IN VITRO EFFECT OF MALAYSIAN *Mikania cordata* LEAVES EXTRACT ON BLOOD COAGULATION FACTORS LEVEL IN PLASMA

By

NUR YUZAIZA BINTI YUSOFF

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DECLARATION

I hereby declare that I am the sole author of this dissertation entitled "*In Vitro* Effect of Malaysian *Mikania Cordata* Leaves Extract on Blood Coagulation Factors Level in Plasma". I declare that this dissertation is submitted to Universiti Sains Malaysia (USM) for the purpose of the award of Master of Science in Transfusion Science. This dissertation is the result of my own research under the supervision of Dr Hafizuddin Mohamed Fauzi except as cited in references. The dissertation has been accepted for the study performed and is not concurrently submitted in candidature of any other degree.

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LIST OF SYMBOLS & ABBREVIATIONS

***et al.,* :**	and others
***etc* :**	*Et cetera*
% :	percentage
°C :	Degree celcius
SD	Standard deviation
g :	gram
ml:	millilitre
mm	millimetre
mg:	milligram
µg :	microgram
µl :	microlitre
µm :	micrometer
min :	minute
h :	hour
nm :	nanometer
w/v :	Weight over volume
sp :	species
dh_2O	Distilled water
NaCl	Sodium chloride
rpm	Rotation per minute
kHz	kilohertz
FGN	Fibrinogen

PPP	Platelet poor plasma
NIST	National Institute of Standard and Technology
PT	prothrombin time
APTT	activated partial thromboplastin time
TT	thrombin time
GCMS	Gas Chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
NRCS	Natural Resources Conservation Services
MSD	Mass Selective Detector
BSTFA + TMCS	Bistrimethylsilyltrifluoroacetamide + Trimethylchlorosilane
TIC	total ion chromatogram

ABSTRACT

Current anticoagulant agent such as warfarin and heparin have demonstrated life-threatening side effect and its efficacy also been doubted. So, exploration of herbal plant been done and one of them is *Mikania cordata. Mikania cordata* (Bum. F.) B.L Robinson or commonly called selaput tunggul, in Malaysia, belongs to family of Asteraceae and it was claimed to prolonged PT (prothrombin time), APTT (activated partial thromboplastin time) and TT (thrombin time) in early study. Thus, aim in this study were to identify the active compound through GCMS analysis and to determine level of factors VII, IX, X, II and FGN in plasma treated *in vitro* with different concentrations of *M.cordata* aqueous leaves extract. As a result, in 25.0 mg/ml of *M. cordata* extract, significant percentage of factor reduced can be observed which were in FII with 90.17%, followed by FGN (89.80%), FX (80.91%), FIX (69.15%) and finally FVII (62.67%) compared to control with $p < 0.001$.This finding was supported by the identification of malic acid which was believed to possess anticoagulant activity. Malic acid also reacts in concentration- dependent manner when 25.0 mg/ml of *M. cordata* extract treated on plasma showed high reduction of factors compared to 12. 5 mg/ml. In conclusion, it was confirmed that malic acid possess anticoagulant activity in concentration dependent manner, by reducing level of factors VII, IX, X, FII and FGN in plasma.

ABSTRAK

Egen anti pembekuan darah yang sedia ada seperti warfarin dan heparin dilaporkan mempunyai kesan sampingan yang membahayakan, nyawa, malah, keberkesanannya juga turut diragukan. Oleh itu, kajian mula dilakukan terhadap tumbuhan herba dan antaranya ialah *Mikania cordata*. *Mikania cordata* (Bum. F.) B.L Robinson atau dikenali sebagai selaput tunggul di Malaysia, tergolong dalam keluarga Asteraceae dan dipercayai mampu melanjutkan masa pembekuan darah dalam ujian PT (masa prothrombin), APTT (pengaktifan separa masa tromboplastin) dan TT (masa thrombin) dalam kajian terdahulu. Oleh itu, kajian ini dijalankan untuk mengenalpasti komponen aktif menggunakan analisis GCMS dan untuk mengkaji tahap faktor VII, IX, X, II dan FGN dalam plasma yang dicampur ekstrak daun *M.cordata* dengan kepekatan yang berbeza. Hasil kajian menunjukkan, dalam 25.0 mg/ml ekstrak, peratusan faktor berkurang sangat ketara dengan $p < 0.001$ iaitu dalam FII sebanyak 90.17%, diikuti FGN (89.80%), FX (80.91%), FIX (69.15%) dan FVII (62.67%) setelah dibandingkan dengan kontrol. Hasil kajian ini diperkukuhkan lagi dengan penemuan asid malik yang dipercayai memiliki aktiviti anti pembekuan darah. Tindakan asid malik sebagai anti pembekuan darah bergantung kepada kepekatan ekstrak yang digunakan dan dibuktikan, dalam kepekatan 25.0 mg/ml, peratus penurunan faktor adalah tertinggi berbanding 12.5 mg/ml. Kesimpulannya, asid malik memiliki sifat anti pembekuan darah dengan menyakibatkan penurunkan tahap faktor VII, IX, X, FII dan FGN dalam plasma.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Blood coagulation cascade involved a series of sequential steps which include intrinsic, extrinsic and common pathways. They are propagated by the formation of complex enzymes which is serine protease (factor II,VII, IX, X, XI and XII) and non-enzymatic cofactor protein which assembled on membrane surface in calcium-dependent manner (Butenas & Mann, 2001). Hence, maintaining of component level in vascular system is essential because this sequential cascade may decide the formation of thrombin thus directs the aggregation of blood via polymerization of fibrin (Hoffbrand *et al*., 2011).

