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Gliricidia Sepium (Jacq.) Kunth ex Walp. Trunk Extract's Hemostatic Property

Rexy Cordial Alvarez, RMT,MSMLS,Ph.D.
Medical Technologist IV
Department of Pathology and Laboratories
Bicol Medical Center - Naga City

Abstract:- Plants have been used in traditional medicine, which has developed into an intriguing but mostly untapped route for the introduction of novel medications. Although *Gliricidia sepium*, also known as Madre de cacao or kawakate, is used to treat coagulation problems in traditional medicine, there is no scientific evidence to support its hemostatic ability as of yet. The hemostatic potential of *Gliricidia sepium* trunk extract was investigated in this work. Utilizing citrated plasma and conventional techniques, extracts of the *Gliricidia sepium* trunk were extracted through squeezing and tested for their hemostatic effects using the prothrombin time (PT), activated partial thromboplastin time (APTT), and clotting time (CT) assays. In the phytochemical analysis of the trunk extract, sterols, alkaloids, saponins, and glycosides were found. Trunk extract markedly reduced PT and APTT (both $p<0.05$), while slightly increasing CT ($p< 0.05$). In comparison to the normal control, the results of the recently completed research demonstrated significant differences in all concentrations of treated samples in the PT and APTT. The same result was seen when treated in a dose-dependent sequence based on concentration. Trunk extract at 4% concentration exhibited the best reduction in PT ($p=0.0003$), whereas the trunk extract at 5.46% concentration showed the best reduction in APTT ($p=0.0007$). This denotes the plant extract from *Gliricidia sepium's* trunk's hemostatic potential as well as its potential application and suitability for future investigation of the plant extract's hemostatic properties.

Keywords:- *Gliricidia sepium, hemostasis, prothrombin time, activated partial thromboplastin time, clotting* time.

I. INTRODUCTION

Total blood volume of a healthy adult comprises about 8% of the body weight or about 5,600 ml in a 70-kg individual. This makes blood a major important fluid of the body (Godkar & Godkar, 2013). Blood is responsible for distributing nutrients and hormones to the body and delivers oxygen to the tissue and eliminates carbon dioxide and other waste products from the tissue. It regulates body temperature and counteracts excessive blood loss from the body through a process known as hemostasis. When deficiency in the blood occurs, weakness, lethargy, fatigue, nervousness, and depression can be manifested. It has three cell types known as eryhtrocyes, leukocytes, and thrombocytes, each with its own functions.

Keeping the blood within the blood vessels during the period of injury is a process called hemostasis. The creation of enzymes from their precursor zymogens, pro-coagulants, and proenzymes is thought to occur through a complicated sequence of cascading mechanisms known as coagulation (Dey & Bhakta, 2012). The hemostatic system is made up mostly of blood vessels, platelets, coagulation factors, coagulation inhibitors, and fibrinolysis (Guyton & Hall, 2000). When a blood vessel is damaged, the blood vessels constrict and thrombin is activated along with platelet adhesion and activation. Primary hemostasis mechanisms are activated by small injuries to the vascular system which seals the affected areas and platelets fill the open space to finally create a platelet plug. Secondary hemostasis is induced directly or by primary hemostasis and is essential to control bleeding from large wounds resulting from trauma, surgical, or dental procedures.

Thrombosis and hemorrhage occur once hemostasis systems are out of balance (Riddel, Aouizerat, Miaskowski & Lillicrap, 2007). Hemostasis can save lives, thus finding alternative treatments that make the process easier is a medical priority. (Weremfo, Adinortey & Pappoe, 2011).

A. The Plant

Fig. 1: The *Gliricidia sepium* plant

Even today, about 40% of medicines that are given in the west are made either directly or indirectly from plants. In developing nations, the use of medicinal plants and traditional medicines is now a recognized and established profession, especially in rural areas where access to highly advanced medical facilities may be limited and in some situations where traditional remedies appear to be favored. (Ike, Nubila, Ukaejiofo, Shu, & Okpalaji, 2010). Historically, both developing and developed nations used medicinal herbs extensively. According to data from the World Health Organization, 80 percent of the world's population relied mostly on conventional interventions that used plant extracts or their active ingredients. (Reddy and Jose, 2010).

