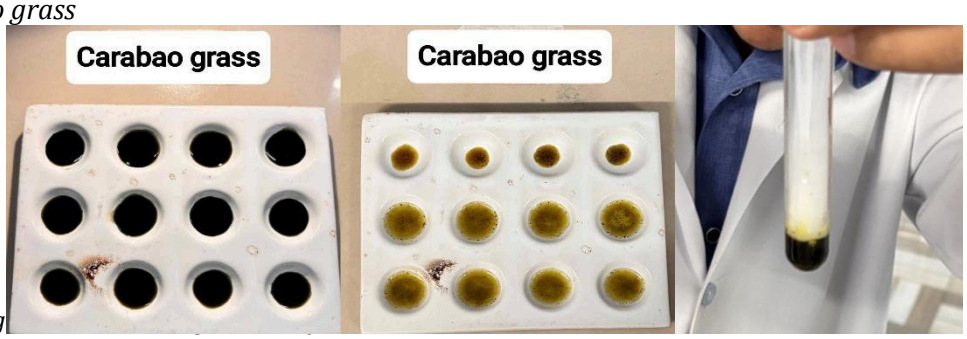
RESULTS AND DISCUSSIONS

Section 1: Phytochemical Screening of Carabao grass and Paragis grass

Figure 1

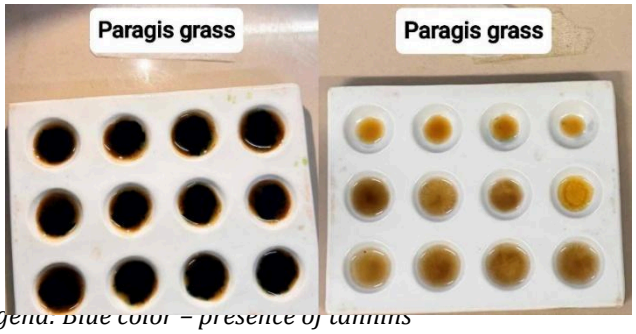
Presence of Tannins and Flavonoids in Carabao Grass

Carabao grass



Legend: Blue color – presence of tannins
Yellow to colorless – presence of flavonoids

Paragis grass



Legend: Blue color – presence of tannins
Yellow to colorless – presence of flavonoids

The figure above shows the presence of flavonoids and tannins in carabao grass and paragis using the tube method. Still, since the result in the test tube method was not that visible, the spot test was used as the confirmation. Point five (0.5) mL of the carabao grass extract and paragis grass extract is mixed with sodium hydroxide (NaOH), followed by the addition of hydrochloric acid (HCl), resulting in a yellow to colorless color that indicates the presence of flavonoids. The use of diluted ferric chloride, mixed with the carabao grass extract and paragis grass extract, resulted in a blue color, which indicates a positive for tannins.

According to Garduque et al. (2019), carabao grass phytochemical constituents are highly abundant in flavonoids, triterpenes, saponins, glycosides, tannins, and traces of alkaloids. Afrose et al. (2024) stated that the ethanolic extract of *Paspalum conjugatum* contains alkaloids, tannins, flavonoids, and glycosides. Adoho et al. (2021) examine the diverse secondary metabolite profile of *E. indica,* which includes alkaloids, terpenes, flavonoids, tannins, anthraquinones, saponins, cardiac glycosides, and anthracene glycosides.

Therefore, carabao grass and paragis grass show positive results for flavonoids, with a yellow to colorless color, and for tannins, with a blue color. Secondary metabolites are responsible for blood clot lysis.

Section 2. Thrombolytic Activity of *Paspalum conjugatum*

Table 1

Thrombolytic Activity of Ethanolic Extract of Paspalum conjugatum

Concentrations	Mean of Weight (g) Before	Mean of Weight (g) After	Mean of Percentage Clot Reduction (%)
20 mg/mL	.259	.089	65.60
10 mg/mL	.301	.152	49.39
5 mg/mL	.402	.291	27.59
2.5 mg/mL	.269	.220	18.09

The table above shows the thrombolytic effect of the ethanolic extract of carabao grass at various concentrations. The results indicate a dose-dependent effect: as the concentration increases, the percentage of clot lysis also rises. Specifically, at 2.5 mg/mL, clot lysis is 18.09%, increasing to around 27.59% at 5 mg/mL, 49.39% at 10 mg/mL, and reaching a steady increase that peaks at 65.60% at the highest concentration (20 mg/mL).