Overproduction of clot then leads to the formation of thrombus. There are two types of thrombosis which are venous thrombosis and arterial thrombosis. Venous thrombosis encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE). Meanwhile major manifestations of arterial thrombosis are cardiac ischaemic, stroke and possibly peripheral vascular disease (thrombosis in leg arteries) (Previtali *et al*., 2011). In order to treat thrombosis diseases, antiplatelet and anticoagulant have been introduced. Antiplatelet can inhibit the production of thromboxane, thus avoid

formation of clot and anticoagulant act by targeting clotting factors of blood coagulation cascade (Büller *et al*., 2004).

Currently, synthetic drugs such as warfarin and heparin are widely been used as anticoagulant agent. However, there are several disadvantages such as the life-threatening side effect and undoubted efficacy of the drug (Jain *et al*., 2014; Manicam *et al*., 2010). Specifically, for vitamin K antagonist (warfarin), the well- known side effect, narrow therapeutic index, unevenness of dose response and the dietary interaction still remains as the limitation (Garcia *et al*., 2009). Therefore, development of novel anticoagulant agent been progressed to overcome the limitation, nevertheless the cost is too expensive and somewhat affect the productivity of study.

As a result, exploration of herbal plant been done. In fact, according to Hammer *et al*. (1999), plant contains abundant resources of chemical compound which can be used for treatment in modern medicines, food supplement and nutraceuticals. To date, billions of dollars have been earned through market trafficking due to augment of herbal medicine every year. However, the true machinery of action of herbal plants on diseases is not truly known. In a period of hundred years, numerous medicinal plants have been utilized by human being across the world for the management of diverse sickness and diseases without knowing the true mechanism (Marcus & Grollman, 2002).

Therefore, the discovery of blood coagulation factor level after treated with *Mikania cordata* is warranted since it have been claimed to prolonged PT (prothrombin time), APTT (activated partial thromboplastin time) and TT (thrombin time) in human plasma. This preliminary result indicated that *M.cordata* would act as anticoagulant

agent such as heparin and warfarin (Shaffee *et al*., 2015). Hence, *in vitro* factor assay was adopted to elucidate the specific mechanism of action of *M.cordata* aqueous extract on blood coagulation system.

1.2 Rationale of Study

M.cordata aqueous extract was found to prolonged PT, APTT and TT in clot-based assays treated to human blood (Shaffee *et al*., 2015). This preliminary finding indicated that, *M.cordata* aqueous extract has a big potential to act as anticoagulant agent which might treat thrombosis diseases. As a consequence, study was conducted to elucidate the specific mechanism of blood coagulation system after treated with *M.cordata* aqueous extract which focus on the factor level that are important as a key player in blood coagulation system.

1.3 Objectives

1.3.1 General Objective

To study blood coagulation factors level in plasma treated *in vitro* with *M.cordata* aqueous leaves extract.

1.3.2 Specific objective

The specific objectives in this study are as follows:

a) To identify active compound in *M.cordata* using GCMS analysis.

b) To determine the level of factors VII, IX, X, II, fibrinogen in plasma treated *in vitro* with different concentration of *M.cordata* aqueous leaves extract.

1.4 Hypothesis

H_O: There are no changes of coagulation factors level in plasma treated *in vitro* with *M.cordata* aqueous leaves extract.

H_A: There are changes of coagulation factors level in plasma treated *in vitro* with *M.cordata* aqueous leaves extract.

1.5 Significance of Study

Result of this study may contribute to the body of knowledge and hence improve understanding about the mechanism of action of *M.cordata* as anticoagulant agent. Further study need to be done completely to elucidate the capability of *M.cordata* before it can be declares as novel anticoagulant agent. Therefore, by understanding the mechanisms of *M.cordata* action on diseases, specific indication of the usage can be made.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of haemostasis

Haemostasis (Greek word; haem refer to blood and stasis is halt) is defined as interruption of blood flow or blood restrain either by natural means (clot formation or vessel spasm) or artificial means (compression or ligation) (Miller-Keane *et al*., 2005). Haemostatic system is a possessive process which represents a mild equity between procoagulant and anticoagulant which stop the bleeding from spots of blood vessel destruction and thus disintegrate the clot formation (Hoffbrand *et al*., 2011).

Following vascular destruction, haemostasis response started. In haemostasis, there are three main mechanisms involved, which are vasoconstriction, platelet aggregation and blood coagulation cascade (Figure 2.1). Synergy between all mechanism lead to the formation of secure haemostasis plug. Nevertheless, development of extensive clot is very dangerous. Therefore, fibrinolysis mechanism is very important in vascular system.