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Many researches have investigated the anticoagulant activity of some plants and found out that some of these plant extracts have anticoagulant activity for treating problems in hemostasis (Al-Saadi, 2013). Hence, it is a need and demand of time to look into alternative anticoagulants (Mahajan & More, 2012). One such herbal plant is *Gliricidia sepium.*

Figure 1 shows *Gliricidia sepium* plant commonly known as Gliricidia. Its common name varies in different countries. In Tamil, they are Matza Raton; Cacao de nance, Cachanance, Vivasayathagarai; Madreado in Honduras, both in the Philippines and Guatemala, they are called Kakawate or Madre de Cacao; and in Nicaragua, it is called Madero negro. It is a herbaceous plant in the Fabaceae subfamily Papilionoideae. Second, only to Leucaena leucocephala in importance, this invasive species is a native of South America and is regarded as the most useful legume tree. The tree has a height of 10 to 12 meters and is unaffected by diseases or pests. It is used as a green manure for paddies in Tamil Nadu. It is called "Madrecacao" (mother of cacao) because cocoa and coffee plantations in Mexico use the tree's shade (Sukumar & Aparna, 2014). According to Nazli, Sohail, Nawab, and Yaqeen (2011), the plant is employed as support plant, green manure, animal feed, fuel wood, shade, and living fences. Trees have spreading crowns. Odd-pinnate leaves typically alternate, subopposite, or opposite, and measure up to 30 cm. length; 2-7 cm long, 5-20 oval or elliptic leaves. 1-3 cm long. wide. Occasionally, the rachis and midrib of leaves have crimson stripes. The mature plant has a bunched raceme that is 5 to 15 cm long, around 15 to 40 blooms per raceme, and is a light pink to violet color with white flecks and a spread pale yellowish spot at the base of the petals. The calyx is glabrous, green, and frequently tinged red. Standing, rounded petals are around 20 mm long; keel petals are 1520 mm long and 4–7 mm broad. The fruit is green in color, occasionally with a reddish-purple tint when unripe, and is slender, 10-18 cm long, 2 cm wide, with twisting valves during dehiscence. The bean-like seed is elliptical, lustrous, pigmented, brown, and 10 mm in length (Parrota, 1992).

B. Therapeutic and Important Uses of the Plant

Gliricidia sepium has multimedicinal uses. It is used against diarrhea and hemorroid and used as hemostatic. It has anti-inflammatory effects that aid control all signs of inflammation of the stomach, esophagus, small intestines, and irritating bowel discomfort. Tannins not only treat burns and halt bleeding, but they help manage infection as the site heals. Tannins have the capacity to create a barrier to prevent tissue from coming into contact with a more complicated infection. (Ashok & Upadhyaya, 2012).

The lardicival activity of *Gliricidia sepium* against *Culex quinquefasciatus* has already been studied and provides new tools for effective control approaches against *Culex quinquefasciatus* larva (Thomas & Shonima, 2012).

Gliricidia sepium was generally effective for the treatment of animal diseases such as in dogs. Perhaps because of its primary constituents, which include tannin, sulfate, glycosides, and fats (Viste, Fontanilla, Agpasa, Tabije & Camalig, 2013).

A methanolic extract of *Gliricidia sepium* leaves inhibits the growth of lettuce seedlings, because of the coumarin that played an important function in the inhibitory activity of *Gliricidia sepium.* This coumarin activity was regarded as one of the key characteristics of an allelopathic plant (Takemura, Kamo, Sakuno, Hiradate & Fujii, 2013).