This finding aligns with the study by Culliao et al. (2020), which demonstrated that the highest concentration of the extract from *Termitomyces eurrhizus* achieved the highest clot reduction percentages, at 35.19 ± 0.13% and 32.41 ± 0.17%, respectively. Additionally, Akbor et al. (2023) reported a concentration-dependent effect of clot lysis with the methanolic leaf extract of Celtis tenuifolia, with maximum clot lysis observed at the highest concentration (198.41 ± 1.87 μg). In comparison, the positive control, streptokinase, used in the study by Afalla et al. (2024) showed a clot reduction of 89.70%, whereas the negative control showed a mere 0.32%.

Thus, the highest concentration (20 mg/mL) of carabao grass exhibits the greatest thrombolytic activity among all tested concentrations, establishing it as the optimal concentration. This suggests that the ethanolic extract effectively enhances clot lysis.

Section 3. Thrombolytic Activity of *Eleusine indica*

Table 2

Thrombolytic Activity of Ethanolic Extract of Eleusine indica

Concentrations	Mean of Weight (g) Before	Mean of Weight (g) After	Mean of Percentage Clot Reduction (%)
20 mg/mL	.394	.068	82.67
10 mg/mL	.355	.145	59.00
5 mg/mL	.370	.216	41.53
2.5 mg/mL	.373	.278	25.38

Based on the above table, the thrombolytic effect of *Eleusine indica* ethanolic extract increases in a dose-dependent manner. The extract shows a 25.38% reduction in clot formation at the lowest concentration (2.5 mg/mL). It rises steadily with increasing dose, reaching 82.67% at 20 mg/mL.

This aligns with the findings of Rahaman et al. (2020), who demonstrated that higher concentrations of *Rudbeckia hirta* leaf extract led to better clot lysis. The study by Culliao et al. (2020) also showed that the highest concentrations of chloroform and ethanol extracts of Termitomyces eurrhizus corresponded to the largest clot reduction percentages, at 35.19 ± 0.13% and 32.41 ± 0.17%, respectively. In comparison, the positive control, streptokinase, used in the study by Afalla et al. (2024) showed a clot reduction of 89.70%, while the negative control showed only 0.32%.

The 20 mg/mL concentration of *E. indica* exhibits the highest thrombolytic activity among all tested concentrations, making it the optimal concentration. Therefore, this suggests that the plant extract possessed significant thrombolytic activity.

The ethanolic extract of *Eleusine indica* demonstrates a clear dose-dependent thrombolytic effect. At the lowest concentration of 2.5 mg/mL, the extract achieves a 25.38% reduction in clot size, which steadily increases to 82.67% at the highest concentration of 20 mg/mL. This finding aligns with the research by Rahaman et al. (2020), which showed that higher concentrations of Rudbeckia hirta leaf extract led to improved clot lysis. Similarly, Culliao et al. (2020) reported significant reductions in clot formation with increasing concentrations of chloroform and ethanol extracts from *Termitomyces eurrhizus*.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study aimed to determine the in vitro thrombolytic activity of ethanolic extracts from carabao grass (*Paspalum conjugatum*) and paragis grass (*Eleusine indica).* Phytochemical spot tests revealed the presence of flavonoids and tannins in both plants, indicating that the plant extracts are good sources for clot lysis. This ethanolic extract of *Paspalum conjugatum* demonstrated the ability to lyse clots, with the highest thrombolytic activity observed at the highest concentration tested. Similarly, the ethanolic extract of *Eleusine indica* showed the highest percentage of thrombolytic activity at its highest concentration while still retaining clot lysis capability at lower concentrations. Thus, both plants could serve as potential sources of natural thrombolytic agents, as their activity demonstrates a significant ability to lyse blood clots. This study represents only a preliminary investigation of their thrombolytic activity; further research into their pharmacological and medicinal properties is essential to fully explore their potential as thrombolytic agents.

Recommendations

1. Utilizing their pharmaceutical development, given their demonstrated safety (non-toxic) and efficacy, these grass extracts could be further investigated for incorporation into pharmaceutical formulations. This could lead to the development of herbal medicines or supplements to improve cardiovascular health.
2. Move from in vitro to in vivo studies to evaluate the thrombolytic activity of these extracts in animal models. This is crucial for assessing their safety and efficacy.
3. If in vivo studies yield positive results, consider planning pre-clinical trials to evaluate the efficacy and safety of the extracts in humans.
4. Collaborate with pharmacologists and medicinal chemists to enhance understanding of the extracts' properties and to facilitate the development of new thrombolytic agents.

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