Blood coagulation cascade is part of the vital mechanism which involved extrinsic, intrinsic and common pathway which connects to form fibrin polymer. Plasma consists of several factors which work together with the aid of many other cofactor,

inhibitor and receptor to produce fibrin polymer. Table 2.1 demonstrates the key protein involved in haemostasis network.

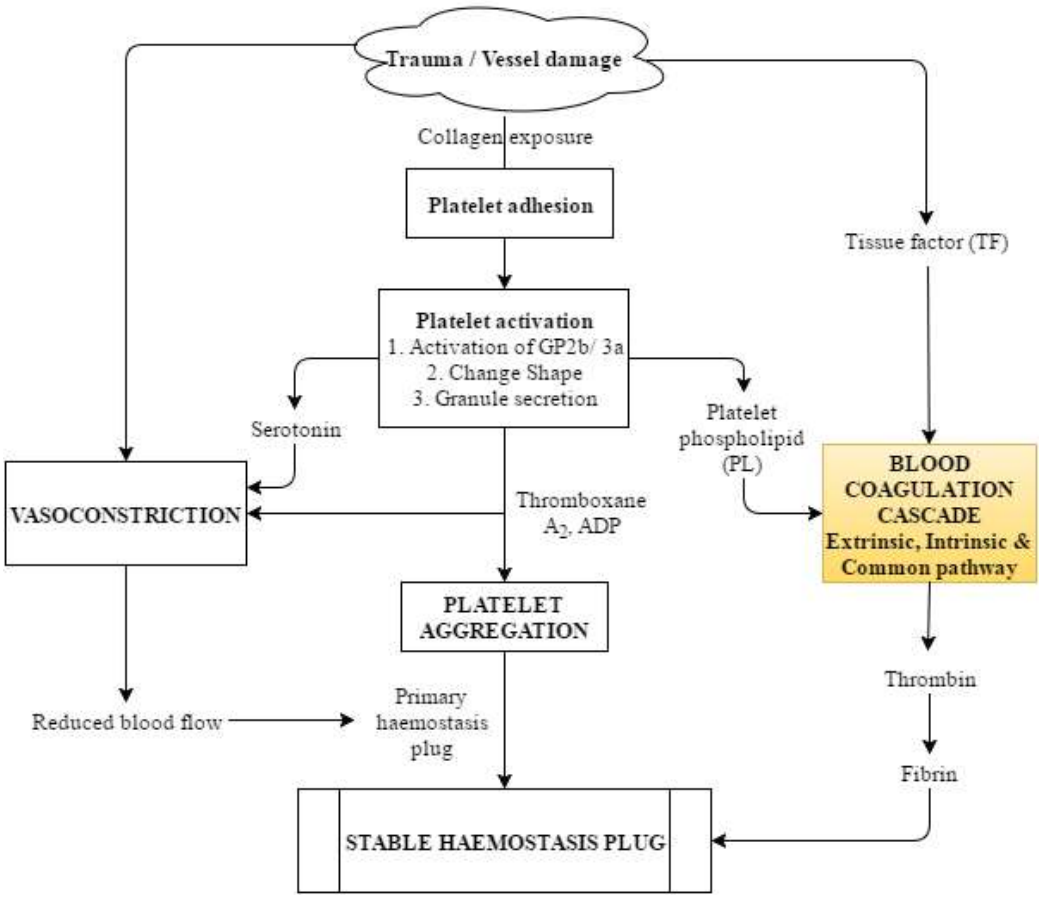

Figure 2.1 Haemostasis networks which involved three main mechanism; vasoconstriction, platelet aggregation and blood coagulation cascade (adapted from Hoffbrand *et al*., 2011)

2.2 Blood coagulation cascade

In vivo, initiation of blood coagulation begins when tissue factors (TF) express on cells is exposed to plasma contain FVII. According to Hoffbrand *et al*. (2011), only one to two percent of total factor VII present in activated formed while the rest are in inactive state whereby only become stimulated after bound to TF. TF or contact system is indirectly contributed to physiological of haemostasis (Peterson *et al*., 1995).

High binding of FVIIa on TF at vascular surface leads to the formation of TF-FVIIa complex. These, in turn, enable the generation of FIXa and FXa. The generation is initiates by serine protease activity (Figure 2.2). The generation of activated formed of factors subsequently allow the formation of thrombin and yet fibrin monomer (Furie *et al*., 2008). However, in the first stage of activation, only small amount of thrombin form from prothrombin. At this stage, it is expeditiously inactivated by tissue factor pathway inhibitor (TFPI). Therefore, second stage of activation is requires to outburst the production of thrombin. In the second stage of activation or so-called amplification, coenzymes FV, FVIII and FXI are back-activate by limiting the proteolysis after insufficient thrombin formed (Gugliemone *et al*., 2001; Chu, 2011).

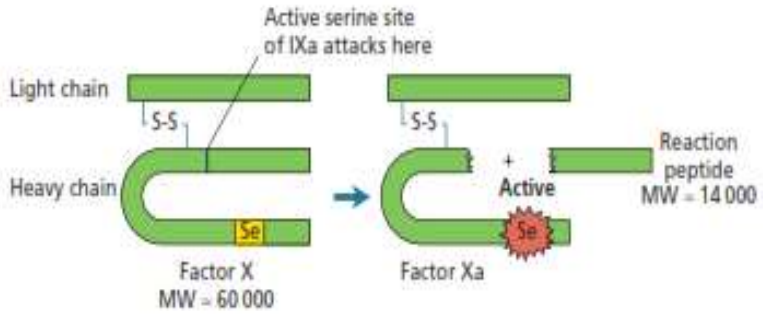

Figure 1.2 Activity of serine protease by cleaving FX to forms FXa.