Flavanoid, glycoside, and isoquecitrin are present in the light pink flowers of *Gliricidia sepium.* The isolated yellow pigment were observed to be antibacterial (Sukumar & Aparna, 2014). The presence of phenolic compounds and flavanoids was attributed to the antimicrobial ability of the *Gliricidia sepium* flower methanol extract. *Gliricidia sepium's* crude extract demonstrated exceptional effectiveness against several harmful microorganisms (Jose, Thomas & Reddy, 2013). It also has the most effective component against bacteria and fungal infection and can be an important source of chemical compounds (Nazli et al., 2011). An in vitro study corroborated the microbial activity of the said plant used in folkloric medicine and was reported effective against three or more pathogenic microorganisms (Rojas, Ochoa, Ocampo & Munoz, 2006).

Ethanolic leaf extract of *Gliricidia sepium* was also used to examine its Nematicidal and antimicrobial properties. Nematicidal effectiveness has shown sixty percent mortality against *Meloidogyne incognita* nematodes; a maximum of seventy-eight percent repellency against mosquito *Aedes aegypti;* and activity against bacteria such as *E. coli, S. aureus, Pseudomonas spp., S. typhi, Klebsillia spp.,* and *V. cholerae* (Nazli, Akhter, Ambreen, Solangi & Sultana, 2008).

Gliricidia sepium also showed potential activities against cancer cell lines and microbes together with inhibitory properties against one or more cancer cell lines (Cates, Prestwich, Innes, Rowe, Stanley et al., 2013). The antioxidant potential of aqueous extracts of *Gliricidia sepium* was also investigated by DPPH assay. Results showed that the water extract of this plant had potent superoxide radical scavenging effect (Akharaiyi, Boboye & Adetuyi, 2012)

In vitro HRBC membrane stabilization assay and an in vivo arrangement-induced paw edema model in albino Wistar rats were used to assess the anti-inflammatory properties of aqueous extract of *Gliricidia sepium.* This confirms the scientific basis of using flowers of *Gliricidia sepium* in controlling inflammation (Kumar, Naik, Chandra, Lavanya, Kumar et al., 2014).

A study by Abdulrahaman, Bamidele & Oladele (2013) revealed that the blood of experimental animals were not affected in taking the fiber of *Gliricidia sepium.* Furthermore, the mixing of wood fibers and wheat flour produces no adverse affect on the physical and baking properties of bread. Hence, it is recommended that *Gliricidia sepium* wood is a potential source of dietary fiber.

C. Phytochemical Properties

Phytochemical analyses of *Gliricidia sepium* sap disclosed the availability of various secondary metabolites like anthraquinones, alkaloids, phenols, saponins, tannins, polyoses, polyuronoids and flavanols in moderate concentrations in the stem, leaf and roots of the plants.

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Flavones and flavanols are present in the root and stems but absent in leaves. Saponin shows very good result in all parts like root, stem, and leaves. The compounds like iridoids, juglon, emodin, anthrocine are totally absent. Cardiac glycosides are likewise absent.

Through GC-MS analysis, the bark oil of Gliricidia sepium was examined. Nineteen compounds were identified and quantitatively analyzed, with the following being the major components: methyl-3 (E) pentenyl ether (11.55%), 3-methyl-2 butanol (10.65%), 3-methoxy-hexane (10.14%), 1-(1-ethoxyethoxy)-2 hexene (9.72%), 2-decanol (8.97%), (Reddy & Jose, 2010).

Based on the study, *Gliricidia sepium* was identified to contain tannins. Tannin cause an astringent dry mouth taste once this substance binds to proteins. The amount of this substance depends on the location of the tree. Afrormosin, medicarpin, and certain isoflavins are three more medicinally active substances that are present in addition to tannins. The majority of Gliricidia sepium research has been on the plant's nutritional value. However, other studies have focused on its effectiveness on soil nematode populations and its capacity to act as an insecticide or fungicide when applied to plants or roots. (Ashok & Upadhyaya, 2012).

Saponin is the most abundant phytochemical present in *Gliricidia sepium*. Saponins are glycosides that are abundantly found in many foods edible for animals and man. There are two Saponin groups: steroidal saponins and triterpenoid saponins. Steroidal occur as glycosides in certain pasture plants while triterpenoid saponins which occurs in soybeans and alfalfa. A lot of pharmacological effects has been reported about saponins which includes its effect on bacteria, fungi, viruses, hepatoprotective, anti-inflammatory, and anti-ulcer (Soetan, Oyenkunle, Aiyelaagbe & Fafunso, 2006).

Gliricidia sepium is used folklorically to stop bleeding due to an exterval cuts in some areas in Caramoan, Camarines Sur. The hemostatic and wound-healing properties of the fresh extract made from the trunk of *Gliricidia sepium* are also used as an emergency intervention for the management of external wounds (Duke & Wain, 1981). Although it is frequently used as an anti-hemorrhagic, no actual research on its hemostatic effects has been done to date. In order to establish a scientific empirical foundation for the hemostatic potential of the trunk extract of *Gliricidia sepium*, the primary objective of this study is to evaluate the hemostatic effect of *Gliricidia sepium* using hemostatic parameters such as clotting time, prothrombin time, and activated partial thromboplastin time.

II. MATERIALS AND METHODS

Fig. 2: The trunk of *Gliricidia sepium* plant

A. Plant Materials

The *Gliricidia sepium* plant was collected from San Felipe, Naga City, province of Camaraines Sur. A voucher specimen of the plant was submitted to the Central Bicol State University of Agriculture, College of Agriculture and Natural Resources in Pili, Camarines Sur for taxonomic identification by agriculturists.

B. Sample preparation and extraction

The whole plant was washed thoroughly with tap water to eliminate particulates and ultimately with sterilized water. The extract was taken from the trunk of a fresh plant (seen in Figure 2) by squeezing with cotton wool. It was strained through Whatman No.1 filter paper and placed in sterile bottles (Darvin, 2013).). For every 100 g. of trunk of the plant yielded 21 ml. of trunk extract. Plant extracts were processed through centrifugation for 5 minutes at 5000 rpm minutes and the supernatant was kept at 4 degrees Celsius in sterile tubes (Kothale, Rothe & Pethe, 2012).

Fig. 3: The bark of *Gliricidia sepium* plant and the collected trunk extract

C. Phytochemical Analysis

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Phytochemical analyses of the trunk extract of *Gliricidia sepium* as seen in Figure 3 was conducted qualitatively by the Industrial Technology Development Institute of the Department of Science in Technology, Bicutan, Taguig, Metro Manila using the method described by Trease and Evans (2002). Responses to various tests were denoted by + and – signs indicating the presence or absence of phytochemical constituents respectively.

D. Participants

The participants in this study were normal and healthy male adult volunteers who were initially screened and found to have a normal results of prothrombin time and activated partial thromboplastin time. Approximately ten milliliters of blood was extracted from each participant. All patients with abnormal PT and APTT results were excluded.

E. Declaration of Conflict of Interest

The researcher declares that they have no known financial conflicts of interest or close personal ties that would have appeared to affect the research findings disclosed in this study.

F. Blood Samples

Using a disposable syringe, blood samples were extracted from healthy male volunteers then placed in an evacuated tube with anticoagulant using 3.2% sodium citrate (9 parts of blood to 1 part of tri-sodium citrate solution for PT and APTT determination (Mahajan & More, 2012) and coagulated blood using red top/plain tube for the determination of clotting time. Ethical approval for the use of human participants was sought.

G. Measurement of Activated Partial Thromboplastin Time (APTT)

Citrated plasma was used for this test. Five tubes were used and labeled as SC, S0, S1, S2, S3, and S4. SC is the control tube, S0 blood sample only, and S1, S2, S3, and S4 contained 25, 50, 75, and 100 ul of trunk extract of *Gliricidia sepium* respectively (Klotoe, Dougnon, Sacramento, Dandjesso, & Edorph, 2012). After that, the blood was centrifuged at 1000 rpm for fifteen minutes to obtain platelet-poor plasma, and a fully automated coagulometer was used to assess the results (Sysmex CA-500, Japan).