During amplification, FXIa activate FIXa which form a complex with FVIIIa. This reaction occurs on vascular phospholipid surface of vessel in the presence of Ca. Formation of tenase complex (FVIIIa-FIXa) thus activates sufficient FXa. Activated FXa finally form complex with FVa and generate FVa-FXa prothrombin complex. Ultimately, enable formation of sufficient fibrin clot after outburst production of thrombin (Hoffbrand *et al*., 2011; Chu, 2011; Dahlbäck *et al*., 2005).

Briefly, the subsequent activation is played by different form of multimolecular complexes. Specifically, tenase complex (FVIIa- FIXa) and prothrombinase complex (FVa- FXa) act as procoagulant complexes which prepared by activated platelet. TF, FVa, FVIIIa known as cofactor, enzymes such as FVIIa, FIXa, FXa and substrate of serine protease (zymogen) are FIX, FX and prothrombin. Formation of complexes is induced by product formed by previous reaction of enzyme (Hoffbrand *et al*., 2011; Chu, 2011; Mcintosh, 2005).

In a typical pathway of coagulation measurement, prekallikrein (PK) and high molecular weight kininogen (HMWK) are believed to activate FXI, yet in latest finding of person with hereditary deficiency of these contact factors, shown the scarcity of abnormal bleeding. Due to that, it is been suggest that, these pathways are not involved in *in vivo* physiological of blood coagulation system (Hoffbrand *et al*., 2011; Bjarne, 1984).

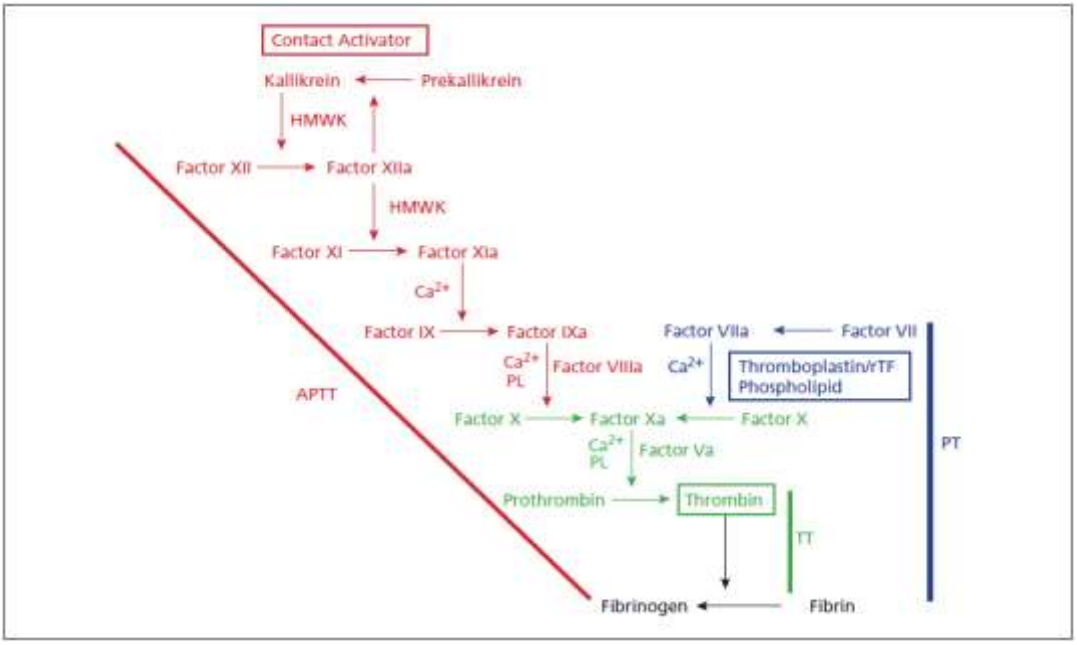

Figure 2.3 Blood coagulation cascade. Red represents intrinsic, blue is extrinsic and green is common pathway (Hoffbrand *et al*., 2011).

2.2.1 Feedback inhibition of the procoagulant response

Inhibition of procoagulant response is initiated by tissue factor pathway inhibitors (TFPI). TFPI are exists on cells surface, plasma, as well as spot of injury due to local platelet activation. TFPI will inactivate TF-FVIIa complex by generation of quaternary inhibited complex (Peterson *et al*., 1995).

Other inhibitors that play a pivotal role in inhibition of procoagulant response are anti-thrombin (AT) and protein C. AT produce inactive complexes with FIX, FX, FXI and impaired thrombin formation. Whereby, activated protein C (APC) together with it cofactor, protein S (PS) will rapidly inactivates the procoagulant cofactors FVa and FVIIIa by specific proteolysis. These reactions are known as negative feedback loop (Friedrich *et al*., 2001).