H. Measurement of Prothrombin Time (PT)

Citrated plasma was used for this test. Five tubes were used and labeled as SC, S0, S1, S2, S3, and S4. SC is the control tube, S0 blood sample only, and S1, S2, S3, and S4 contained 25, 50, 75, and 100 ul of trunk extract of *Gliricidia sepium* respectively (Klotoe et al., 2012). The blood was then processed through centrifugation at 1000 rpm for 15 minutes to obtain platelet poor plasma and analyzed using a fully automated coagulometer (Sysmex CA-500, Japan).

I. Measurement of Clotting Time (CT)

Five tubes were used and labeled as S0, S1, S2, S3, and S4. S0 is the sample tube and S1, S2, S3, and S4 contain 25, 50, 75, and 100 ul of the trunk extract of *Gliricidia sepium*, respectively. 500 ul of recently collected blood were added to each tube after each tube spent 60 seconds in a water bath at 37C. The timer was instantly set off, and each tube's CT was recorded. (Klotoe et al., 2012).

J. Statistical Analysis

Results were stated as ± standard error of the mean (S.E.M) to show the variation in groups. to demonstrate group variation. In order to show differences between the control and treatment groups, analysis of variance (ANOVA) was used in the data analysis. Multiple comparisons were tested using Dunett and Tukey method. The data were computed using PASW version and GraphPad Prism 6.

III. RESULTS

Constituents	Result
Sterols	(+)
Triterpenes	(-)
Flavonoids	(-)
Alkaloids	(+)
Saponins	(+)
Glycosides	(+)
Tannins	(-)

Table 1: Qualitative phytochemical analyses of the trunk extract of *Gliricidia sepium*

Note: (+) Presence of constituents
(-) Absence of constituents

Table 1 displays the results of the phytochemical tests. Four phytochemical components, including sterols, alkaloids, saponins, and glycosides, were found in the trunk extract of Gliricidia sepium, but triterpenes, flavanoids, and tannins were not present. The presence of the said phytochemicals correlates with reports of previous studies (Reddy & Jose, 2010; Soetan et.al. 2006). Noticeably, tannin previously reported as present in the root, stem, and leaves of *Gliricidia sepium* (Ashok & Upadhyaya, 2012; Kothale et al., 2012) were absent in the plant extract used in this study. This is may be because of the different methods employed in the process of collecting the extract and use of other parts of the plant.

The effects of the extract on coagulation time were evaluated by looking at clotting time, PT, and APTT in order to explore the hemostatic properties of the trunk extract of this plant.

Group	Result (in seconds)
Normal Control	11.63±0.088
1800ul of blood	9.57±0.033

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25 ul extract + 1800 ul of blood (1.377 %)	9.13±0.033
50 ul extract + 1800 ul of blood (2.7 %)	8.57±0.033
75 ul extract + 1800 ul of blood (4 %)	8.40±0.058
100 ul extract + 1800 ul of blood (5.26 %)	8.17±0.088

Table 2: Effect of the trunk extract of *Gliricidia sepium* on Prothrombin Time

Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group.

The impact of the Gliricidia sepium extract on the PT is shown in Table 2. The PT value in the adverse control group was 11.63 0.088 sec. For the 1800 µl of blood sample, the patient control's PT were 9.57 0.033 sec (p=0.0026). At concentrations of 1.377% (p=0.0013), 2.7% (p=0.0012), 4% (p=0.0003), and 5.26% (p=0.0016), there was a noticeable drop in PT, with the concentration of the extract demonstrating the most significant decrease in PT, as shown in Figure 4. The result reflected that the concentration of 4% revealed the optimum concentration with the highly significant decreased (p=0.0003) on the effect of trunk extract on PT. Noticeably, the decrease in PT was dose-dependent with shorter PT with increasing concentration of the extract (inverse relationship).

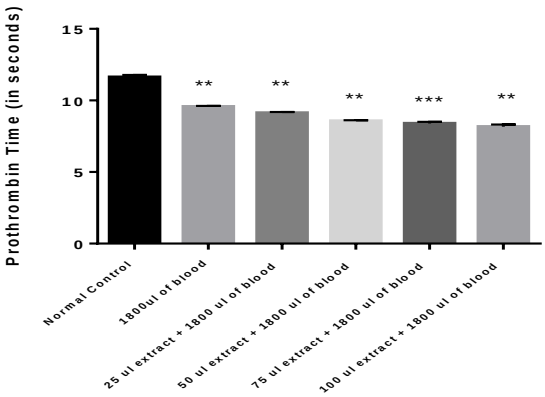

Fig. 4: Effect of treatment with trunk extract on Glirici dia sepium at varying concentrations (25, 50, 75, and 100 µl) on prothrombin time.

Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group.

Table 3: Effect of the trunk extract of *Gliricidia sepium* on Activated Partial Thromboplastin Time

Table 3 presents the effect of the *Gliricidia sepium* extract on the APPT. In the negative control group, APTT was 37.03 ± 0.088 sec. The APTT of the patient control was 36.17 ± 0.088 sec (p=0.0936) for the blood sample of 1800µl. According to Figure 5, there was a noticeable decrease in the APTT at concentrations of 1.377% (p=0.0133), 2.7% (p=0.0015), 4% (p=0.0033), and 5.26% (p=0.0007), with the biggest decrease in the APTT being observed at the extract's highest concentration. Result reflected that the concentration of 5.26% revealed an optimum concentration with the highly significant decreased (p=0.0007) on the effect of trunk extract on APTT. Similar to the effect of the PT, the effect of the extract on the APTT was likewise dose-dependent in an inverse relationship with shorter APTT with increasing concentrations of the extract. As can be observed from the results, each concentration showed a substantial difference from the negative control. This was also noted when treated per group and mixture using Tukey method.

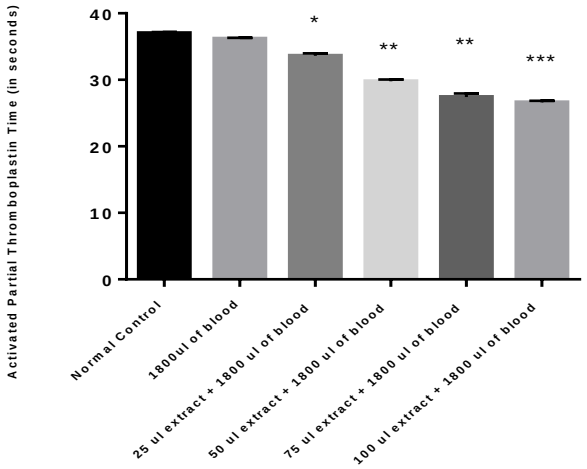

Fig. 5: Effect of treatment with trunk extract on *Gliricidia sepium* at varying concentrations (25, 50, 75, and 100 µl) on activated partial thromboplastin time.

Group	Result (in seconds)
500 ul blood	248.3±10.14
25 ul extract + 500 ul of blood (4.7%)	192.7.3±3.71
50 ul extract + 500 ul of blood (9.0%)	210±5.77
75 ul extract + 500 ul of blood (13.0%)	228±6.01
100 ul extract + 500 ul of blood (16.6%)	241.7±10.14

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Groups	Result (in seconds)
Normal Control	37.03±0.088
1800ul of blood	36.17±0.088
25 ul extract + 1800 ul of blood (1.37%)	33.63±0.203
50 ul extract + 1800 ul of blood (2.7%)	29.80±0.153
75 ul extract + 1800 ul of blood (4%)	27.43±0.296
100 ul extract + 1800 ul of blood (5.26%)	26.60±0.153

Table 4: Effect of the trunk extract of *Gliricidia sepium* on Clotting Time

Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group.

Table 4 shows the effect of the trunk extract on the clotting time. In the patient control group, the clotting time (CT) was 248 ± 10.14 sec. Administration of the extract resulted in a decrease in CT to 192.70 ± 3.71 sec. at a concentration of 4.7% (p=0.0666). It was also insignificantly increased for the concentration of 9.0% (p=0.2257), 13.0% (p=0.3734) and 16.6% (p=0.8978), as shown in Figure 6.