PC becomes activated by complex formation of thrombin with thrombomodulin (TM), an endothelial cell receptor. Activation of PC also is enhanced by another cellular receptor, called endothelial cell protein C receptor (EPCR) (Vanderwouwer *et al*., 2004). APC also will reinforce fibrinolysis by inactivate tissue plasminogen activator inhibitor (t-PAI) (Figure 2.4).

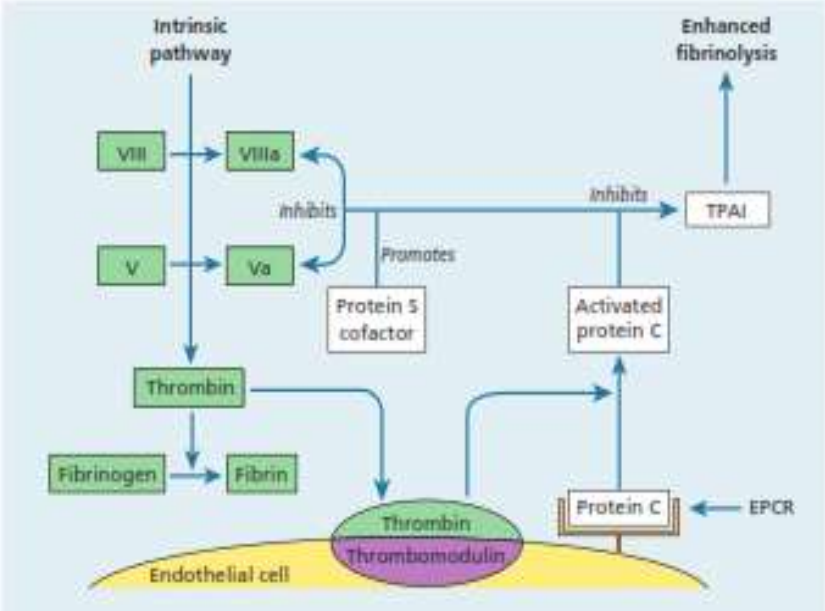

Figure 2.4 Mechanism involve in inhibition of procoagulant response (Hoffbrand *et al*., 2011).

2.2.2 Fibrinolysis

After formation of clot, removal of insoluble fibrin deposition is in urgent needs. The system involves plasminogen (PLG) and plasmin, PLG activator and several inhibitors of PLG activators and plasmin. PLG activator can be divided into two types which are endogenous (tissue or plasma derived) and exogenous (e.g. bacterial or venom derived) (Table 2.1) (Hoffbrand *et al*., 2011).

Fibrinolysis starts after plasminogen converted to plasmin, whereby plasmin would degrade at least 50 cleavage site of fibrinogen and transformed it into heterogenous mixture or so-called fibrin degradation products (FDPs). Plasmin also has a capability in hydrolyzing other substrate such as FV and FVIII (Thorelli *et al*., 1999). Conversion of plasminogen to plasmin is aids by intrinsic (vessel wall) and extrinsic (tissues) activation. Specifically, in intrinsic FVIIa and Kallikrein are involved, meanwhile in extrinsic, tPA and urokinase-like-A are involved (Figure 2.5) (Dahlbäck *et al*., 2004).

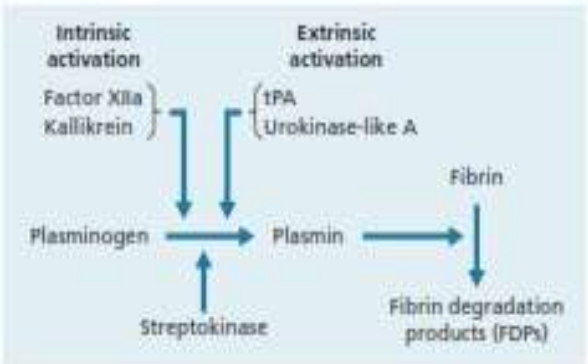

Figure 2.5 Mechanism of fibrinolysis. Formation of plasmin from plasminogen with the aids of PLG activators to formed FDPs (Hoffbrand *et al*., 2011).

In clinical practice, fibrinolytic agent is widely been used such as recombinant tPA, incorporated using recombinant DNA technology, streptokinase produce by streptococci and urokinase which is taken from human urine. The formation of plasmin at the spots of injury restraint the growing thrombus and the ruptured products of fibrinolysis also act as inhibitor to thrombin and fibrin polymerization (Dahlbäck *et al*., 2004). On the other hand, fibrinolytic system also may be destruct by inhibit the tissue plasminogen activator (tPA-i) and α_2- Antiplasmin (α_2-AP) (Hoffbrand *et al*., 2011).

Table 2.1: Key proteins involved in haemostasis network (Hoffbrand *et al*., 2011)

Common name	Main action
Tissue factor (TF)	Cofactor for FVII/ FVIIa
Prothrombin (FII)	Clots FBG, activates PC, FXI, TAFI
Factor V (FV)	Cofactor for FXa
Factor VII (FII)	Activates FIX and FX
Factor VIII (FVIII)	Cofactor for FIXa
Factor IX (FIX)	Activates FX
Factor X (FX)	Activates prothrombin
Factor XI (FXI)	Activates FIX
Prekallikrein (PK)	Anti - angiogenic, profibrinolytic
Factor XIII (FXIII)	Cross - links fibrin
Fibrinogen (FGN)	Mechanical stabilization of clot
Von Willebrand Factor (VWF)	Cell adhesion and FVIII carrier
Thrombomodulin (TM)	Cofactor in PC/ TAFI activation
Endothelial protein C receptor (EPCR)	Cofactor in PC activation
Protein C(PC)	Inactivation of FVa and FVIIIa
Protein S (PS)	Inactivation of FVa and FVIIIa
Tissue factor pathway inhibitor (TFPI)	Inhibition of coagulation initiation
Antithrombin (AT)	Inhibits thrombin, FIX, FX, FXI
Plasminogen (PLG)	Dissolution of clot in wound repair
Tissue Plasminogen Activator (tPA)	Plasma activator of plasminogen
Prourokinase (UK)	Tissue activator of plasminogen
Tissue Plasminogen Activator Inhibitor (t-PAI)	Inhibition of tPA and uPA
α_2- Antiplasmin (α_2-AP)	Inhibition of plasmin
Thrombin – activatable fibrinolysis inhibitor (TAFI)	Inhibition of fibrinolysis

2.3 Abnormalities of Coagulation

Haemostasis is vital in providing stable physiology of vascular system. This is because hemostasis able to balance formation of clot and thus cleaved it off to produce FDPs. Nonetheless, inherited and acquired disorder may disrupt the system and thus lead to hypocoagulation (bleeding) or hypercoagulation (thrombosis) of vascular system. Coagulation disorder can be passed down through genetics or develop later in life. According to James and Thomas (2008), coagulation disorder can be divided into two; thrombosis (hypercoagulation) and bleeding (hypocoagulation).

Thrombosis is related to deficiencies of antithrombin III and protein C or S cofactors, inhibition of fibrinolysis and related consumptive coagulopathies (disseminated intravascular coagulation [DIC]). Thrombosis can be divided into two; arterial thrombosis (form in artery) and venous thrombosis (form in vein). Arterial thrombosis can cause stroke, cardiac ischaemic and possibly peripheral vascular disease (thrombosis in leg arteries). Common cause of arterial thrombosis is artherosclerosis which narrowing the artery and thus block the blood flow to vital organ. After acute myocardial infarction and stroke, venous thromboembolism normally arose. It is represented in two events; acute pulmonary embolism (PE) and deep vein thrombosis (VTE) (Previtali *et al*., 2011).

Meanwhile, bleeding is associated with thrombocytopenia (immune mediatedor acquired), toxicosis (warfarin toxicity), inherited disorders (haemophilia A, von Willebrand's disease), primary fibrinolysis (hyperplasminemia), and disseminated intravascular coagulation [DIC]).

2.3.1 Anticoagulant of Thrombotic Disorder

Anticoagulant agent is a drug that has been developed to treat thrombotic disorder other than antiplatelet since 20 years ago (Garcia *et al*., 2009). The use of vitamin K antagonist is very effective and had contributed to the remarkable achievement for the prevention and treatment of most thrombotic disorder. However, still there were considerably limitations of the drug (Table 2.4) (Gresele *et al*., 2002**).** Due to that, development of novel anticoagulant has been keep progressing (Table 2.2).

Table 2.2: Antithrombotic agent used to treat thrombotic disorder (Gresele *et al*., 2002)

	Anticoagulant agents
First generation	✓ Heparin ✓ Warfarin
Second generation	✓ Low-molecular-weight heparins ✓ Hirudin
Novel approaches	✓ Inhibitors of tissue-factor–factor-VIIa pathway ✓ Selective factor Xa inhibitors ✓ Selective thrombin inhibitors ✓ Human activated protein C ✓ Soluble recombinant thrombomodulin ✓ Novel vitamin K antagonist

Warfarin, a coumarin derivative, dicoumarol (bishydroxywarfarin) is isolated from spoiled sweet clover by Prof Karl Link in 1939. This compound has proved to cause haemorrhagic deaths in cattle. Later, on 1955s warfarin was introduced as an anticoagulant agent and been used in clinical practice to treat thrombotic disorder.

Dicoumarol able to interfere in cyclic interconversion of vitamin K and 2,3 epoxide (vitamin K epoxide). The capability of dicoumarol highly contributed to the coagulation activity since FII, FVII, FIX and FX are vitamin K dependent protein, whereby vitamin K is required to perform γ- carboxylation to become activated. Hence, by inhibiting vitamin K conversion cycle, dicoumarol induced partially decarboxylated protein and finally reduced the anticoagulant activity (Hirsh *et al*., 2003).