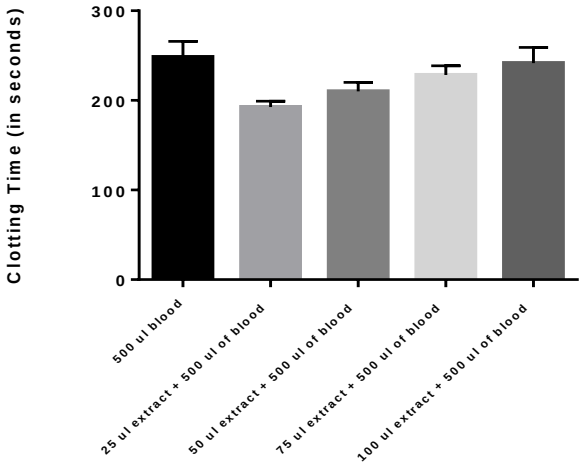

Fig. 6: Effect of treatment with trunk extract on *Gliricidia sepium* at varying concentrations (25, 50, 75, and 100 µl) on clotting time.

IV. DISCUSSION

Hemostasis plays a vital role in the prevention of uncontrolled loss of blood in the system. The hemostatic system signifies a fragile balance between pro-coagulant, anti-coagulant mechanisms allied to a process of fibrinolysis (Lewis & Decie, 2002). Hemostasis is a series of shifting activities whereby blood coagulation is initiated and terminated quickly and precisely organized (Nathan et al., as cited by Riddel et al., 2007). Hemostatic mechanisms guard against the risk of fatal bleeding. A protective hemostatic occlusion is produced by platelet and clotting factors, and its purpose is to maintain blood flow at the wounded location. The fibrinolytic process has evolved to recanalize blocked vessels as healing progresses to restore introduction through a damaged channel in which the protective clot has formed in order to maintain equilibrium. Despite the existence of numerous fail-safe mechanisms used to restore hemostasis, many illnesses that are linked to either bleeding tendencies or prothrombotic diseases still exist. (Riddel et al., 2007). Utilising hemostatic variables like clotting time, prothrombin time, and activated partial thromboplastin time, this study analyzed the hemostatic effect of the trunk extract of *Gliricidia sepium* and identified the mechanism underlying the hemostatic properties of the trunk extract of *Gliricidia sepium.* Additionally, the phytochemical components of the *Gliricidia sepium* trunk extract were identified. Based on the outcomes and additional statistical analysis of the *Gliricidia sepium* trunk extract.Its procoagulant effects on blood coagulation were discovered to have significant hemostatic potential through a significant reduction in prothrombin time (PT) and activated partial thromboplastin time (APTT).

The results of this study indicate that the PT and PTT of both hematologic parameters were changed by the trunk extract of *Gliricidia sepium*. Therefore, by encouraging coagulation factors (II, V, VII, VIII, IX, X, XI, and XII), it impacts both the extrinsic and intrinsic routes (Klotoe et al., 2012). The trunk extract of *Gliricidia sepium* appears to have little effect on fibrin synthesis but instead primarily affects the coagulation components of both the extrinsic and intrinsic routes, leading to a considerable reduction in both prothrombin time (PT) and activated partial thromboplastin time (APTT). Additionally, the effect of the trunk extract on both PT and APTT is dose-dependent, with larger doses significantly reducing PT and APTT. Thus, it suggests that the procoagulant effect of the extract yields more significant effects in higher concentrations of the trunk extract of *Gliricidia sepium.* Results of this study correlate with the findings on the activities of bitterleaf *(Vernonia amygdalina)* extract on induced male diabetic albino rats where the extract of the plant demonstrated hemostatic activity by shortening the PT. It was discovered that applying V. amygdalina squeezed leaves directly to an injury would stop the bleeding from the afflicted arteries. (Oguntola, 2013 as cited by Oguwike et al., 2013). This finding is similar to the effect of *Gliricidia sepium* where squeezed trunk extract likewise decreased the PT. In a similar manner, the results of this study also correlated with the finding of Bamilede et al. (2010) on the assessment of the hemostatic effects of the methanolic leaf extract of *Ageratum conyzoides* in albino rats which showed a significant decrease in PT .