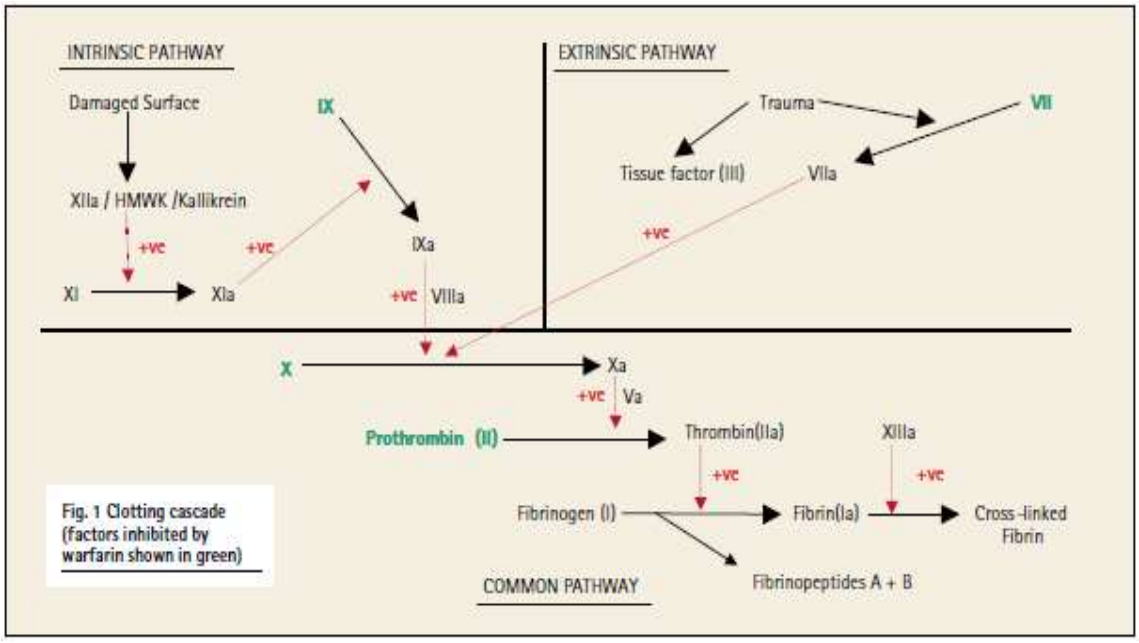

Figure 2.6 Warfarin actions on coagulation cascade which inhibit production of FVIIa, FIXa, FXa and FIIa (shown in green) (Rice *et al*., 2003).

Another first generation drug invented is heparin. Unfractionated heparin (UFH) with molecular weight approximately 15000 Da is a sulphated polysaccharide which binds to antithrombin (AT) and coagulation enzyme to inactivate thrombin. By inactivating thrombin, fibrin cannot be formed and also inhibit thrombin-induced activation of platelet, FV and FVII. Besides that, for inhibition of FXa, only binding to AT is required. Thus, in second generation, low molecular weight heparin (LMWH) is

invented. Small fragment of heparin with mean molecular weight of 4500 to 5000 Da is produced through chemical or enzymatic depolymerization and thus can only fit to bind at AT (Hirsh *et al*., 2001).

Hirudin is an anticoagulant agent that was taken from salivary gland of blood-sucking leeches (Hirudo medicinalis). It is a natural potent inhibitor of thrombin formation and is high quality anticoagulant agent (Markwardt, 2002). However, natural source of hirudin is inadequate thus limit it usage in clinical practice. Therefore, development of recombinant hirudin such as lepirudin and desirudin through gene technology is been developed. Lepirudin inhibit thrombin formation by blocking the active site pocket or catalytic site of thrombin. In contrary to heparin, lepirudin can bind to both free and clot-bound thrombin and is a direct thrombin inhibitor (Petros, 2008; Greinacher & Lubenow, 2001).

In third generation of anticoagulant agent, all of them are novel and some still warrant further study before release. Under each target, there are many agents have been progressed (Table 2.3). For inhibition of the tissue-factor–factor-VIIa pathway, modulating of inhibitor is aimed to invent the anticoagulant agent. Specifically, selective Xa inhibitor can be classified into two, which are direct which bind directly to FXa and indirect such as pentasaccharides which need AT to initiate the action (Gresele *et al*., 2002).

Whereby, selective thrombin inhibitor can react directly by binding to thrombin and thus prevent it interaction with substrate and indirectly, activates AT and cause inhibition of thrombin formation. Finally, by increasing soluble thrombomodulin, lead to activated protein C and subsequently formation of fibrin polymer (Gresele *et al*., 2002).

Table 2.3: Example of novel anticoagulant agent for each target (Garcia *et al*., 2009; Gresele *et al*., 2002)

Target	Agent
Inhibition of the tissue-factor–factor-VIIa pathway	✓ Monoclonal antibody against tissue factor ✓ Soluble inactive tissue factor ✓ Factor VIIai ✓ Recombinant nematode anticoagulant protein (rNAPc2) Yes ✓ Small synthetic inhibitors
Selective factor Xa inhibitors	✓ Direct natural inhibitors (tick anticoagulant peptide, antistasin, lefaxin) ✓ Direct synthetic inhibitors (DX9056, YM416, SK549) ✓ Direct factor Xa inhibitor (Rivaroxaban, Apixaban, Betrixaban, YM150, Edoxaban (U-176b), Tak-442, Otamixaban) ✓ Indirect factor Xa inhibitor (Idraparinux, Idrabiotaparinux & Pentasaccharide)
Selective thrombin inhibitors	✓ Dermatan sulfate ✓ Oral heparin ✓ Noncovalent active-site blockers
Human activated protein C and soluble	✓ Human recombinant activated protein C ✓ Recombinant soluble thrombomodulin
Vitamin K Antagonist (VKA)	✓ ATI-5923

Table 2.4: Anticoagulant agent available in market with its side effect (Jain *et al*., 2014)

Available drugs	Side effects
Warfarin	• Most Common – Tingling sensation, headache, chest, abdomen, joint, muscle pain, dizziness, shortness of breath, difficulty in breathing and swallowing, weakness, low blood pressure and shock. Severe active bleeding during pregnancy; documented hypersensitivity - fever, rash and hair loss. • Gastrointestinal - Nausea, vomiting, diarrhea and abdominal pain. • Central Nervous System - Fatigue, tiredness, uneasiness, weakness, headache, dizziness, loss of consciousness, fainting, coma and taste perversion
Coumarins	• Coumarins is a vascular purpura that causes skin necrosis. • This is associated with protein C deficiency and Malignancy. • Coumarins cross the placenta and cause spontaneous abortion and specific embryo abnormalities if administered in the first trimester of pregnancy.
Heparin	• Major bleeding. Heparin-induced thrombocytopenia (HIT), Paradoxical Thrombosis.
Fondaparinux	• Most Common- Mild bleeding, reduced platelet levels • (Thrombocytopenia), irritation, rash or itching at the injection site. • Blood- Bleeding, anemia, blood clot formation, postoperative bleeding and bruising. • Central Nervous system- Sleeplessness, dizziness and confusion. • Miscellaneous- Low blood pressure (hypotension), low potassium in blood, increase in liver enzymes (elevations of hepatic enzymes) and no excess of cardiovascular events.

Dalteparin	• Skin- Hair loss, skin necrosis. • Genitourinary- Blood in urine.(Haematuria) • Blood- Any bleeding event, blood clot in the spine. • Hypersensitivity- Allergic reactions, including itching, rash, fever, injection-site reaction, hypersensitivity reactions. • Local- Injection-site blood clot, wound hematoma, injection-site pain
Lepirudin	• Heart- Heart failure. • Skin- Bleeding in injection site, wounds and allergic skin reactions. • Eye and ENT- Nosebleed. (Epistasis) • Gastrointestinal- Gastrointestinal and rectal bleeding. • Genitourinary- Abnormal kidney function, blood in urine and vaginal bleeding. • Blood- Anemia and sepsis, Liver- Abnormal liver function. • Respiratory- Pneumonia. • Miscellaneous- Fever and infection
Urokinase	• Most Common- Severe bleeding. (Haemorrhage) • Heart - Heart attack, pulmonary embolism. • Blood- Decreased red blood cells and platelets. • Miscellaneous- Excess sweating.

2.4 *Mikania cordata*

2.4.1 Taxonomical classification and Nomenclature

Mikania cordata (Bum. F.) B.L Robinson belongs to family of Asteraceae. Generally, through the classification, *M. cordata* can be describes as vascular, seed, flowering, dicot and hempvine plant (Figure 2.7).

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnolipsida
Subclass	: Asteridae
Order	: Asterales
Family	: Asteraceae
Genus	: *Mikania*
Species	: *Mikania cordata*

Figure 2.7 Taxonomical classification of *M. cordata* (Adapted from NRCS, United States Department of Agriculture).

Scientifically, it is also called as *Eupatorium cordatum* Burm. F. It is commonly known as heartleaf hempvine due to its shape and trailing characteristic. In Malaysia, it is known as ulam tikus, akar lupang and selaput tunggul. Belows is the common or vernacular name of *M. cordata.* It is called differently according to particular language and region (Table 2.4).

Table 2.5: Nomenclature of *M. cordata* (Al Nayeem *et al*., 2011; Zerin *et al*., 2012; Biswas *et al*., 2011; Ahmed *et al*., 2008)

Country/Language	Common / Vernacular Name
English	Mile-a-minute
Bangladesh	Assamlata, Germanlata Taralata Dubainna lota and Refugee
Philippine (Tagalog)	Bikas
China	Mi Gan Cao
Indonesia	Sembung rambat
Thailand	Khikaiyanna
Malaysia (Malay language)	Ulam tikus, Akar lupang and Selaput tunggul

2.4.2 Distribution and Botanical Description

M. cordata is a creeping woody perennial which grow in tropical regions of Africa, Asia (Malaysia, Bangladesh, India, Philippine, China, Indonesia, Thailand), Brazil and South America (Argentina, Paraguay and Uruguay) (Chowdhury *et al*., 2011; Aguinaldo *et al*., 2003).

Physically, *M. cordata* is a plant with smooth vines and consist of 4 to 10 cm length of green leaves. The shape of leaves is deltoid-ovoid or so-called ovate heart-shaped. Apparently, it has long –petioled with pointed tip, truncate base and toothed margins. *M. cordata* also produce many white flowers (Ahmed, 2008) with length between 6 to 9 mm, cylindrical in shape which is borne in compound inflorescences (Figure 2.8). Typically, *M. cordata's* flower bloom during dry season (Al Nayeem *et al.*, 2011; Rahman *et al*., 2008).