The phytochemical testing of the trunk extract of *Gliricidia sepium* revealed various phytochemicals namely: sterols, alkaloids, saponins, and glycosides. However, of these detected phytochemicals, saponins are the most abundant phytochemicals present in *Gliricidia sepium* (Soetan et al, 2006). The observed effect on PT and APTT can be putatively attributed to saponins. This is due to the fact that several studies have already reported the hemostatic potential of saponins from other plants (Gao et al., 2014; White, Fan & Chow, 2000). However, because this study used crude extract only, this purported activity can only be established through further studies using purified saponins from *Gliricidia sepium*.

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Majority of previous coagulation studies conducted on other plants showed anticoagulant properties of the extracts used while this study indicated procoagulant properties of the trunk extract of *Gliricidia sepium.* In contrast to this study, Ike et al. (2010)'s investigation on crude neem leaf acetone water led to a statistically significant rise in PT and APTT. This may be attributed to the absence of anticoagulant components of the crude neem leaf acetone water extract. Another reason may lie in the difference in the methods used in the collection of the extract and the phytochemical constituents present in the trunk extract. In addition, unlike our work, which was conducted in vitro, the study by Ike et al. (2010) used an in vivo analysis in which Wistar rats were forced fed for four weeks with graded dosages of acetone water-fractionated neem leaf extract. Similar to this, Mahajan et al. (2012) found no correlation between the anticoagulant activity of aqueous and ethanolic extracts and their separated phytochemicals of various medicinal plants, specifically Enicostemma littorale, Acheranthgus aspera, Abutilon indicum, and Tridax procumbens.The aqueous leaf extract of Abutilon indicum showed better efficacy in the aforementioned investigation, extending the CT and PT. In vitro research on the anticoagulant activity of several plants employing garlic oil extract contradicted this study in a similar manner (Al-Saadi, 2013). Garlic's chemical components can actually slow down the production of new fibrin and aid in removing any fibrin that may already be present in the blood. Of note is the presence of tannins in the extracts used in the aforementioned studies (Ike et al., 2010; Mahajan et al., 2012), which is absent in the *Gliricidia sepium* trunk extract used in this study. However, the addition of sap to the plasma had no impact on PT or PTT in the study by Klotoe et al. (2012) on the hemostatic potential of Musa sapientum sap. Therefore, sap has no effect on the intrinsic or extrinsic pathway clotting factors. (Klotoe et al., 2012). The differences in the results on the coagulative properties of aforecited plants as opposed to the hemostatic effect of *Gliricidia sepium* as revealed in this study may be due to the differences of the methods of extraction and the variation in the phytochemical contents of the plants.

V. CONCLUSION

The present study demonstrated that the trunk extract of *Gliricidia sepium* has significant procoagulant properties that can significantly decrease the PT and APTT. Study reflected that the result from (4%) concentration of trunk extract revealed the optimum concentration with significant decreased (p=0.0003) on PT while the result from 5.46% concentration of trunk extract revealed the optimum concentration with significant decreased (p=0.0007) on APTT. The effects of the trunk extract on the said parameters are dose-dependent with increasing reduction in both PT and APTT in higher concentrations.

VI. RECOMMENDATIONS

Further in-depth studies on the trunk extract of *Gliricidia sepium* as an alternative procoagulant are recommended. Additional studies and researches must be further conducted using different parts of the plants as well as fractionation of the plant constituents to determine the exact effect of each phytochemical content.

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DATA AVAILABILITY

Visit https://orcid.org/0000-0001-6996-4498 for the data that support this study. In order to protect data privacy, access to the data is restricted and requires authorization and a data sharing agreement.

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